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# Ruby River Arctic Grayling Restoration Project Completion Report

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**Project Background:** Montana Arctic grayling (grayling) were patchily distributed throughout Montana's upper Missouri River drainage prior to the mid-1850s; however, by the late 1900s, one of the last self-sustaining fluvial grayling populations resided in the Big Hole River (Liermann 2001). Grayling declines are attributed to overfishing, introductions of non-natives, and habitat degradation (Kaya 1990). By the mid-1980s, the Big Hole River grayling populations began to sharply decline which led to the creation of the interagency Montana Fluvial Arctic Grayling Workgroup (MAGWG 1995). In 1994, fluvial grayling in the upper Missouri River Basin were found to be warranted for listing under the Endangered Species Act but precluded by higher priority listings (USFWS 2014) and reduced abundances necessitated efforts to restore fluvial grayling (Kaya 1990). Previous attempts at restoring self-sustaining grayling populations in Montana were unsuccessful and stocking lacustrine fish in riverine habitats, stocking too young of fry, and the presence of non-native salmonids were hypothesized as causative factors (Kaya 1990). The upper Ruby River (Ruby River) was selected for grayling restoration due to size (~41 unfragmented miles of habitat), low gradient (mean of 0.7%), abundance of pool habitat, and low density of non-natives (Figure 1; Kaya 1992, Liermann 2001). This report summarizes restoration of grayling to the Ruby River.

**Project Goals:** The goal of the original Montana Fluvial Arctic Grayling Restoration Plan (restoration plan; MAGWG 1995) was:

> "The restoration goal for Montana grayling is the presence, by the year 2020, of at least five stable, viable populations distributed among at least three of the major river drainages (Big Hole, Jefferson, Beaverhead, Madison, Gallatin, Sun, Smith) within the historic range of Montana grayling in the Missouri River system upstream from Great Falls including those upper Missouri Basin waters within Yellowstone National Park. A population will be considered stable and viable in a stream when monitoring confirms that, for at least 10 years, successful stock recruitment exceeds mortality of reproductive adults to successfully compensate for stochastic factors and perpetuate the species within suitable habitats."

The restoration goal for the Ruby River reintroduction was to establish a stable, naturally reproducing population above Ruby Reservoir by 2005 (Byorth 1996). Three objectives were created to help achieve the goal:

1. Monitor survival, movements, and densities of introduced grayling to determine factors affecting success of reintroduction.
2. Through monitoring, document natural reproduction by 2002.
3. Attain stable to increasing population densities in sampling sections where natural reproduction equals or exceeds annual mortality for three consecutive years.

**Stocking/RSIs:** Grayling reintroduction to the Ruby River began in 1997 and continued through 2008. Stocked grayling were progeny of Big Hole brood populations at Axolotl and Green Hollow lakes. Grayling were first reintroduced by stocking age-0 (~1.8") to age-1 (6 to 9") grayling from 1997 to 2005, except in 2002 (Table 1). Remote site incubators (RSI) were used from 2003 to 2008 (Table 1). Stocking of age-1 grayling was discontinued after 2005 due to high mortalities and limited reproduction of stocked grayling compared to RSI-reared grayling (Cayer, pers. comm.). RSI reintroductions ceased after 2008 when FWP determined grayling had reached abundance, distribution, and age-class structure levels to presumably support a stable, viable population (Magee et al. 2012). No population augmentation occurred following 2008.

**Figure 1.** Grayling reintroduction area in the upper Ruby River. Electrofishing monitoring sections are shown in red and positive eDNA samples collected in the Sweetwater Bridge and Greenhorn sections are shown as green dots.

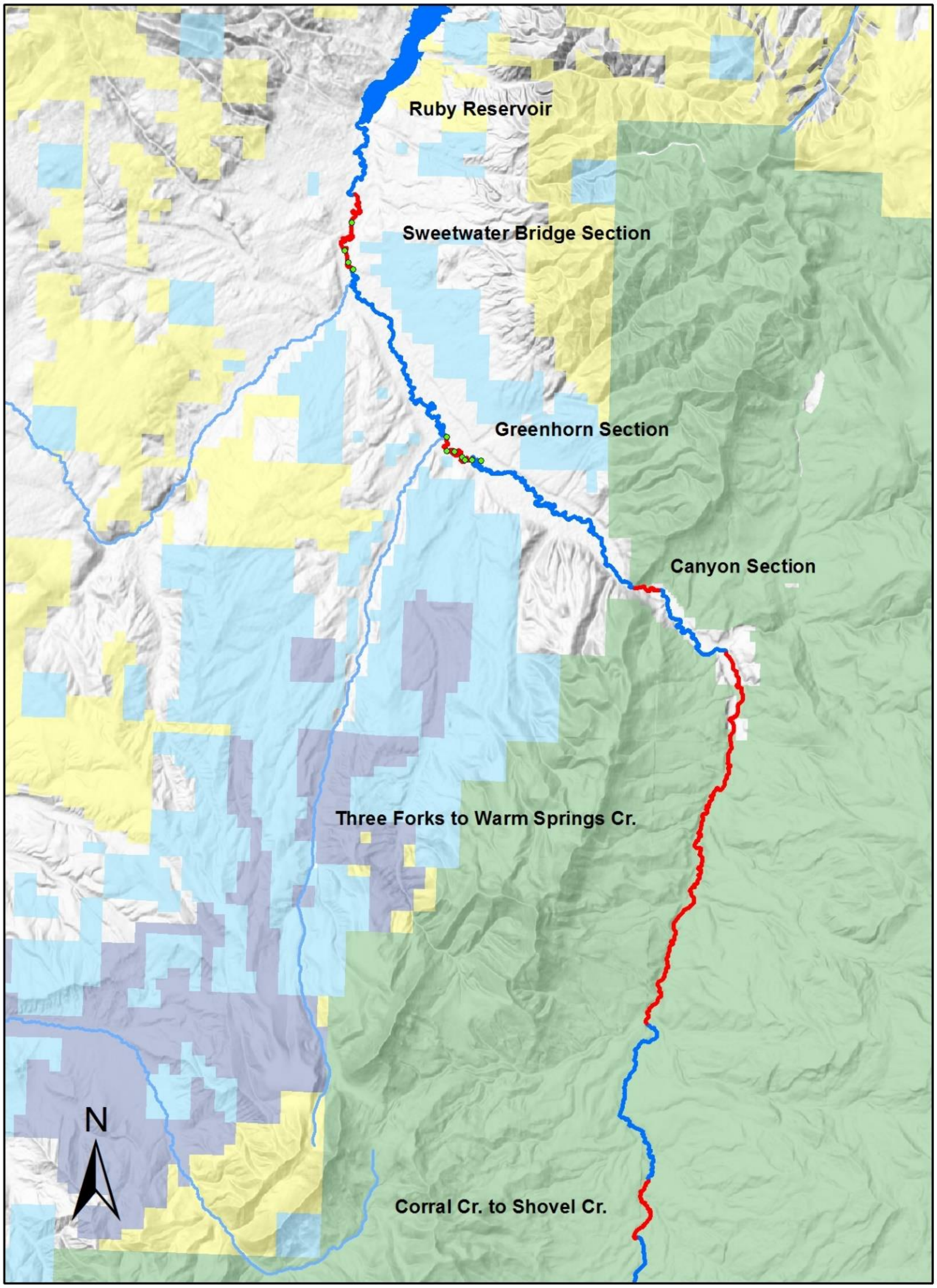

**Table 1.** Stocking rates and locations of grayling in the upper Ruby River, 1997-2008.

| Year | Number of Grayling/Eggs Stocked | Average Length (in.) | River Mile Range |
|---|---|---|---|
| 1997 | 29805 | 1.8 | 72.0-86.7 |
| 1998 | 9804 | 9.1 | 76.0-87.8 |
| 1999 | 7339 | 9.2 | 77.5-87.8 |
| 2000 | 10668 | 6.1 | 77.6-87.8 |
| 2001 | 1177 | 9.2 | 62.1 |
| 2002 | 0 | - | - |
| 2003 | 37183 | 8.1 | 77.6-96.5 |
| 2003 | 225600 | RSI | 90.0-96.6 |
| 2004 | 29863 | 8.2 | 77.6-87.2 |
| 2004 | 40320 | RSI | 80.0-96.6 |
| 2005 | 14470 | 8.2 | 45.0-96.3 |
| 2005 | 78000 | RSI | 80.0-96.6 |
| 2006 | 37200 | RSI | 68.2-96.6 |
| 2007 | 86800 | RSI | 68.2-96.6 |
| 2008 | 314000 | RSI | 68.2-96.6 |

**Electrofishing Surveys:** Natural reproduction of grayling was documented by electrofishing surveys in each of the past 10 years. We surveyed the Ruby River each autumn following cessation of stocking (2009-2018). Mobile-anode electrofishing equipment mounted on a drift boat, crawdad, or tote barge was used to survey various reaches, typically from the Middle Fork of the Ruby River to the Vigilante Bridge (Figure 1). Naturally-produced young-of-year grayling (YOY) were captured in all sampling events from 2009 to 2018 (Table 2). Recent electrofishing surveys suggest grayling densities are highest between Three Forks Cow Camp and the confluence with Warm Springs Creek.

**Table 2.** Grayling electrofishing survey results from the upper Ruby River.

| Year | Number of YOY Grayling | Total Number of Grayling |
|---|---|---|
| 2009 | 61 | 232 |
| 2010 | 39 | 96 |
| 2011 | 5 | 65 |
| 2012 | 11 | 98 |
| 2013 | 9 | 36 |
| 2014 | 1 | 35 |
| 2015 | 24 | 76 |
| 2016 | 5 | 70 |
| 2017 | 55 | 131 |
| 2018 | 3 | 37 |

**eDNA sampling:** Downstream grayling distribution was assessed using environmental DNA (eDNA) sampling in 2016. Electrofishing efficiency downstream of Warm Springs Creek is low and may not reliably be used to assess grayling presence or describe distribution. To assess grayling presence, environmental DNA (eDNA) samples were collected every 500 meters from the Sweetwater Bridge and Greenhorn survey sections by filtering 5L of river water through a paper filter, which were submitted to the National Genomic Center to analyze for the presence grayling DNA. Grayling presence was detected in multiple samples from each section. The furthest upstream positive sample for grayling DNA was 0.4 miles downstream of the Ledford Creek mouth, and the most downstream positive sample was 4.2 miles upstream of Ruby Reservoir (Figure 1).

**Genetic monitoring:** We collected tissue samples from grayling sampled by electrofishing to assess genetic diversity and demography of the restored population. Average expected heterozygosity ($H_e$) and allelic richness ($A_r$) were quantified with mixed age samples collected in each given year. Effective number of breeders ($N_b$), a measure of demography and surrogate for the genetic effective population size, was estimated for each cohort with adequate sample size using a combination of YOY, which could be assigned to cohort based on size, and older fish that were assigned to cohort by aging scales. Tissue samples (0.25 $cm^2$) were collected from the pelvic fin and placed in 2 ml screw cap vials with 95% non-denatured ethanol. Several scales were removed from the left side of the body between the lateral line and the dorsal fin and placed in an envelope. The scales were washed in the laboratory and three scales were pressed between microscope slides for each fish. Scales were aged using the Leica LAS Interactive Measurement module by two independent readers and, when differences in age occurred between readers, they were assigned an agreed upon age. The genetic data necessary to quantify $H_e$, $A_r$, and $N_b$ were produced by the University of Montana Conservation Genetics Laboratory following protocols described in Leary et al. (2015).

Measures of genetic diversity ($H_e$ and $A_r$) were relatively high and stable from 2010 to 2018 (Table 3). $H_e$ was similar to the Big Hole population, but $A_r$ was somewhat lower, which likely reflects the relatively small number of families used for reintroduction (Leary et al. 2015). 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. As such, monitoring should occur within one to two generations (3-6 years) and, if declines in $A_r$ and $H_e$ are observed, infusion of genetic diversity from the Axolotl and Green Hollow broods are management options.

**Table 3.** 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 |

**Conclusion:** Restoration of grayling to the Ruby River has satisfied all project goals and is considered successful and complete. Natural reproduction of grayling was documented by electrofishing surveys for ten consecutive years (2009-2018) following cessation of reintroduction efforts. Electrofishing and eDNA surveys indicate grayling are distributed over at least 37 miles of the Ruby River, from Three Forks Cow Camp to 4.2 miles from the reservoir. Although genetic diversity of the restored population was high and stable, genetic diversity of future generations should be monitored.

**Future management:** Future management of the Ruby River grayling population will consist of periodic monitoring and, if needed, genetic infusion. Because project goals have been met, intensive annual monitoring of this population is no longer required. Ruby River grayling will be monitored every three to six years to assess genetic diversity, unless survey results indicate more frequent assessment is warranted. Genetic monitoring data will be used to make decisions regarding genetic infusion, which would occur by introducing a relatively small number of fish from the Axolotl and Green Hollow grayling broods via RSIs.

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