EFFECTS OF HABITAT FRAGMENTATION BY TOSTON DAM ON GENETIC STRUCTURE, NATAL ORIGINS, AND HOMING OF BROWN TROUT IN THE UPPER MISSOURI RIVER

by

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ABSTRACT

Brown Trout became a key coldwater sportfish after their establishment in southwest Montana, where maintaining abundant Brown Trout populations is a priority for Fish, Wildlife & Parks. Toston Dam was built in 1940, blocking upstream fish passage, and creating a run-of-river, irrigation storage reservoir. The Upper Missouri River below Toston Dam was among 12 Montana rivers designated as Blue-Ribbon Trout Streams in 1969 but currently supports a Brown Trout population at low abundance. Biologists have been concerned with the decline in Brown Trout abundance in this reach of the Missouri River since the early 1990s. The effects of Toston Dam on Brown Trout in the Upper Missouri River sub-basin are not well understood. We therefore assessed how habitat fragmentation by Toston Dam affects Brown Trout in the Upper Missouri River to improve management of the species. Genetic analyses were used to assess whether Brown Trout above and below Toston Dam differ genetically. We used strontium isotopic and elemental ratios in water and otoliths to identify natal origins of Missouri River Brown Trout. We used radio telemetry to determine if migrating adults translocated above Toston Dam were attempting to return to Sixteenmile Creek, a presumed recruitment source for Brown Trout below Toston Dam. Results showed no genetic difference between Brown Trout above and below Toston Dam, but genetic structure within the sub-basin resembled patterns observed in native populations. Otolith microchemistry indicated only 11 of 80 Missouri River Brown Trout originated there. Fourteen fish were from tributary creeks, emphasizing the importance of tributaries for recruitment. Only three fish originated in Sixteenmile Creek, and just 1 of 60 telemetered fish ascended it, suggesting it is less important in sustaining Brown Trout below Toston Dam than previously thought. In contrast, the Gallatin River was important for Missouri River Brown Trout as 37 of 80 fish were from the Gallatin River and 32 telemetered fish ascended it. Brown Trout translocated above Toston Dam made extensive use of upstream waterways. Our multifaceted approach produced three complementary lines of evidence that will inform management aimed at improving connectivity, recruitment, and the sustainability of the Brown Trout fishery.

INTRODUCTION

As anthropogenic pressures on freshwater resources continue to grow, concern for the long-term persistence of freshwater organisms increases. Freshwater ecosystems occupy just a small fraction of the Earth (< 1%) but are crucial in supporting a large and diverse group of organisms (Strayer and Dudgeon 2010; Su et al. 2021). Freshwater fishes are the most diverse vertebrates and among taxa at the greatest risk of species extinction (Strayer and Dudgeon 2010; Burkhead 2012; Tickner et al. 2020). Leading risk factors impinging on aquatic ecosystems include invasive species, habitat loss and degradation, and climate change (Sala et al. 2000; Grooten and Almond 2018). These factors are often confounded and require focused research to understand a particular effect. This holds true in southwest Montana, USA, where the declining population abundance of Brown Trout *Salmo trutta* has prompted Montana Fish, Wildlife & Parks (FWP) to conduct an evaluation of habitat fragmentation effects on this important sport fish.

Invasions of nonnative fishes are one of the top threats to freshwater fish biodiversity worldwide (Sala et al. 2000; WWF 2018) but stocking and managing non-native sportfish to create and sustain recreational fisheries is a common management practice. Human assisted introductions have led to a rise in the occurrence of these invasions (Loewen et al. 2020). Non-native salmonids have long been stocked in Montana to bolster angling opportunities (FWP 2022). The Brown Trout, a salmonid originally from Europe, North Africa, and western Asia (Holton 1990), was a primary species stocked historically in Montana. The original introduction occurred in the Madison River drainage in Yellowstone National Park in 1889 (Brown 1971). Brown Trout tend to be successful in adapting to new environmental conditions (Valiente et al.

2009) because of their flexible life history traits (Jonsson and Jonsson 2011; Birnie-Gauvin et al. 2019). They often become naturalized and one of the dominant species present. After their establishment in Montana, Brown Trout became a key coldwater sportfish. They are particularly important in southwest Montana fisheries, where FWP manages wild Brown Trout across the region (FWP 2019).

Brown Trout can be effective competitors with native fishes (McIntosh 2000; Townsend 2003; McHugh et al. 2006) but the absence of Brown Trout does not necessarily lead to the success of native salmonids. Brown Trout and Rainbow Trout *Onchorhynchus mykiss* were introduced in Montana at the same time, often outcompeting native trout in large, mainstem rivers. However, the large decline in Westslope Cutthroat Trout *Onchorhynchus lewisi* distribution is mostly attributed to introgressive hybridization with Rainbow Trout (Allendorf and Leary 1988). While promoting Brown Trout success, FWP also aims to conserve the limited distributions of native Westslope Cutthroat Trout in southwest Montana (Jaeger et al. 2024). Managing for persistence of a competing non-native trout species might seem counterintuitive, yet interspecific competition is not the sole factor to be considered in most systems. Water temperature is key in dictating physiological processes of all fishes (Moyle and Cech 2004), as well as distribution of native salmonids (Keleher and Rahel 1996; Dunham et al. 2003). Brown Trout tend to have higher thermal tolerance and optimal growth temperature ranges than native Montana species such as Westslope Cutthroat Trout and Bull Trout *Salvelinus confluentus* (Bear 2005; Wehrly et al. 2007), which allow Brown Trout to thrive in low elevation systems, instead of being restricted to high elevation headwaters that are associated with native coldwater species. Despite differences in thermal tolerance, areas of species overlap between native and non-native

trout inevitably lead to concerns about interspecific competition. However, Brown Trout are replacing native Bull Trout, rather than displacing them, in places no longer thermally suitable for Bull Trout (Al-Chokhachy et al. 2016). Also, Brown Trout affect persistence of Westslope Cutthroat Trout the least among the three most common non-native trout in western Montana (Rainbow Trout, Brook Trout *Salvelinus fontinalis*, and Brown Trout; Bell et al. 2021). As climate change continues to alter habitat, non-native species are predicted to show greater resistance to it than native fauna (Sorte et al. 2013). Brown Trout in southwest Montana are expected to show lower levels of decadal declines in occupied stream length than native trout (Bell et al. 2021). Although non-native, preservation of Brown Trout will therefore help to maintain recreational fishing opportunities in Montana.

Manmade impoundment and diversion structures are common obstacles in waterways worldwide, creating fragmented habitat in over half of all rivers (Nillson et al. 2005). Only a small fraction of streams in the United States are not fragmented by dams or diversions (Benke 1990). The altered river systems fragment many freshwater fish populations (Nilsson et al. 2005; Liermann et al. 2012). The Montana Department of Natural Resources and Conservation (MTDNRC 2018) reported that more than 64,000 reservoirs exist in the State. Among these reservoirs, 3,007 have dams that meet the storage capacity criteria (> 7.62-m tall or > 1,233.48-m^3 water storage) to be listed in the National Dam Inventory (U.S. Army Corps of Engineers 2024); therefore, most dams in Montana are small but even small structures in the landscape can change connectivity and decrease gene flow within salmonid populations including those of Brown Trout, leading to interpopulation genetic differentiation (Costello et al. 2003; Moccetti et al. 2023). Dams can also make feeding and spawning grounds unproductive through alterations

of water depth, current, and sediment deposition (Kruk and Penczak 2003), compounding the negative effects of fragmentation on movements. Reduced spawning habitat in impounded mainstems makes access to associated small tributaries important (Kruk and Penzcak 2003). In large river basins, small tributaries often provide ideal spawning and rearing habitat, whereas the mainstems provide feeding and refuge habitat for adult fish (Klemetsen et al. 2003). Complete barrier removal is often the best way to increase river connectivity among high-quality feeding, refuge, and spawning habitats. Abundances of Brown Trout increase significantly after hydroelectric dam removal (Birnie-Gauvin et al. 2017).

Although dams are detrimental to riverine fish populations, options for mitigating their effects are limited. Barrier removal is typically not feasible, as many of the structures are still in use and provide valuable benefits to society. Fishways reconnect fragmented reaches but can be expensive, ineffective, and overly selective within and among local species requiring thorough assessment for each situation (Mallen-Cooper and Brand 2007). Fishways are generally not considered in situations where a structure halts further expansion of illegally introduced species. Physically moving fish to reconnect subpopulations might be warranted in such cases. Westslope Cutthroat and Bull Trout transported upstream of Milltown Dam, Montana, made extensive use of upstream spawning grounds (Schmetterling 2003) thereby diminishing and potentially reversing the declines of fragmented populations until the dam was removed. Fish translocation, despite its labor intensity, enables a modest annual reconnection of spawning fish to upstream habitat when dam removal or passive fish passage is not achievable.

Toston Dam was built in 1940 to create a run-of-river, irrigation storage reservoir on the Missouri River 24 km southeast of Townsend, Montana. The state-owned gravity-overflow dam

is made of concrete and has 7 inflatable rubber flashboards. At 15.7 m in height and 214.9 m in width, the dam impounds 5,057,275 m^3 of water at full pool. The gravity overflow section is 7.3 m high (MTDNRC 2021). A 9.66-megawatt Kaplan hydropower turbine retrofit was completed in 1989, creating the Broadwater Hydroelectric Project (Figure 1). Although Toston Dam allows for no upstream fish passage, downstream passage is possible. Water spills over the dam when discharges exceed turbine capacity (187.7 m^3/s). Downstream survival of passage through the turbine is unknown.

The effects of Toston Dam on Brown Trout in the Upper Missouri River drainage by impeding upstream passage for 84 years are not well understood. During autumn, upstream movements by spawning Brown Trout are halted by the dam. Local fishery managers have questioned whether these fish are inclined to return to their natal origins within the headwaters of the Missouri River or Sixteenmile Creek, a tributary 7.3 river kilometers (rkm) upstream of the dam. Based on its extensive drainage area and its abundant Brown Trout population, Sixteenmile Creek was presumed to be an important recruitment source of juvenile Brown Trout to the Upper Missouri River (R. Spoon, FWP, and B. Rehwinkel, retired FWP, personal communications). Whereas habitat fragmentation caused by Toston Dam raises concerns for Brown Trout, the dam also prevents the unwanted upstream invasion of other nonnative fishes such as illegally introduced Walleye *Sander vitreus* and Smallmouth Bass *Micropterus dolomieu* from below Toston Dam. The presence of invasive fishes, hydropower production, and agricultural use makes removal of Toston Dam for improved river connectivity unrealistic.

Sparse information exists about the genetic variation within and among Brown Trout populations in southwest Montana but lake and stream dwelling Brown Trout populations in their

native ranges are highly genetically structured, often showing significant genetic differences between neighboring subpopulations (Ferguson 1989; Moeller-Hansen et al. 1993; Östergen and Nilsson 2012). These distinct patterns of genetic variation indicate an overall genetic structure (Roberts 1993), whereas a population with completely random mating and dispersal would lack detectable genetic structure. Distance alone can be enough to create genetic variation in longitudinally connected Brown Trout populations (Carlsson and Nilsson 2000). Gene flow and genetic drift caused significant genetic variation between neighboring non-native Patagonian subpopulations over short timescales (Valiente et al. 2009). The effect of genetic variability on fitness of salmonids is not always clear, but decreased genetic variation can lead to a reduced probability of persistence (Wang et al. 2002). A better understanding of genetic structure could lead to improved management of Brown Trout populations in Montana, especially in fragmented habitat. Because loss of genetic diversity limits the potential of populations to adapt and persist, preservation of genetic variation within and among populations should be a primary goal of their management (Wang et al. 2002). Individuals from genetically diverse populations might be candidates for translocation, whereas populations with low genetic diversity could be prioritized for genetic enhancement efforts. This concept aligns with the current emphasis on incorporating conservation genetics into management, especially in preservation of small, isolated populations (Ralls et al. 2018; Kovach et al. 2021).

Our goal was to determine if habitat fragmentation by Toston Dam affects Brown Trout genetics, natal origins, and homing in the Upper Missouri River. Our objectives were to (1) determine if genetic variation of Brown Trout above and below Toston Dam differs, (2) identify natal origins of Missouri River Brown Trout from above and below Toston Dam, and (3)

determine if migrating adults below the dam are attempting to return to Sixteenmile Creek to spawn. Our focus was on the Upper Missouri River from Canyon Ferry Reservoir to the headwaters of the Missouri River including the associated Brown Trout spawning tributaries within that reach. We hypothesized that Brown Trout below Toston Dam would have distinguishable levels of genetic variation compared to fish above the dam, reflecting the extent of restricted gene flow caused by the dam (Objective 1). We hypothesized that over half of Missouri River Brown Trout originate from tributaries, highlighting the importance of mainstem-to-tributary connections above and below Toston Dam and determining if the dam prevents the return of spawning adults to their natal origins (Objective 2). We also hypothesized that migrating adult fish halted below the dam would use Sixteenmile Creek to spawn when moved upstream of the impassable barrier, demonstrating that Toston Dam prevents the return of Brown Trout to an important spawning tributary (Objective 3).

Multiple established techniques were used to address the research objectives, each supporting the goal of assessing the effect of Toston Dam on Brown Trout. The potential genetic consequences of Toston Dam on Brown Trout were explored by quantifying the amount of genetic variation and differentiation in Brown Trout above and below the dam. Genetic variation refers to the overall diversity of genes within a population, whereas genetic differentiation is a measure of how distinct populations are from each other. Characterization of the genetic variation of trout populations is common (Allendorf and Leary 1988; Ferguson 1989; Carlsson and Nilsson 2000; Wang et al. 2002; Whiteley et al. 2004; Östergen and Nilsson 2012). However, investigating Brown Trout genetics in Montana required the development of four microsatellite multiplexes to efficiently process Missouri River Brown Trout DNA. These multiplexes, which

allow for the examination of multiple microsatellite regions simultaneously, were developed at the University of Montana Conservation Genetics Laboratory (Missoula, Montana) in support of our study. Analyses of otolith microchemistry were used to identify natal origins of Brown Trout from the Missouri River in relation to Toston Dam. Strontium (Sr) elemental and isotopic composition (^{87}Sr:^{86}Sr) of Brown Trout otoliths were determined and compared to corresponding values from study-area waters. Juvenile Brown Trout typically spend the first year of life in their natal stream (Klemetsen et al. 2003; Cook and Bourret 2022) where alkaline earth metals are incorporated into discrete layers of calcium (Ca) carbonate of their inner ear bones (otoliths) as they grow. This process, combined with enough water chemistry variability throughout a basin and fish residency in targeted areas through the larval stage, makes this method a reliable way to link adult fish to their natal origin (e.g., Walther et al. 2008; Olley et al. 2011). Radio telemetry was used to determine the spawning destinations of Brown Trout translocated upstream of Toston Dam. Radio telemetry has frequently been used to document salmonid spawning movements in Montana (Schmetterling 2003; De Rito 2004; Grisak et al. 2012) and was effective in the Upper Missouri River sub-basin (A. Strainer, FWP, unpublished data).

We planned this research to understand the effects of fragmentation by Toston Dam on this Brown Trout population and to help guide future fisheries management in the area. A new Federal Energy Regulatory Commission (FERC) license for Toston Dam was finalized in 2022 and went into effect in 2024. The findings from our work will be used to guide the allocation of hydropower mitigation funds generated by the license effectively. Additionally, our project may serve as a foundational step for a broader regional or statewide assessment of the genetic structure of Brown Trout populations in Montana, addressing pressing questions regarding

population dynamics. Given the growing concerns about declining Brown Trout abundances in the state, fishery managers could benefit from identifying genetic patterns that expose evolutionary differentiation, variation, gene flow, and potential adaptation and selection among Brown Trout (Vieira et al. 2016). The application of this information could lead to improved management practices. Furthermore, examining Brown Trout populations in areas downstream of the Missouri River headwaters, where Brown Trout have experienced prolonged declines, may yield insights applicable to preventing population declines upstream.

Study Area

The Missouri River originates 46 km northwest of Bozeman, Montana, at Headwaters State Park where the Jefferson, Madison, and Gallatin rivers converge to form the longest river in Montana. The Missouri River flows 30.6 km before flowing through Toston Dam (Figure 2), the first of many dams constructed along its route. The location of Toston Dam drains an area of about 37,993 km^2. The U.S. Geological Survey (USGS) has operated a stream gauging site downstream of the dam since 1910. The annual mean flow for the 82-year period of approved record (1942 - 2024) was 140.2 m^3/s (USGS 2025). The study area included the 67 km of the Missouri River upstream of Canyon Ferry Reservoir and the tributaries that enter the Missouri River within that reach (Figure 2), including parts of the headwater sub-basins of the Madison, Gallatin, and Jefferson rivers.

The Missouri River fish species assemblage above Toston dam consists of native and non-native species. The native species include Westslope Cutthroat Trout, Mountain Whitefish *Prosopium williamsoni*, Longnose Sucker *Catostomus catostomus*, White Sucker *C. commersoni*, Plains Sucker *Pantosteus jordani*, Longnose Dace *Rhinichthys cataractae*, Stonecat *Noturus*

flavus, Burbot *Lota lota*, and Rocky Mountain Sculpin *Cottus bondi*. The non-native species include Rainbow Trout, Brown Trout, Brook Trout, Rainbow × Westslope Cutthroat hybrid trout *O. mykiss* × *O. clarki lewisi*, Common Carp *Cyprinus carpio*, Redside Shiner *Richardsonius balteatus*, Flathead Chub *Hybopsis gracilis*, Fathead Minnow *Pimephales promelas*, Yellow Perch *Perca flavescens*, and Northern Pike *Esox lucius*. The Missouri River species assemblage below Toston dam is the same as above with the addition of introduced Walleye and Smallmouth Bass.

The 36.4 km reach of the Missouri River from Toston Dam downstream to Canyon Ferry Reservoir was among 12 Montana rivers designated as Blue-Ribbon Trout Streams under the provisions of Section 89-801 passed by the Montana legislature in 1969 (FWP 1979). This area once supported abundant Brown and Rainbow trout populations, creating substantial angler activity (Fredenberg 1979). The river was managed by FWP exclusively for wild trout from 1973 to 2009. The department added Walleye as a low-end management priority in 2010 (FWP 2010). Since 2015, Walleye from Canyon Ferry Reservoir have migrated annually into the Missouri River. This colonization has been declared a foraging run and is not believed to involve spawning activity or result in resident Walleye (Strainer 2018). However, the increased abundance of Walleye is probably causing shifts in the fish assemblage of the river above Canyon Ferry Reservoir. The presence of Walleye raises concerns about their potential negative effects on river trout populations, especially naturally reproducing Brown Trout.

Brown Trout population abundance in the Missouri River below Toston Dam declined during the 1980s and remains low (R. Spoon, unpublished data). River population monitoring indicated a significant decrease in abundance, with spring abundance estimates of fish age two

years and older (age-2+) decreasing from 200 fish/km in 1979 to as low as 34 fish/km in 1993 (Figure 3). A similar trend was observed in Canyon Ferry Reservoir, where the total Brown Trout catch from sinking gill nets declined from 77 fish in the summer of 1984 to 26 fish in 1994 and has persisted at only 1 to 2 fish per summer in recent years (Troy Humphrey, FWP, unpublished data). Subsequently, Brown Trout abundance has declined sufficiently in the river that it is considered too labor-intensive to estimate, and autumn catch per unit effort (CPUE) is now used as an index of abundance instead. The CPUE for Brown Trout below Toston Dam was 0.41 fish/min in autumn 2024, with an average of 0.37 fish/min from 1994 to 2024. Brown Trout numbers in this area were formerly augmented with hatchery raised fish from a wild Missouri River brood. These fish stockings occurred only sporadically and the last comprised 62,272 age-1 fish in 1998 (FWP 2022). This self-sustaining Brown Trout population is thought to now rely heavily on recruitment of juveniles from the Missouri River tributaries (FWP 2020).

Limiting factors to Brown Trout survival and recruitment in the Upper Missouri River below Toston Dam include interspecific competition with unlawfully introduced species, predation by piscivorous birds, poor flow regimes, limited high-quality spawning habitat, and habitat fragmentation by Toston Dam (FWP 2020). Walleye were probably introduced into Canyon Ferry Reservoir in the early 1980s and first appeared in gillnets in 1989 (Yerk 2000). Northern Pike were first observed in nets in 1985. The first American White Pelican *Pelecanus erythrorhynchos* nests were discovered on islands in Canyon Ferry Reservoir ponds in 1989 (Hendricks and Johnson 2002), where a robust pelican nesting colony now exists (Fred Jakubowski, FWP, unpublished data). The addition of a hydropower component to Toston Dam in 1989 introduced the potential for turbine-related fish mortality, compounding any habitat

fragmentation effects already present. Additionally, occasional (once per month) hydropeaking at Toston Dam caused downstream water level fluctuations of less than 15 cm from 1989 to 2024 (J. Beck, retired MTDNRC, personal communication). Even small fluctuations can alter movement and holding patterns of juvenile Brown Trout (Naudascher et al. 2024), introducing another potential stressor to the Missouri River population. Fluctuations from hydropeaking were generally minimal and indistinguishable from natural variation, but early operational challenges occasionally caused large (~1.5 m) fluctuations in water level downstream of the Toston Dam (J. Beck, retired MTDNRC, personal communication). The timing of major fluctuations in the early 1990s may have exacerbated other emerging stressors to Brown Trout in the system.

Six perennial creeks flow directly into the Missouri River within the study area: Deep, Dry, Crow, Marsh, Warm Springs, and Sixteenmile creeks (Figure 1). Big Springs also flows into the Missouri River 1.4 km below Toston Dam, contributing up to 1.1 m^3/s of cold spring water to the river. Of these streams, Sixteenmile Creek has the largest drainage area (1,375.8 km^2) and is the only one to enter the river above Toston Dam (USGS 2022). Sixteenmile Creek is 111 km long and originates in the Crazy Mountains in the Gallatin National Forest at an elevation of 1,981 m. Sixteenmile Creek flows through varied geography consisting of timbered forest, sagebrush flats, and hay fields before culminating in limestone canyons and making its confluence with the Upper Missouri River. This confluence is 6.4 km upstream of Toston Dam, and 25.8 km downstream of Headwaters State Park (FWP 1989). Outside the study area, Beaver, Confederate, Duck, and Magpie creeks flow into Canyon Ferry Reservoir, but habitat degradation and limited connectivity have made them unproductive for Brown Trout spawning and rearing (R. Spoon, personal communication).

Sixteenmile Creek flows mainly through private land, which, along with few roads and rough terrain, makes the stream remote and difficult to access. The major anthropogenic effect on Sixteenmile Creek is agriculture, consisting of cattle grazing and irrigation for hay. Various levels of stream bank degradation and channel instability are present along much of the lower half of Sixteenmile Creek. In addition to normal negative effects associated with grazing cattle, a "Cattle Dip" insecticide spill in October 1969 led to the destruction of fish populations on the lowest 35.6 km of Sixteenmile Creek. Both Rainbow and Brown trout populations returned to previous abundances and size structure after 4 years (Workman 1981). The mean density of age-1+ Brown Trout at river kilometer 36.6 was 663 fish/km from 1972 to 1974; mean density in 2023 was 476 age-1+ fish/km. Monitoring the population status of fish in Sixteenmile Creek has been minimal and largely constrained by access opportunity.

METHODS

Fish Sampling

Four different electrofishing systems were used to collect fish for genetic analyses, otolith microchemistry, and radio telemetry (Reynolds and Kolz 2012) depending on stream size. All sampling on the Upper Missouri and Jefferson rivers was completed using a jet-boat-mounted electrofishing system, which included a fixed-boom electrode system on a 5.5-m aluminum boat. The system was powered by a 3,500-W, 120/240-V AC Honda gasoline powered generator. A Coffelt model VVP-15 rectifying unit was used to convert AC to DC. Optimum settings were 300 V with an output current of 7 A. Given its intermediate stream size, Sixteenmile Creek was sampled with a mobile-anode system. This system included a canoe that housed the same power source and rectifying unit as the jet-boat system. Instead of a fixed-boom positive electrode, a hand-held throwable electrode was attached to an extension cord to reach all areas of the stream. The negative electrode was suspended from the boat. A similar system was deployed from a drift boat to sample fish from the Madison and Gallatin rivers. Fish from Deep, Dry, Warm Springs, Marsh, and Crow creeks were collected using a backpack electrofisher (Smith-Root, Vancouver, Washington; Model 12-B) with one operator and one netter.

Genetics

Fin clips for genetic analyses were collected from 275 Brown Trout throughout the target rivers and streams (Figure 4) when most migrant fish were absent, and water conditions were conducive to high capture efficiencies. Sampling at the 5 river locations occurred from May 23 to June 28, 2022. Fin clips were collected from Brown Trout in 6 spawning streams from July 6,

2022, to April 28, 2023. Sampling in Warm Springs and Marsh creeks required two sampling events, leading to pooled samples (Table 2). Low sample size from Crow Creek (n = 2) was the result of poor habitat and high discharge during sampling. Sampling in Deep, Dry, Crow, Marsh, Warm Springs, and Sixteenmile creeks focused on juveniles to avoid including river migrants and ensure the samples represented progeny of fish that spawned in these streams. We included juvenile fish of multiple sizes in each sample to minimize bias from sampling full siblings or families. Sampling locations were selected based on access and habitat separation. The probability of detecting genetic differences among tributaries is greater within well-defined, distant habitats as fish there are less likely to interbreed with neighboring populations compared to more connected habitats. In contrast, sampling where fish can move frequently between connected or transitional habitats can obscure localized genetic differentiation (Morán et al. 1995).

Patterns of population genetic variation were evaluated using 16 polymorphic microsatellite loci (Keenan et al. 2013) to genotype Brown Trout. Samples were analyzed by staff at the University of Montana Conservation Genetics Laboratory. Staff extracted DNA using a detergent-based cell lysis buffer and ammonium acetate protein precipitation followed by isopropyl alcohol DNA precipitation. The extracted DNA was re-suspended in 100 µL TE (Tris-ethylenediaminetetraacetic acid) buffer. The DNA was diluted 1:10 and polymerase chain reaction (PCR) amplified in a PTC-200 thermocycler (MJ Research, Waltham, Massachusetts) using the QIAGEN Multiplex PCR Kit (QIAGEN, Valencia, California). Multiplex reactions used a volume of 10 µL and followed the QIAGEN Microsatellite protocol. In the PCR process, the DNA was heated to 95°C for 15 min to separate its two strands, 8 cycles of heating to 95°C

for 30 s, annealing at 57°C for 30 s with a gradual decrease in temperature, heating to 72°C for 2 min to extend the DNA strands, 32 cycles of heating to 95°C for 30 s, annealing at 60°C for 30 s, and again heating to 72°C for 2 min to amplify the DNA. Visualization of PCR products was performed on an ABI3130xl Genetic Analyzer (Applied Biosystems, Foster City, California) in the Murdoch DNA Sequencing Facility at the University of Montana. Allele sizes were determined using the ABI GS600LIZ ladder (Applied Biosystems). Chromatogram output was viewed and analyzed using Geneious Prime Version 2021.1.1.

Population summary statistics including average observed heterozygosity (H_O), average subpopulation expected heterozygosity (H_S), allelic richness (A_R), and pairwise genetic differentiation (G_{ST}) (Östergen and Nilsson 2012; Kovach et al. 2022) were used to investigate the genetic structure of Brown Trout populations in the Missouri River sub-basin. These were calculated using R packages adegenet v2.1.10 and hierfstat v0.5.11, and GenoDive (Meirmans 2020). STRUCTURE (Pritchard et al. 2000), a model-based clustering method that does not require predetermined grouping of individuals by population, was used to further evaluate population genetic structure among sub-populations in the study area. The model assumes K populations, where each population is defined by a set of allele frequencies at each locus. Each population (cluster) is assumed to be in Hardy-Weinberg equilibrium, and marker loci are assumed to be in linkage equilibrium with one another (i.e., alleles at different loci are independent). Individuals are assigned to the population to which they most closely belong. If their genotype indicates that they are admixed, they are assigned to two populations. In addition to the sample-based population genetic evaluation, STRUCTURE was used to demonstrate the presence of population structure and to assign individuals to populations.

Otolith Microchemistry

Water chemistry sites (n = 16) were selected to characterize spatial variability of trace elements and isotopes in the study area (Figure 5). Logistically, collection of samples from every one of the hundreds of possible upstream Brown Trout recruitment sources was not feasible. However, existing water chemistry (M. B. Duncan, FWP, unpublished data) of potential natal waterways upstream of our study area offered context on how water chemistries there compare to ours and indicated that sufficient variation probably existed to support using this method. Water samples were collected in the six potential spawning creeks that flow into the Missouri River, as well as the lower reaches of the Gallatin, Madison, and Jefferson rivers. Two water samples were collected from both Deep and Sixteenmile creeks because of their lengths: near their confluences with the Missouri River in both streams and downstream of known spawning sites at rkm 15.3 in Deep Creek and rkm 12.6 in Sixteenmile Creek. Samples were collected from 4 locations within the mainstem of the Upper Missouri River, as well as in Big Springs. Water samples were collected immediately before spring runoff during the emergence period of juvenile Brown Trout at 15 sites on May 23, 2022, and at Dry creek on May 19, 2023. Samples (50 mL) were collected and filtered using 50-mL syringes with 0.45-µm sterile filters. Each sample was preserved by adding 2 mL of a 35% nitric acid (HNO3) solution in an acid-washed polyethylene container. Water samples were stored in a refrigerator at above-freezing temperatures.

Water chemistry differences were assessed by determining Sr:Ca and ^{87}Sr:^{86}Sr ratios. Samples were processed at the W. M. Keck Collaboratory for Plasma Spectrometry at Oregon State University. Elemental-signature samples were diluted 5-fold with 250 µg/mL Cs from cesium carbonate in 2% (volume/volume) ultrapure nitric acid. Elemental concentrations were

measured on a Spectro Arcos II Inductively Coupled Plasma Optical Emission Spectrometer configured in end-on position. A blank of 2% HNO_3 was analyzed at the beginning of the run. To test procedural accuracy, two replicates of certified river water standard SLRS-5 were prepared in the same way as the samples and were run at the beginning and end of the analysis. These showed good agreement with certified values (e.g., Ca = 10.3 Ca µg/mL [2 SD ± 0.2] compared to an accepted value of 10.5 ± 0.4 µg/mL).

Water ^{87}Sr:^{86}Sr ratios were determined by isolating strontium from other elements using Eichrom Sr-spec resin to prepare for isotopic analysis on a Nu Plasma 3D Multi-Collector Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Approximate concentrations were determined so that all samples and standards were run at an equivalent concentration of ~20 ppb Sr. Contribution of krypton (Kr) on mass 86 was estimated using measured ^{83}Kr, and contribution of rubidium (Rb) on mass 87 was monitored using measured ^{85}Rb (no significant contribution of ^{87}Rb was observed). All ^{87}Sr:^{86}Sr were corrected for mass bias using the ^{88}Sr:^{86}Sr; all data were also adjusted post-run by normalizing to NIST NBS987 standard. The validity of this approach was verified through repeated measurements of secondary standards: EMD, with an accepted value of 0.70819 (measured as 0.708183 ± 0.000010, n = 7) and an in-house standard Hydrate Ridge, with an accepted value of 0.70917 (measured as 0.709167 ± 0.000008, n = 6).

Otolith microchemistry was used to identify the natal origins of 80 Missouri River Brown Trout captured from Trident, Montana, downstream to the river delta at Canyon Ferry Reservoir (Figure 5). The river was divided into four 8.3-km sections above and four 9.1-km sections below Toston Dam for sampling to avoid analyzing a disproportionate number of fish from one

section. The heads of nine to thirteen fish from each section were removed, bagged, and frozen until otolith removal. Fish ranged in size from 130 to 605 mm in length and 18 to 2,059 g in weight. Both sagittal otoliths from each fish were extracted using forceps, scrubbed, triple rinsed with ultrapure Milli-Q water, and dried under a laminar flow hood for 24 hours before storage in polyethylene vials. All instruments and containers were nonmetallic and acid-washed. One sagittal otolith from each Brown Trout was selected for laser ablation. Preparation for laser ablation involved mounting (sulcus side up) and sanding (Duncan et al. 2021). Otoliths were mounted on acid-washed microscope slides using cyanoacrylate glue and sanded to the nucleus with 320, 600, and 1500-grit sandpaper. Mounted otoliths were scrubbed with a nylon brush, triple-rinsed, and soaked in water overnight to break the mounting glue bond. The sanded otoliths were triple rinsed, dried overnight, and then randomly remounted with cyanoacrylate glue among four slides to minimize systematic bias from instrument drift.

Otolith microchemistry analysis was completed using laser ablation inductively coupled plasma mass spectrometry. Trace elements and isotopes were measured in separate assays with unique equipment settings and data reduction methods. Elemental concentrations were obtained using a Thermo Fisher Scientific iCAP RQ quadrupole ICP-MS whereas strontium isotopes were measured using a Nu Plasma 3 multi-collector ICP-MS. The spectrometers were coupled to an Applied Spectra RESOlution-SE 193 nanometer excimer laser. Elemental values were obtained by ablating otoliths along a vertical transect using a beam diameter of 50 μm with a repetition rate of 12 Hz for 30 s. The analyte of interest was calcium-normalized ^{86}Sr. A strontium ^{87}Sr:^{86}Sr isotopic ratio was obtained by ablating otoliths along a “U” shaped transect, wrapping the spot from the elemental analysis. Brown Trout otolith chemical concentrations from vertical transects

are highly correlated with those from horizontal transects and reveal similar values (Veinott et al. 2013). The beam diameter was 50 μm with a repetition rate of 12 Hz and transects advanced at 5 μm/s. The core of each otolith was ablated, with each transect being placed at the edge of the primordium, because natal origins were of interest. Mass bias and krypton interference were addressed using methods similar to those used in the water chemistry analyses.

Otoliths were analyzed in two runs of 40, with certified reference materials measured throughout each run for calibration and to correct for instrument drift (Munro 2004). Reference materials for elemental analysis were NIST-612 and MACS-3, which were measured every 10 otoliths. The NIST-612 glass standard was used as the primary calibration standard for data reduction. To verify the accuracy of the calibration, MACS-3 was analyzed as a secondary standard, and the recorded strontium concentration was compared with accepted values. The mean strontium concentration for MACS-3 was 7,689.2 μg/g ± 664.3, which falls within two standard deviations of the accepted value of 6,640 μg/g. The reference material for isotopic analysis was Batbjerg Clinopyroxene (Neumann et al. 2004), NanoSr (Weber et al. 2020), and AP gastro (Padilla et al. 2015). Analyses yielded average measured ^{87}Sr:^{86}Sr values of 0.70473 ± 0.00003 for Cpx (accepted value = 0.70447), 0.70788 ± 0.00006 for NanoSr (accepted value = 0.70756), and 0.70950 ± 0.00003 for AP gastro (accepted value = 0.70917).

Raw ICP-MS data were processed to exclude non-representative measurements recorded during and in between ablation intervals, ensuring accurate elemental and isotopic profiles. Elemental and isotopic concentrations were derived from raw counts of elemental data that were processed using LaserTRAM-DB v.1.0.0 (Lubbers et al. 2021), whereas raw isotope data were processed using a macro-enabled Microsoft Excel workbook developed by the W. M. Keck

Collaboratory. We used a conservative approach to data processing to ensure that comparable natal materials from each otolith were analyzed in each run, focusing on ablation depth to maintain consistency in the elemental and isotopic measurements. The maximum depth of the ablation craters on 16 otoliths were measured using a 3D laser profiler (KEYENCE, Itasca, Illinois; VK-X3000 Laser Confocal Microscope). The average maximum depth of the elemental ablation craters was 114.9 μm (SD ± 13.4 μm) and the average depth for the isotope transects was 50.8 μm (SD ± 10.6 μm). The isotopic:elemental depth ratio of 0.44 μm was applied to the 30 s elemental vertical transects, leaving the first 13.3 s for evaluation. To avoid surface contamination, the first 2 s of ablated material were not used (Veinott et al. 2012), leaving the next 11.3 s of ablated material for evaluation. Only the middle 1/3 of the calculated length of the "U" shaped isotope transect was used (90.4 μm ± 3.65 μm) to determine ^{87}Sr:^{86}Sr ratios. Srontium:Ca (mmol:mol) ratios were calculated from elemental concentrations.

We used strontium isotopic and elemental ratios in water and otoliths to enhance the accuracy of stream assignment for fish, leveraging the strengths of both metrics rather than relying on one. Strontium isotope composition provides a high degree of accuracy in assigning fish to individual streams because otolith uptake of isotopic strontium is nearly 1:1 (Muhlfeld et al. 2012; Cook and Bourret 2022). We also relied on elemental concentrations of calcium and strontium because a significant positive correlation between Sr:Ca ratios in water samples and Brown Trout otoliths exists. The relation among barium (Ba), magnesium (Mg), and manganese (Mn) concentrations in otoliths compared to concentrations in water is not as clear as that of Sr (Bath et al. 2000; Wells et al. 2003; Matetski et al. 2022). Therefore, Ba, Mg, and Mn were not used. Although positively correlated, trace elements in ambient water are higher than the

corresponding values in otoliths. To adjust for the difference in concentration, a partition coefficient ($D_{element:Ca} = (element:Ca)_{otolith}/(element:Ca)_{water}$) was applied to the element:Ca ratios from ambient water to predict the expected values in the associated otoliths (Muhlfeld et al. 2012). Partition coefficients for element:Ca incorporation into fish otoliths are species and element specific, but the process of material uptake into otoliths is mostly consistent among salmonids (Munro 2004). Therefore, a linear equation (otolith Sr:Ca [mmol/mol] = 0.3829x – 0.0314, where x is water Sr:Ca [mmol/mol]) that relates Sr:Ca in water and Brown Trout otoliths from the Clark Fork River Basin, Montana (Cook and Bourret 2022), was used in place of a locally developed coefficient.

We used simple bivariate plots and Euclidean distances to assign natal origins. Euclidean distance measures how different the chemical signature of an otolith is from the signatures of known water sources in a standardized multivariate space. A shorter distance indicates a closer match to a specific location. Estimation of natal origins using otolith microchemistry often relies on known-origin fish from probable juvenile recruitment sources to model expected chemical signatures, which are then used to assign the natal origins of unknown-origin fish (Veinott et al. 2012; Muhlfeld et al. 2012; Mikheev et al. 2020; Cook and Bourret 2022; Mikheev et al. 2022) but capturing known-origin fish from all of the many potential upstream recruitment sources in the Upper Missouri River basin was impractical. Instead, Euclidean distance was calculated for each otolith relative to each water body using the equation

$$d = \sqrt{(x_{otolith} - x_{water})^2 + (y_{otolith} - y_{water})^2}$$

where d = Euclidean distance, x = the Sr:Ca ratio, and y = the ^{87}Sr:^{86}Sr ratio. Fish were assigned

to the corresponding water body based on the shortest distance between otolith and water signatures in the standardized space. Strontium:Ca and ^{87}Sr:^{86}Sr ratios for otoliths and water samples were standardized to a mean of zero and a standard deviation of one to make them comparable on the same scale. Fish captured upstream of Toston Dam were only analyzed against the seven upstream water samples. The analyses of the 40 fish sampled from below Toston Dam included comparison to water samples from all 16 sampled waterways from above and below the dam. This approach ensured that fish caught above Toston Dam were not incorrectly assigned natal origins from below the dam. Ideally, water signatures differ enough to allow assignment of natal origins to individual or groups of streams with high certainty. However, Sr:Ca and ^{87}Sr:^{86}Sr ratios from sampled waterways overlapped enough that grouping did not allow for clear natal origin assignment for some fish. Therefore, a threshold was established to mitigate low-confidence assignments and reduce the risk of errors. The average Euclidean distance for all assignments was 0.460 (SD ± 0.307). Given the uncertainty of assigning fish to natal origins without accounting for all potential juvenile recruitment sources, the upper threshold was set at 0.767 (0.460 + 0.307). Any Euclidean distance exceeding the distance threshold value resulted in no assignment for that fish.

Radio Telemetry

Radio telemetry was used to determine if migrating adults would spawn in Sixteenmile Creek when given the opportunity. Specifically, 60 spawning-capable fish were equipped with radio transmitters to define the importance of Sixteenmile Creek as an upstream recruitment source for Brown Trout below Toston Dam. In autumn of both 2022 and 2023, 15 female and 15 male Brown Trout were captured in the 1.0 km reach immediately below Toston Dam and tagged

with radio transmitters. Tagging and translocation first took place on October 26, 28, and 31, 2022. The second group of fish was tagged on October 13, 17, 19, 20, and 23 and November 1, 2023. Each fish was anesthetized with tricaine methanesulfonate (MS-222), photographed, measured (total length, mm), and weighed (g). Mean length of telemetered Brown Trout was 538 mm (± 87.2 mm) and mean weight was 1,604 g (± 921.7 g). The average length and weight of males were 594 mm (± 78.5 mm) and 2,145 g (± 999.8 g), respectively. Tagged females averaged 482 mm (± 53 mm) in length and 1,063 g (± 350.1 g) in weight. Ultrasonography was used to confirm stage of maturity of female fish using a Lumify linear transducer (L12-4 iOS; 4-12 MHz; Philips, Bothell, Washington). The soft tissue exam was performed at a scanning depth of 4.0 cm, and gain was adjusted to 70-80. The transducer was placed in a Whirl-pak with lubricant for protection and was placed transverse on the abdomen. Females were classified as non-reproductive or spawning capable based on the presence of discernible ovarian follicles observed by means of ultrasonography (Crossman et al. 2022). Only spawning capable fish were tagged. Stage of maturity of male fish (non-reproductive or spawning capable) was determined by physical traits and manual expression of gametes (Crossman et al. 2022). All non-reproductive fish (n = 20) were excluded from tagging. Fish were placed upside down in a V-shaped operating table for surgery and gill irrigation. A 2-cm incision was made anterior to the pelvic girdle on the linea alba, and a transmitter was surgically implanted into the peritoneal cavity of each fish. The external antenna was routed using the shielded needle technique (Ross and Kleiner 1982), which places the transmitter and antenna exit away from the incision for better healing. The incision was closed with 2 or 3 simple interrupted sutures using Ethicon 3-0 PDS-II violet monofilament with FS-1 24-mm reverse cutting needles. Surgery time averaged 299 s (180 to 544 s). All 60

fish visibly recovered, and no immediate mortality occurred. Fish were held in an instream live car until full recovery before translocation 3.0 km upstream of Toston Dam. Average recovery time before release was 136 min (32 to 288 min). The release location was chosen for ease of access and to be at the upstream end of river inundation, where slight water velocity potentially helped fish orient upstream.

Lotek MST series tags (Lotek Wireless, Newmarket, Ontario) were used to allow a variety of options for tracking fish movements. The MST series tags have a motion/tilt sensor option that sends an active code when moving. If movement stopped, an inactive code was transmitted, which allowed for possible mortality detection. These tags had detection ranges of about 300 to 600 m. Transmitter frequency of 148.340 MHz was used as attenuation caused by deep (> 5 m) or high conductivity water (> 500 µS/cm) was of minimal concern (Cooke et al. 2012). Conductivity in the study area was typically ≤ 450 µS/cm. Battery life was extended by taking advantage of full schedule programming. The 30 transmitters deployed in 2022 were programmed to transmit at 10-s pulse intervals for 15 weeks after activation. This period of transmission was followed by 13 weeks of deactivation. The radio tags then transmitted again for the life of the tag, allowing for tracking into the second spawning cycle in autumn of 2023. Programming MST-930-M tags with these parameters created an estimated tag life of 486 d. The tags were expected to have transmitted until at least February 24, 2024. Transmitters used in the second autumn of tagging were activated before implantation, programmed at 5-s pulse intervals, and set to run uninterrupted for the life of the tag. Tags did not exceed 2% of the weight of the implanted individual to limit physiological effects (Adams et al. 1998; Zale et al. 2005). These MST-930-M tags weighed 4.5 g such that fish as small as 225 g could be tagged. The lightest fish

tagged was 531 g. Tag to body weight ratio was not limiting because spawning-capable adults were targeted. We chose unnecessarily small tags because smaller transmitters require smaller incisions and fewer sutures, leading to reduced handling and recovery time (Swanberg et al. 1999). MST-930-M transmitters are 9.5 mm in diameter and 32 mm in length. Fish were captured during an already physiologically demanding spawning period, so effort was made to reduce the additional stressors of capture and surgery, including by use of smaller tags.

Five fixed-station receivers (Lotek model SRX400 and SRX800) were positioned in strategic locations (Sites 1 through 5) in the study area on October 14, 2022 (Table 1; Figure 6). Site 1 was upstream of Sixteenmile creek near Trident, Montana, at rkm 3,718.2 of the Upper Missouri River (measuring from its confluence with the Mississippi River). The receiver at Site 1 recorded fish moving upstream into the headwaters and out of the focal study area. The fixed-station receiver at Site 2 was placed in lower Sixteenmile Creek at rkm 3.7 (0 rkm point is the confluence with the Missouri River) and recorded fish migrating into Sixteenmile Creek. Site 3 was placed upstream on Sixteenmile Creek at rkm 11.7 and was used to assess movements farther upstream. Site 4 was located immediately downstream of Toston Dam. This receiver detected fish that returned to the dam. Remote site 5 was placed upstream of Canyon Ferry Reservoir at rkm 3,654.2 on the Missouri River to detect fish emigrating downriver into the reservoir. The receiver at Site 3 was moved from Sixteenmile Creek to the Gallatin River at rkm 0.2 (measuring from its confluence with the Missouri River) in autumn of 2023 to monitor movements into the Gallatin River. Although our primary focus was on Sixteenmile Creek, fish relocations in 2022 indicated that positioning the fixed-station receiver in the Gallatin River

would detect more fish than leaving it in Sixteenmile Creek, thereby maximizing the number of fish relocations.

Fixed-station receivers were configured identically except that stations on Sixteenmile Creek and the Missouri River at Trident used SRX400 (Lotek) radio receivers, whereas sites on the Missouri River below Toston dam used the newer SRX800 data logging devices (Lotek). Both receiver models collected the same type of data but required different versions of the Lotek Host software. Pole-mounted 4-element Yagi antennas (Wade Antenna, Brantford, Ontario) were used at all sites. The power supply for each fixed-station receiver included a 100-W solar panel (Ameresco Solar, Dallas, Texas) top mounted on a pole, a 30-A, 12/24-V Prostar charge controller (Morningstar Corporation, Newtown, Pennsylvania), and a group 27M 12-V 90-Ah flooded deep cycle marine and RV battery (Duracell, Bethel, Connecticut). Components were housed in weatherproof security containers. Receivers were set up and installed using one frequency (148.430) with recording intervals of 10.5 s. Antennas detected radio frequencies from tags, each uniquely coded to correspond to an individual fish. Gain at each site was set using a transmitter held underwater at the shoreline opposite the station. Fixed stations were checked and downloaded periodically based on the amount of data collected.

Mobile, manual tracking supplemented fixed-station data collection. Manual tracking with a handheld 3-element folding Yagi antenna and a Lotek SRX1200 mobile receiver was conducted on foot and by vehicle. We manually tracked 2 to 3 times a week throughout the winter of 2022 and concluded on 6 February 2023 when the tags shut down for 13 weeks. Mobile tracking effort in the second year was reduced to 1 to 2 days a week throughout February and March. Most fish were detected beyond the most upstream fixed station at Trident, leading to

frequent mobile tracking along the Missouri River headwater rivers. Relocations were generally limited to river reaches accessible by roadways or public access points. Three fixed-wing flights were used to cover the study area quickly, extending the geographical range of tracking. Two flight surveys documented fish locations during December of 2022 and 2023. A 2.5-h flight on December 10, 2022, made a 354.4 km loop that covered the Missouri River from the mouth of Sixteenmile Creek to Headwaters State Park, the Gallatin River from its mouth to rkm 66.6 and rkm 60 on the East Gallatin River, the Madison River from rkm 0 to below Bear Trap Canyon (rkm 46.4), and the Jefferson River below Waterloo Bridge (rkm 87.7) down to its confluence with the Madison River. A subsequent aerial survey on December 20, 2023, covered the same areas with the addition of the Missouri River from Sixteenmile Creek to Canyon Ferry Reservoir. This 4-h flight spanned 555.7 km. Additionally, a 2-h and 45-min flight that covered 228.7 km was made on September 6, 2023, to document pre-spawn locations of telemetered Brown Trout.

All relocations included the tag code, geographic coordinates (decimal degrees), antenna gain, and associated signal power. Fish tag IDs were referred to using ‘F’ (female) or ‘M’ (male) to indicate sex, followed by a hyphen and the tag number (e.g., F-11 or M-48). Each relocation was classified as mobile (airplane, vehicle, triangulated, fish in hand) or fixed station. Fixed-station data were reduced to a single relocation per day, based on the highest power value detected from that tag. Power values were individually evaluated and filtered out if confidence in the detection was low. Fish could have two relocations in one day only if detected by both fixed-station and mobile antennas on the same day. Inactive tags were relocated multiple times throughout the study, but for analysis, each was counted only the first time it was found inactive. All remote-site data logging and mobile relocations were included in total relocation counts.

Locations were overlaid on a map with a river kilometer layer to determine distances traveled. Fish were assumed to have spawned at their uppermost location if they ascended a stream with suitable spawning habitat during the autumn spawning period.

RESULTS

Genetics

Allele counts and heterozygosity measures reflected varied genetic diversity of Brown Trout among locations (Table 2). All 16 microsatellite loci were polymorphic, with *Omy1001* being the only locus to have just 2 alleles. Locus *CoCl_Lav_4* had 49 null alleles across samples and was removed from analysis. A total of 180 alleles was found, ranging from 2 to 27 (*Ssa410*) alleles per locus. The average number of alleles per locus was 12 (SD ± 8.3). The average H_O ranged from 0.507 to 0.717, whereas average H_S ranged from 0.531 to 0.700 across loci. The average observed heterozygosity among all populations was 0.658 (SD ± 0.057), and the overall average expected heterozygosity was 0.665 (SD ± 0.050).

Fish in tributaries generally had lower average allelic richness (A_R) and higher average pairwise genetic differentiation (G_{ST}) than fish in mainstem sites. Allelic richness averaged 7.280 (SD ± 1.513) alleles per locus across all samples and ranged from 3.867 in Dry Creek to 8.698 in the Missouri River above Toston Dam. Average A_R across populations was 6.212 (SD ± 1.494) in the five creek tributaries and 8.348 (SD ± 0.264) in mainstem rivers. Average pairwise G_{ST} ranged from 0.067 in the Missouri River below Toston Dam to 0.296 in Dry Creek (Table 2). Commonly used interpretive thresholds suggest that values between 0.05 and 0.15 indicate moderate genetic differentiation, 0.15 to 0.25 indicate high differentiation, and values greater than 0.25 indicate very high differentiation (Hartl and Clark 1997). Genetic differentiation was significant for all but four pair-wise comparisons. Brown Trout from above and below Toston Dam in the mainstem Missouri River had the lowest differentiation (G_{ST} = 0.001) among all pairwise combinations (Table 3). Missouri River Brown Trout from above and below Toston

Dam also had nearly identical A_R (Mann-Whitney U test: U = 114, $P = 0.967$) (Figure 8). Missouri River Brown Trout from below Toston Dam also showed no apparent genetic differentiation from fish sampled in the Gallatin ($G_{ST} = 0.014$, $P = 0.096$) and Madison ($G_{ST} = 0.007$, $P = 0.232$) rivers, but they differed moderately from fish collected in the Jefferson River ($G_{ST} = 0.045$, $P = 0.001$). Although genetic differentiation of Brown Trout from the Missouri River above Toston Dam was statistically different from those sampled in the Madison ($G_{ST} = 0.019$, $P = 0.044$) and Gallatin ($G_{ST} = 0.025$, $P = 0.023$) rivers, these divergence levels are considered low (Wright 1965; Hartl and Clark 1997). Genetic divergence of Sixteenmile Creek and Missouri River above Toston Dam fish was also significant ($G_{ST} = 0.018$, $P = 0.066$) but considered low. Notably, Dry Creek had the lowest average A_R (3.867) and highest average pairwise G_{ST} (0.296), indicating that Dry Creek may be largely or completely genetically isolated from other Brown Trout populations in the study area.

Distinct tributary groups and a larger mainstem population were revealed when using allele frequencies to assign individuals to sub-populations rather than relying on predetermined grouping by sample location. Brown Trout from Dry, Warm Springs/Marsh, and Deep creeks each stood out as distinct clusters (populations). In contrast, fish from Sixteenmile Creek and the Madison, Gallatin, Jefferson, and Missouri rivers formed one large genetic cluster (Figure 9). The most informative partitioning was evident when 2 to 5 sub-populations were assumed to exist in the study area (i.e., K = 2 to 5); no biologically meaningful grouping occurred when K > 5. Assuming two populations (K = 2), Dry Creek fish were assigned as their own population. Assuming 3 sub-populations (K = 3), the groupings were Dry Creek fish, Warm Springs/Marsh Creek fish, and all other fish. With the addition of two more assumed populations, fish from

Deep Creek were grouped separately, along with a more ambiguous group containing the rest of the fish. Therefore, genetic structure was consistent regardless of whether sub-populations were determined by sample or assigned based on allele frequencies. Small, genetically distinct tributary groups were present, along with a larger group that resided in the expansive and well-connected waterways.

Otolith Microchemistry

Water Chemistry

Distinct water chemistry was present in some, but not all waterbodies. Strontium isotope ratios ranged from 0.70781 to 0.71593. Original Sr:Ca ratios ranged from 1.38 to 4.03 mmol/mol, and the adjusted values ranged from 0.50 to 1.51 mmol/mol (Table 4). Differences in water chemistry among streams were generally greater than within individual streams. Longitudinal differences were low in Sixteenmile Creek, Deep Creek, and the Missouri River below Toston Dam, whereas more variation existed between the sites in the Missouri River above Toston Dam, particularly in Sr:Ca ratios (Figure 10). The Trident Sr:Ca ratio was similar to those of the Gallatin and Madison rivers, whereas the Missouri River Sr:Ca below the confluence of Sixteenmile Creek was similar to that of the Missouri River below Toston Dam. Dry Creek Sr:Ca and ^{87}Sr:^{86}Sr ratios (1.38 mmol/mol and 0.71593) were most similar to those of the Madison River (1.91 mmol/mol and 0.71512) despite their spatial separation, and differed notably from other waterways in the study area. Water chemistry of Sixteenmile Creek was distinct when considering only the waterways sampled above Toston Dam. Strontium:Ca and ^{87}Sr:^{86}Sr ratios of Sixteenmile Creek were similar to those of Marsh and Warm Springs creeks, probably due to the influence of underlying and nearby geology, which includes sedimentary

sandstone and metasiltstone, as well as strontium-rich limestone and volcanic materials (Capo et al. 1988; USGS 2024a). Crow Creek water also showed distinct water chemistry, having the lowest $^{87}Sr:^{86}Sr$ ratio among all water samples (0.70781).

Otolith Chemistry

Seventy Missouri River Brown Trout of unknown origin were each assigned to one of 10 natal locations and 10 fish were unassigned (Figure 11). Otolith strontium isotope ratios ranged from 0.70854 to 0.71923, and strontium:Ca ratios ranged from 0.13 to 1.33 mmol/mol. Eleven (13.8%) fish were assigned to the Missouri River mainstem, and only 2 of those assignments were to below Toston Dam. Five fish were from the Missouri River below Sixteenmile Creek, and another four fish originated in the Missouri River near Trident. Forty-five (56.2%) fish originated from the headwater river tributaries of the Missouri River. The Gallatin River was the origin of 37 (46.2%) fish, comprising half of the fish collected from above the dam and nearly half (42.5%) of those from below (Table 6). The Madison River contributed an additional 8 (10%) fish. The small tributaries (creeks) were assigned 14 fish (17.5%). Big Springs, Dry, Crow, and Sixteenmile creeks were each assigned three fish. Marsh Creek was assigned two fish. Ten (12.5%) fish were of unknown origin. All but two fish were captured in sections of the Missouri River downstream from their natal origin. Six water sample locations did not have any fish assigned to them (Jefferson River, Missouri River at Indian Campground, Warm Springs, Sixteenmile Upper, and both Deep Creek locations). Therefore, the only waterways without any fish assigned to them were the Jefferson River, Warm Springs Creek, and Deep Creek. Notably, Warm Springs Creek was a migration corridor for the two fish from Marsh Creek.

The Euclidean distances between otolith and water chemistry signatures, which were used to assign natal origins, varied and led to differing confidence levels in these assignments (Table 5). The average Euclidean distance used for all assignments was 0.378 (± 0.189). The average Euclidean distance for fish assigned to the Missouri River was 0.257 (± 0.121), the lowest among all waterways (Table 5), allowing for high-confidence assignments to this location. In contrast, fish assigned to Dry Creek had the highest average Euclidean distance of 0.521 (± 0.163), leading to some uncertainty in assignment. The 10 fish that could not be assigned had an average Euclidean distance of 1.038 (± 0.355), well above the assignment threshold. Nearly half of the sampled fish were confidently assigned to the Gallatin River, with an average Euclidean distance of 0.343 (± 0.191).

The chemical signatures of the 10 unassigned fish most closely matched those of the Gallatin River (6 fish), Madison River (2 fish), and Dry Creek (2 fish). Notably, applying an assignment threshold did not affect Missouri River assignments, supporting the assumption that shorter Euclidean distances correlated with higher-confidence assignments. Assignments to Dry Creek and the Madison River had the highest average Euclidean distances, and each had fish excluded from assignment.

Radio Telemetry

Telemetered Brown Trout were relocated 1,020 times from autumn 2022 through spring 2024; 236 relocations were mobile. Each trout was relocated with a mobile receiver an average of 3.9 (± 3.7) times. The first aerial survey on December 10, 2022, revealed the location of 27 of 30 Brown Trout. During the second flight, on December 20, 2023, we located 18 tags from 2023 and 7 tags from 2022. We relocated 10 tags on the September 6, 2023, flight that targeted pre-

spawn locations of telemetered Brown Trout. The most-upstream locations of 27 fish were documented during aerial surveys, with 18 in 2022 and 9 in 2023. Mobile surveys resulted in the detection of the most-upstream locations of 22 fish (9 in 2022 and 13 in 2023) and fixed stations documented the most-upstream locations of 11 fish (3 in 2022 and 8 in 2023).

Brown Trout translocated above Toston Dam made extensive use of upstream waterways (Tables 6, 7, and 8). However, only 1 out of 60 fish ascended Sixteenmile Creek. Fish F-66 ascended Sixteenmile Creek on November 4, 2023, and was found near a riffle with newly made redds on November 16, 2023. Fish F-66 travelled at least 24.5 rkm (20.1 rkm within Sixteenmile) from the release point before its tag was found inactive onshore among scattered fish scales on December 19, 2023, likely the result of a predation event. Most fish ascended the Gallatin River in both 2022 (16 fish) and 2023 (16 fish) including 11 Brown Trout in the mainstem Gallatin River, 8 in the Gallatin River above the confluence with the East Gallatin River, and 5 in the East Gallatin River. Brown Trout entered six Gallatin basin tributaries: three fish entered Smith Creek, and single fish ascended Dry, Thompson, Camp, Baker, and Rey creeks. Fish F-47 briefly entered the Gallatin but did not move past the fixed-antenna array, instead returning to the Missouri River. Seven Brown Trout moved into the Jefferson River in 2022 (Figure 13), and three were recorded there in 2023 (Figure 14). All relocations within the Jefferson River sub-basin occurred in the Jefferson River, except for one fish (F-63) that ascended the North Boulder River. Two and four fish ascended the Madison River in 2022 and 2023, respectively. Only 3 fish did not leave the Missouri River, but an additional 8 fish were not documented outside of the Missouri River sub-basin (Figure 12 and 14); these included 7 trout that were detected at the Trident fixed station (Site 1) and not located farther upstream. In 2022,

three fish (M-16, M-29, and F-30) were located at Trident and then were not located upstream but probably ascended one of the Missouri River headwater tributaries. Likewise in 2023, four fish (F-50, M-56, M-59, and M-60) were located at Trident and then were not located upstream but could have ascended either the Madison or Jefferson rivers. They could not have ascended the Gallatin River, as the fixed-station receiver there would have captured their movement into the river. The farthest upstream location in the Gallatin River sub-basin was reached by F-28 in the East Gallatin River, 84.5 rkm from the release point. The farthest upstream location among all telemetered fish was reached by M-21 found in the Jefferson River, 124.2 rkm upstream from the initial release point (Table 8 and Figure 13). The most upstream relocation of a fish ascending the Madison River sub-basin was 48.3 rkm from the release point (Table 9), reached by F-67.

Only four fish (M-17, F-33, F-46, and F-66) did not swim past Trident. Fish M-17 travelled 19.5 rkm above the release point and stopped near Clarkston, Montana. Fish F-33 made it to the Trident fixed station before either dying or expelling its tag, resulting in nearly 2,000,000 inactive tag readings. Fish F-46 was the only fish released and then not seen again until it returned to Toston Dam on March 12, 2024. Fish F-46 stayed in a location where mobile receiver detection was difficult or was missed by the Trident fixed station. The inactive tag near the Trident station caused the data logger memory to fill up quickly, creating some data gaps. Fish F-46 may have passed upstream of the Trident site on November 2 or December 12, 2023, and returned downstream on March 11, 2024, when the logger memory was full and not recording data. Fish F-66 was the fish that ascended Sixteenmile Creek.

Most fish made deliberate movements to spawning locations and returned downstream of Toston Dam after the spawning season. Within four days of translocation, 43 Brown Trout

travelled 28.6 river kilometers in the Missouri River to the Trident remote site. All but 10 fish did so within nine days of being moved. No immediate return downstream of the dam occurred, but 33 out of 60 fish eventually returned to below the structure. The exact timing of the return of fish tagged in 2022 could not be determined because the tags were powered off when fish returned downstream of the dam. Most fish tagged in 2023 that returned below Toston Dam did so at about the same time of year as those tagged in the previous year. No active tags were located above Toston Dam when we targeted pre-spawn locations of Brown Trout in September 2023, indicating fish did not stay near their upstream spawning sites. Four inactive tags were located in the Missouri and Jefferson rivers and Baker and Rey creeks (one tag in each). Five active tags were found in the Missouri River below Toston Dam, and one was found in the south end of Canyon Ferry Reservoir; it was later found inactive on the pelican nesting islands.

Thirteen telemetered fish tagged in 2022 were located immediately below Toston Dam in the autumn of 2023. They were detected at the dam anywhere from 1 to 105 days in the period leading up to and during the spawning period. One female (F-18) was relocated in the tailrace of the dam on 117 days, arriving in July 2023 and descending to Canyon Ferry Reservoir on January 17, 2024. Fish F-18 was detected on all but 5 days from October 10, 2023, to January 16, 2024. Conversely, four females (F-14, F-22, F-30, and F-32) were each detected at the dam for just one day in the months of June, September, and October. Three males (M-21, M-29, and M-36) tagged in 2022 returned to the dam in the autumn of 2023 and stayed there for extended periods. Fish M-36 was detected on 56 days from July 29, 2023, through September 24, 2023. Fish M-29 was detected on 105 days through August 11, 2023, to December 8, 2023. In contrast, one male (M-27) was detected at the dam on just one day (November 11, 2023).

DISCUSSION

Genetics

We documented population genetic structuring within Missouri River Brown Trout. The species has been present in Montana for just 135 years, which is a brief span in genetic terms. However, some patterns of genetic structure similar to those seen in populations within their native range have already developed in Upper Missouri River Brown Trout. Fish in larger, more connected waterways tended to have greater allelic richness and lower differentiation (Figure 7 and 8), especially in Missouri River fish from above and below Toston Dam, which had the highest A_R and lowest G_{ST}. Samples from neighboring tributaries showed discernable allelic differences, but no differences were found in Missouri River Brown Trout above and below Toston Dam, despite the dam preventing upstream fish migration for 84 years. Conversely, some native Brown Trout populations were genetically isolated longitudinally by distance within a river, especially when a migration barrier was present (Carlsson and Nilsson 2000; Carlsson and Nilsson 2001; Östergen and Nilsson 2012). The lack of genetic differentiation between fish from above and below Toston Dam indicates that downstream gene flow through the dam is occurring at some level, or the genetic effective population size (N_e) is large and genetic drift (random changes in allele frequencies) is minimal. The genetic effective population size is the evolutionary equivalent to demographic abundance (i.e., the evolutionary "size" of the population) and dictates the rate at which genetic variation decreases, and genetic differentiation increases due to finite population size (Waples 2013; Waples 2024). Effective population size often correlates with the number of breeding individuals contributing alleles to a population, but it is not strictly the count of those individuals in the actual population. Instead, N_e reflects the

size of a hypothetical, idealized population that would experience the same rate of genetic drift as the actual population that experiences real-world complexities including uneven sex ratios, variable reproductive success, and population fluctuations (Wang et al. 2016). A small population typically has a proportionately small N_e, making it more susceptible to genetic drift and the resulting random changes in allele frequencies. Brown Trout density in the Upper Missouri River below Toston Dam is low, which could suggest that genetic drift is mitigated by genetic contributions from upstream individuals.

In contrast to the mainstem populations, many of the creek populations appeared to be genetically divergent from others in the study area. Brown Trout in Dry Creek have probably been genetically isolated since their arrival in the drainage. A similar pattern of increased genetic divergence and decreased genetic variation has been observed in local Westslope Cutthroat Trout populations that are isolated in the Elkhorn Mountains. Although physically reconnecting these fragmented Cutthroat Trout populations is unlikely, genetic rescue through the introduction of a small number of individuals has been shown to improve genetic diversity and survival (Bell 2022). Dry Creek flows out of a mountainous and forested canyon before entering a lowland agricultural area, where it is diverted into an irrigation ditch. The 11 water rights associated with Dry Creek include 4 senior rights with the earliest priority date of 1868, totaling 0.34 m^3/s, which regularly dewaters the stream. Remaining outflows, if any, are joined by irrigation water from Big Springs Ditch just above its confluence with the Missouri River. Stream alterations and dewatering appear to have contributed to genetic isolation by preventing fish movement into the stream, strongly influencing the genetic variation of Brown Trout in this population. If Brown Trout isolation continues in Dry Creek, genetic rescue may become the only option for

maintaining genetic diversity and long-term population viability. Across the sub-basin, fish from each of the creeks had the five lowest levels of allelic richness (Figure 8), and the four highest levels of average differentiation. The one exception was Sixteenmile Creek, which had the sixth highest average pairwise G_{ST} (0.094), a value that was closer to those observed for Brown Trout collected in the well-connected Missouri (0.067 and 0.077), Madison (0.75), and Gallatin (0.082) rivers. The low differentiation in Sixteenmile Creek could be due to its proximity and good connection to the Missouri River and its headwater rivers.

Brown Trout in the Jefferson River were an exception to our general finding that large river populations tended to have low differentiation, as they showed the fifth highest average pairwise G_{ST} (0.114). Jefferson River fish also showed the fourth highest allelic richness (8.103), which indicates that isolation by distance does exist for Brown Trout in Montana, but perhaps at larger geographic scales than observed within the Upper Missouri River sub-basin. Jefferson River fish genes may also be influenced more by upstream populations than by downstream groups, explaining the maintained genetic variation but elevated average pairwise differentiation.

Our results show promise for using genetics as a management tool for Brown Trout in Montana. Robust genetic information is already available for native Montana species such as Cutthroat Trout and Bull Trout, facilitating targeted management actions, but much remains to be uncovered about nonnative sport fish such as Brown Trout. Brown Trout have variable straying rates (Frank and Baret 2012; Mikheev et al. 2020) that affect genetic variation. Despite these variable straying rates, we demonstrated that genetic structure (i.e., differentiation) exists, at least at certain spatial scales, among Brown Trout in the Upper Missouri River sub-basin. However, a comprehensive genetic database will be needed to fully leverage genetic structure for effective

management. Limitations in funding, laboratory capacity, and labor currently hinder the development of such a database, but a genetically informed fisheries management plan has considerable potential to conserve diversity and increase resilience and productivity of Brown Trout populations in Montana. Microevolutionary processes such as genetic drift, selection, and gene flow play a critical role in shaping the genetic structure of populations across a landscape (Manel et al. 2003). These genetic processes appear to be influencing Montana Brown Trout populations, even within the relatively short period since their introduction. Genetic drift may have already led to reduced genetic diversity in smaller, more isolated populations such as those in tributaries. Migration and gene flow through larger waterways such as the Missouri River can counteract these effects, placing emphasis on the importance of connectivity.

Otolith Microchemistry

Most Brown Trout sampled from the Missouri River originated from other waterways, highlighting the importance of tributary contributions to the Brown Trout population in the river. Juvenile recruitment has long been considered a limiting factor in this section of the Missouri River (R. Spoon, personal communication), and these findings reinforce this idea. With only 14% of sampled fish having natal origins within the Missouri River, connections to other sources of Brown Trout recruitment are important. Brown Trout populations in large Montana rivers (Cook and Bourret 2022; M.B. Duncan, unpublished data) and within their native range (Olley et al. 2011) often rely heavily on tributaries for juvenile recruitment. The reliance on tributary contributions of trout is particularly true for the river below Toston Dam where only 2 out of 40 fish received assignments to the reach. The headwaters of the Missouri River appear to be significant recruitment sources for fish in the mainstem. Our data indicate that tributary

populations, especially those in the Gallatin and Madison rivers, play a substantial role in sustaining the Brown Trout population in the Missouri River, particularly in the absence of significant recruitment within the mainstem itself.

Density may influence trout migration into the Missouri River, but it is probably not the primary factor. Brown Trout emigration from densely populated headwater rivers can be density-dependent (Olsson et al. 2006; Chapman et al. 2012), but the 10-year mean age-1+ densities in the Madison (420 fish/km) and Gallatin rivers (169 fish/km) do not support this assumption. Despite the lower density of Brown Trout in the Gallatin River compared to the Madison River, the Gallatin River appears to contribute more fish to the Missouri River. The East Gallatin River might be the source of some of these Brown Trout, as the 10-year mean age-1+ density is 163 fish/km. Rainbow Trout are also abundant in the Madison and Gallatin rivers and probably influence density-dependence dispersal as well. Stream discharge is a significant driver of recruitment throughout the entire range of Brown Trout (Richard et al. 2015; Warren et al. 2015; Lobón-Cerviá et al. 2018). Annual precipitation and discharge in the lower reaches of the Gallatin, Madison, and Jefferson rivers are variable, yet the stable flows from tributaries and springs in the Gallatin sub-basin may play a crucial role in adult spawning, egg incubation, juvenile rearing, and outmigration to downstream waters (Kawai et al. 2013). Groundwater sources are particularly beneficial, as they are generally associated with optimal water temperatures for development and growth of salmonids at multiple life stages (Hansen 1975; Crisp 1988; Elliot and Hurley 2000). The first tributary to enter the Gallatin River, a spring-fed stream, is located 0.8 rkm from the mouth of the river. The first tributaries to enter the Jefferson and Madison rivers are at rkm 25.6 and 37.5, respectively. The proximity of the first tributary in

the Gallatin River to the mouth, compared to the more distant first tributaries of the Jefferson and Madison rivers, may provide a distinct flow and temperature advantage for Brown Trout recruitment. Regardless of the mechanism, the Gallatin River currently plays a key role in the recruitment of Brown Trout to the Missouri River.

Fourteen out of eighty Brown Trout had natal origins in five different creeks within the study area. Three fish were assigned to Sixteenmile Creek, which was fewer than expected given the streams anticipated importance as a recruitment source for the Missouri River. The other 11 fish were assigned to tributaries below the dam. The assignments to Big Springs and Marsh Creek are plausible, as Brown Trout spawn in both streams (C. Pipinich, personal observation), indicating these areas may contribute Brown Trout to the Missouri River. Crow and Dry creeks were assigned three fish each but the average Euclidean distances for fish assigned to these creeks were the highest among all waterways, equating to lower confidence in their assignment than among fish assigned to other streams. Crow and Dry creeks have been altered extensively and experience seasonal dewatering in certain reaches (R. Spoon, personal communication). However, fish could still originate from these streams. The lowest 0.4 km of Crow Creek remains connected to the Missouri River and might support minimal spawning and rearing activity. An irrigation diversion structure restricts upstream migration during certain periods of the year. The habitat just upstream of the diversion is low-gradient and inundated with fine sediment, resulting in poor spawning conditions for trout (Reiser and Bjornn 1979). Dry Creek flows out of a mountainous and forested canyon before entering a lowland agricultural area, where it is diverted into an irrigation ditch. Remaining outflows, if any, are joined by irrigation water from Big Springs Ditch just above its confluence with the Missouri River. Alterations to Dry Creek for

irrigation have disrupted its connectivity with the Missouri River, making upstream migration unlikely. However, downstream emigration may still occur during high-flow periods outside the irrigation season.

The absence of assigned natal origins to certain waterways does not preclude those streams from contributing Brown Trout to the Missouri River. For instance, no fish were assigned to the Jefferson River, despite a good connection to the Missouri River. The lower Jefferson River near Willow Creek, Montana, has a 10-year mean age-2+ Brown Trout density of 81 fish/km. Although Brown Trout density in the Jefferson River is lower than in the Gallatin and Madison rivers, a proportion of these fish could still emigrate downstream to the Missouri River (Chapman et al. 2012). No fish were assigned to Deep Creek, despite documented juvenile Brown Trout emigration into the Missouri River during springtime rotary-screw-trapping (R. Spoon, unpublished data, 2003-2022). Deep Creek was the focus of the first project conducted under the original Toston Dam FERC license agreement to mitigate the increased trout mortality after turbine installation (FERC 1991). Deep Creek was severely dewatered formerly but now remains wetted due to changes in irrigation infrastructure, water-user cooperation, and an in-stream flow lease. These improvements have enabled Brown Trout to reach the Missouri River (Snelson 1996). However, heavy predation would further reduce the likelihood of Brown Trout from Deep Creek being represented in our sample. Seasonally high Walleye abundance in the river, along with their occasional presence in Deep Creek (C. Pipinich, personal observation), increases predation on small out-migrating trout.

Limited otolith sample size, incomplete representation of water sources, and the limitations of our assignment methods prevented us from assigning fish to certain waterways and

determining natal origins of 10 fish. The sample of 80 fish may not have been fully representative of all nearby natal sources. Some fish originating from the Jefferson River may inhabit the Missouri River but were simply not represented in our small sample. Additionally, the waterways included in the evaluation represent only a subset of the many possible upstream recruitment sources. Fish may have originated from sources not included in the study, which could explain why 10 fish remained unassigned to an origin. Six of ten unassigned fish have relatively low Sr:Ca ratios (< 0.5 mmol/mol), indicating they probably come from a stream with underlying geology similar to Ray Creek, where few strontium-rich limestone or carbonate constituents exist (Capo et al. 1998; USGS 2024a). The water chemistry of more than 70 waterways among the Big Hole, Beaverhead, Ruby, and Madison river sub-basins has been characterized as part of ongoing research into the natal origins of Brown Trout in southwest Montana. Preliminary data from these upstream locations indicate that some fish in our sample may have natal origins far upstream of the Missouri River (N. Hudson, Montana State University, unpublished data). In fact, when all available upstream water chemistry was included in our analysis, only two fish were not assigned an origin.

Another potential factor affecting our ability to assign fish to natal origins is the limitations of the assignment methods, which may not have been sensitive enough to detect subtle differences in the otolith signatures of fish from these streams. Limited differentiation among waterways makes it challenging to assign natal origins to individual streams, especially in large and complex systems (Gillanders 2001; Munro 2004). In the Missouri River near Craig, Montana, Munro (2004) was unable to differentiate between two major spawning tributaries, preventing the assignment of fish to individual streams. Studies using known-origin fish from

local streams have achieved high accuracy in assigning natal origins to adult fish (Veinott et al. 2012; Muhlfeld et al. 2012; Mikheev et al. 2020; Cook and Bourret 2022; Mikheev et al. 2022). Capturing fish from each of the hundreds of potential upstream recruitment sources was not feasible, but sampling juveniles from the six spawning streams that flow into the Missouri River in our study area could have helped create more precise assignment groups. This approach would also have enabled the development of a local partition coefficient for adjusting water Sr:Ca. Using early juvenile growth to establish baseline site differences could have also helped minimize potential assignment errors related to maternal influence on otolith core chemistry (Hegg et al. 2013; Veinott et al. 2013; Bourrett et al. 2022).

Despite uncertainties in assigning some fish to individual streams, the research objective of determining the natal origins of fish in the Missouri River was met. A significant proportion of these fish originate from tributaries. The failure to assign natal origins to every fish sampled remains important, because it highlights the limitations of the techniques in a large basin such as the Upper Missouri River (Munro 2004). Otolith microchemistry remains a valuable tool for natal origin assignment, but its application should be thoroughly thought out before use in some systems. Also, the productivity of a tributary varies over time (Nicola et al. 2008), and some recruitment sources considered to be critical contributors are not always productive, particularly during extended periods of drought. Our study included fish and water from just one year, and results could be much different on an interannual basis (Walther and Thorrold 2009).

Radio Telemetry

Instream flow, water temperature, and fish translocation timing could have hindered trout from ascending Sixteenmile Creek. Only one telemetered Brown Trout ascended Sixteenmile

Creek and the others entered other waterways, indicating that Sixteenmile Creek was a minor spawning destination of trout from below Toston Dam and not as important as originally thought. Slight day-to-day variations in environmental factors enhance spawning runs of Brown Trout in their native range, but no single cue is enough to initiate migration (Ovidio et al. 1998). Sudden increase in flows trigger Brown trout migrations but if flows are not sufficient for easy entry into a tributary, Brown Trout will either stage at the mouth of the creek or move on to another location (Campbell 1977; Schulz and Berg 1992), as any hinderance to entry into a spawning location can lead to straying (Healy et al. 2023). The instream flow of Sixteenmile Creek during the duration of the study period is unknown, but the mean annual discharge in 2022 for nearby USGS gauging sites on the Missouri River at Toston (109.3 m^3/s) and the Shields River near Livingston (4.4 m^3/s) were well below the long-term averages at each site (USGS 2024b, 2024c). Annual discharge was above average in 2023, but this improvement in local flow conditions did not cause a large increase in fish migration into Sixteenmile Creek. Water temperature and dissolved oxygen concentration play key roles as well, which may be why the Gallatin River sub-basin emerged as the primary destination. High Brown Trout redd densities occur below instream groundwater upwelling areas (Hansen 1975). The higher prevalence of spring tributaries in the lower Gallatin River compared to the nearby Madison and Jefferson rivers probably contributes to its use as spawning habitat. We observed less channel freezing in the lower Gallatin River sub-basin compared to the lower Madison and Jefferson river basins during aerial surveys in December 2022 and 2023, probably due to the mixing of relatively warm groundwater with cooler surface water. The timing of migration is also important. Most Brown Trout begin spawning movements in September and October, which coincides with when we

moved fish but some Missouri River Brown Trout move into tributaries as early as June (A. Strainer, unpublished data). My sampling design would probably have precluded such individuals from being tagged.

Annual differences in autumn temperatures probably influenced the spawning movements of Missouri River Brown Trout. Cold temperatures slow fish metabolism and reduce movements compared to warmer conditions (Cook and Bergersen 1988; Jonsson 1991; Schulz and Berg 1992). The autumn of 2022 was much colder than that of 2023. Reflecting air temperatures, the average monthly water temperature at Toston Dam was 1.1°C in November and 0.1°C in December 2022, both of which were below the long-term averages of 2.8°C and 0.3°C, respectively. In contrast, the mean water temperatures in November (3.1°C) and December (0.8°C) 2023 were warmer than their respective long-term averages (USGS 2025). The much colder temperatures during the 2022 spawning season may have restricted fish movements, limiting their range and making them easier to relocate. The farthest upstream relocation among all fish occurred in 2022, but overall fish movements appeared reduced compared to 2023. The aerial survey conducted in December of 2022 revealed the locations of 27 fish, whereas only 18 tags from the 2023 fish were detected during the December flight in 2023. Nine fish in 2023 probably moved beyond our effective monitoring range, using upstream reaches of mainstem rivers or tributaries such as Sixteenmile Creek and the North Boulder River. The observation that fish moved farther in 2023 under more optimal swimming temperatures is supported by the higher number of fish (8) in 2023 than in 2022 (3) detected near Trident that were located only once and then not detected again until they returned downstream after spawning. The number of fish whose most upstream location was the Trident fixed station increased slightly from three in

2022 to four in 2023, but the most upstream location of an additional four fish was the Gallatin fixed station in 2023.

Mobile tracking and fixed stations effectively determined where fish from below Toston Dam migrated to spawn but had limitations. The fixed stations collected important information on fish movements within and outside of the Upper Missouri River sub-basin. However, most fixed relocations (423 of 784) were the result of four fish that remained near the Toston Dam fixed station during their second season with a tag, and one fish that stayed near the Trident station for weeks at a time while returning downstream post-spawning season. Little can be inferred about the spawning location of a fish for which the uppermost location was a fixed station because only fish presence at that specific location is recorded. Without repeated detections at the fixed station, or detections beyond the station, inference about the true spawning site is limited. Mobile ground tracking improved documentation of upstream movements beyond the focal 67 km reach of the Upper Missouri River, but inconsistent fish detection limited analysis options (Roger and White 2007). Flights provided comparable surveys but were too expensive to do frequently, and the timing of each flight date was inconsistent. Overall, the findings provided by radio telemetry were descriptive, limiting inferences to movements and not the reasons for them. Many telemetry studies on trout migration used higher relocation occurrences (i.e., once daily to once every two weeks) than we did, allowing inference to be made about fine-scale movement patterns, habitat use, and behavioral responses to environmental changes. However, the drainages examined in those studies were smaller than ours (Meyers et al. 1992; Young 1994; Brown and Mackay 1995; Ovidio et al. 1998; Schmetterling 2003; Rustadbakken et al. 2004). Greater resources would have been necessary to

achieve a similar level of precision in tracking movements in our much larger study area by increasing mobile tracking efforts, aerial surveys, and the number of fixed telemetry receivers placed upstream of the Missouri River.

Toston Dam physically prevents access to upstream spawning habitats, potentially influencing the straying rates and spawning success of Brown Trout. Brown Trout translocated above the dam made extensive movements to spawn. Sparse information exists about the extent of spawning migrations made by non-native potamodromous Brown Trout, but some movements exceeding 100 km were comparable to those made by anadromous Brown Trout in their native range (Jonsson 1985; Thorstad et al. 2016). Although none of the translocated fish returned downstream immediately, most eventually did, much the same as native Brown Trout in Belgium (Frank and Baret 2012). Some upstream-migrating fish were halted by the dam in both years, remaining there for variable amounts of time in the second spawning season. Fish that remained at the dam for less than a day probably left after they encountered the barrier, as physical obstacles can promote straying (Keefer and Caudill 2014; Healy et al. 2023). Other fish that stayed at the dam throughout the spawning season may have spawned below the barrier or persistently attempted to pass it. High discharge from power station outlets often attracts migrating salmonids and can lead to extended delays in upstream movement, even when fish passage is possible (Thorstad et al. 2008). These delays, combined with physical and chemical changes in the river, can impose additional stress on fish, detrimentally affecting spawning success through physiological changes such as follicular atresia and decreased fecundity (Corriero et al. 2021). Limited data suggest that Toston Dam has minimal effects on water quality parameters from upstream to downstream, except for decreased total suspended solids

and turbidity during runoff and increased total dissolved gas pressure (Hydrometrics 2022). No major increase in total dissolved gas pressure has been documented from above to below Toston Dam during the autumn spawning period. However, emerging fry and other resident Brown Trout below the dam could be at risk of gas bubble disease (Maynard 2008) in the spring when water can exceed 108% gas saturation. The physical effects of Toston Dam on the Missouri River and its Brown Trout are more apparent than the potential chemical and physiological effects, which would require focused research to fully understand.

Comprehensive Perspectives

Genetic evidence did not indicate that Toston dam fragments the Upper Missouri River Brown Trout population, as fish from upstream and downstream of the dam had nearly identical genetic variation. The Brown Trout population remains genetically connected in a downstream direction. Individual fish entering the Brown Trout population below Toston Dam increases the likelihood that the alleles needed to persist and adapt in the highly altered river are present within the population (Newman and Pilson 1997; Frankham 2003). Promoting strong connectivity between the mainstem Missouri River and its tributaries should be a high priority. Reconnecting small and isolated populations, such as the genetically divergent population in Dry Creek, are important for maintaining genetic diversity. Accelerated rates of genetic change through drift in small populations often lead to the development of unique genotypes (Ferguson 1989; Frankham et al. 2004). These populations frequently inhabit environmental extremes (e.g., altered instream flow regimes and degraded habitats) and may acquire specialized adaptations that could provide valuable contributions to the genetic resilience of the mainstem Missouri River population. Without maintained genetic diversity, either through sufficient habitat connectivity or genetic

rescue, small populations face an elevated risk of reduced fitness and eventual local extirpation (Bell 2022).

Most Brown Trout did not have natal origins within the Missouri River as judged by otolith microchemistry, and it was unclear how Toston Dam affects the proportion of Missouri River fish that originated in the mainstem. The lack of fish originating in the Missouri River emphasizes the importance of mainstem-to-tributary connections, especially given the limited availability of spawning habitat in the mainstem. Tributary connectivity is particularly important for the Missouri River below Toston Dam, as only 2 out of 40 fish sampled from below the dam originated there. This finding supports the thoughts of managers that minimal spawning takes place in the Missouri River mainstem below the dam (R. Spoon, personal communication). The findings of our genetics and otolith microchemistry components indicate good connectivity in a downstream direction through Toston Dam, as over half of the Missouri River fish caught below the dam originated from upstream sources (Table 6). The high proportion of fish from the Gallatin River (46.2%) suggests that it is an important recruitment source for the Missouri River Brown Trout population, both above and below Toston Dam.

Toston Dam disrupts substantial upstream spawning migrations of Brown Trout and has unquantified effects on downstream fish passage and survival. The low proportion of telemetered fish that migrated into Sixteenmile Creek (1.7%) suggested that the creek has a more limited role as a spawning site for Brown Trout from the Missouri River than expected. Conversely, the telemetry findings underscored the importance of the Gallatin River sub-basin as a spawning location for Missouri River Brown Trout. Telemetry also provided further evidence that fish can pass through Toston Dam in a downstream direction, as most telemetered fish translocated above

Toston Dam returned downstream to below the dam (Table 7). Some fish passed through the turbine, indicating passage is not limited to discharges exceeding 187.7 m^3/s when water flows over the dam spillways. Additionally, two trash screens with 76-mm clear bar spacing at the turbine inlet are not highly restrictive, enabling the passage of large adult trout up to an estimated 724 mm in length (HDR Inc. 2021). Total turbine-related mortality remains uncertain, but larger fish usually face a greater risk of blade-strike mortality than smaller fish (Vikström et al. 2020; Mueller et al. 2022). Kaplan turbines with four blades can cause blade-strike mortality rates up to 12% for salmonids ≤ 270 mm in length (Calles and Greenberg 2009; Vikström et al. 2020) and as high as 30% for fish up to 370 mm (Mueller et al. 2022). Similar blade-strike mortality rates have been estimated for Toston Dam based on site-specific characteristics (HDR Inc. 2021). The predicted blade-strike probability was relatively low (7.0 to 11.8%) for fish 384 to over 762 mm long. The low blade-strike probability for large fish at Toston Dam was attributed to the large turbine runner diameter, slow rotational speed, and the few blades.

The multifaceted approach of our study produced three complementary lines of evidence to assess whether habitat fragmentation by Toston Dam affects Brown Trout in the Upper Missouri River. Collectively, our findings indicate that Toston Dam imposes partial fragmentation on the population of Brown Trout. Genetic, otolith, and telemetry data indicate downstream connectivity is good despite the presence of the turbine and limited spill period. Whereas genetic analyses revealed exchange between fish from Sixteenmile Creek and the broader Missouri River population, telemetry and otolith findings indicated low levels of interchange. The overall high-quality habitat and favorable water conditions in Sixteenmile Creek probably support all life stages of Brown Trout, enabling year-round residency. Prolonged

or permanent residency may explain the limited movement we detected, despite genetic signals of historical or occasional gene flow. Therefore, disruption of migration of Brown Trout by Toston Dam into Sixteenmile Creek does not appear to be of major consequence, whereas maintaining connectivity to the Missouri River headwaters is probably more important for population success. Our evidence also suggests presence of a metapopulation structure, where source-sink dynamics may be involved. The Missouri River below the dam probably functions as a population sink that is dependent on recruitment from tributary sources such as the Gallatin River, which our evidence indicates is a major contributor to the sustainability of the Missouri River Brown Trout population. Small creeks contributed the second highest number of fish to the Missouri River and play an essential role in supporting the population, as reproduction in the mainstem is minimal. Habitat fragmentation caused by Toston Dam exacerbates source-sink dynamics by disrupting connectivity and altering potential spawning habitats, compounding the challenges for mainstem reproduction. Definitively labeling a location as a “source” or “sink” for a population based on short-term sampling is difficult (Dias 1996). However, our findings emphasize the importance of preserving key tributary habitats downstream of Toston Dam and exploring measures to recreate upstream connectivity with headwater rivers to avoid detrimental effects of a potential source-sink dynamic.

Our research provides new information on gene flow, natal origins, and spawning movements in a fragmented river where we determined whether those population dynamics are affected by Toston Dam. However, our study design did not evaluate the magnitude of population-level effects. For example, we determined that Toston Dam disrupts extensive spawning movements, but we do not know how well the population would respond if upstream

migration opportunity was restored. The fate of spawning fish not moved above the dam is also unknown. Our work focused on how fish that can persist in the system are affected by fragmentation from genetic, natal origin, and migration standpoints. The extent to which altered connectivity affects the long-term resilience and sustainability of the Upper Missouri River Brown Trout population is confounded by other limiting factors, such as interspecific competition below the dam and low flow regimes.

Future research could expand on our findings by incorporating multi-method approaches to address knowledge gaps in Upper Missouri River Brown Trout population dynamics. Coupling genetic and otolith microchemistry data to investigate natal site fidelity, dispersal patterns, and connectivity in river systems is increasingly common (Honda et al. 2012; Humston et al. 2021; Bourret et al. 2022; Källo et al. 2023). We identified genetic structure in a Brown Trout population, but we did not investigate how genetic diversity directly related to natal origins. We collected tissue samples from each of the Brown Trout we removed otoliths from. Genotyping these fish could provide a clearer understanding of the relationship between genetic diversity and natal site fidelity. Given the genetic structuring present among Brown Trout populations in the Upper Missouri River subbasin, genotyping these fish would allow us to assess whether genetically distinct groups correspond to different natal sources, providing further insight into dispersal and straying. Future managers could use genetics and otolith microchemistry data to assess restoration success by determining whether restored habitats are more productive and if gene flow is enhanced. As managers continue to collect genetic information, the Brown Trout genetic database could become increasingly representative and valuable as a Brown Trout management tool, providing a more comprehensive understanding of

genetic diversity, identifying potential barriers to gene flow, and informing adaptive management strategies. Similarly, as more water chemistry data is paired with geological composition, future otolith microchemistry studies could involve development of a southwest Montana map with a superimposed ^{87}Sr:^{86}Sr isoscape. This approach would help estimate the origins of fish with otolith signatures that do not directly match water sample values, as demonstrated by Humston et al. (2021) for Smallmouth Bass in Virginia.

Integrating genetics findings with movements derived by telemetry could enhance our understanding of Brown Trout dispersal and spawning by determining if observed movements correspond to genetic population structure. For example, Finlay et al. (2020) combined telemetry with population structure analysis to examine dispersal and straying behaviors in an adfluvial Brown Trout population. Telemetry can identify movement patterns and spawning sites, but combining telemetry with genetic analysis can reveal whether individuals return to natal sites with genetically similar fish or mix with more divergent populations. Such integration would involve minimal additional sampling effort, as tissue can be easily collected during device implantation. We could have integrated genetic data from telemetered fish, but the results would have probably been inconclusive because of low levels of genetic differentiation among populations above Toston Dam. Conversely, integrating spawning movement information with genetic structure analysis below Toston Dam could reveal how spawning movements below the dam affect local genetic makeup and whether fish halted at the dam spawn there, find alternative sites, or fail to spawn. The outcomes could help managers make decisions about whether spawning trout should be translocated above the dam. Integrating methods can provide greater

insight than using either method alone (Müller et al. 2023), but their effectiveness still depends on the specific environmental and ecological conditions of the study area.

Management Recommendations

The disruption of Brown Trout migration and dispersal caused by Toston Dam underscores the importance of acknowledging the effect of the dam on Brown Trout, particularly in the context of ongoing mitigation efforts outlined in the most recent FERC license. Our findings prove existence of a substantial fisheries connection to areas up to 124 rkm upstream. Previously, mitigation efforts from the Broadwater Hydropower Project extended 48 rkm from the dam site. Given our findings, using project resources in locations farther from the dam site than in the past should be considered an appropriate option. The location of new habitat improvement projects is an important consideration as FWP and MTDNRC collaborate on future mitigation efforts, which should aim to promote diverse habitat, genotypes, species, and communities to build resilient local fisheries (Cline et al. 2022). Maintaining diverse habitats while enhancing Brown Trout recruitment sources will support varied life history strategies and genetic diversity, promoting trout persistence and abundance in the Upper Missouri River sub-basin.

Managers should continue to devote resources to tributary creeks with a focus on improved habitat, streamflow, and connectivity. The habitat goal of the 2020-2029 Upper Missouri River Reservoir Fisheries Management Plan (FWP 2020) is to “Aggressively protect and enhance fish habitat as a management tool; enhance fish spawning opportunities in plan area reservoirs, river and tributaries.” The habitat rehabilitation efforts and connectivity improvements made in Deep Creek should be emulated in other streams between Canyon Ferry

and Toston Dam. Although no fish from the Missouri River were assigned natal origins in Deep Creek, Brown Trout in the creek exhibit only moderate genetic divergence from the mainstem Missouri River population, suggesting some level of connectivity. It is possible that genetic divergence is even lower in fish residing farther downstream in Deep Creek than at our sampling site. In contrast, Dry Creek has a highly divergent Brown Trout population and presents an opportunity to enhance connectivity to the Missouri River by modifying irrigation infrastructure. The findings from our genetic and otolith microchemistry components also suggest that Missouri River and Marsh Creek are connected, despite relatively low juvenile fish production from the creek. Fine sediment loading in Marsh Creek hinders juvenile production but does not prevent migrant Brown Trout from spawning there. Targeted habitat restoration and water use improvements in Warms Springs Creek as well as in its Marsh Creek tributary could further improve connectivity to the Missouri River and increase their productivity as sources of Brown Trout. However, an improved understanding of the complex irrigation water interchanges that occurs in each creek is needed first.

The North Boulder River is a known Brown Trout spawning stream outside the Upper Missouri River sub-basin where project resources could be applied. A significant Brown Trout spawning area, as indicated by redd counts, exists downstream of Cold Springs (C. Pipinich, personal observation, 2018–2024). In 2023, one telemetered fish used this groundwater-influenced reach, providing a connection to Toston Dam. Ongoing habitat enhancements in the North Boulder River include the removal of irrigation diversions to improve connectivity and reduce juvenile loss caused by ditch entrainment. Our telemetry findings highlight the importance of such removals in improving connectivity to a known spawning stream, which

could serve as a model for other locations connected to the Upper Missouri River facing similar challenges. The effect of habitat improvements in the North Boulder River on fish abundance in the Missouri River below Toston Dam is unknown. However, higher Brown Trout abundances upstream may promote downstream dispersal into larger habitats through density-dependent displacement, the search for better feeding opportunities, and forced movements caused by competition, high discharge, and habitat quality (Jonsson and Jonsson 2011; Birnie-Gauvin et al. 2019).

We recommend that genetic sampling of Brown Trout be continued (as resources allow) to build a genetic database that accurately represents genetic diversity, population differentiation, and longitudinal variation to support more informed management decisions. Increasing the sample size of a Brown Trout genetics database would enhance the accuracy of observed differences and reduce errors caused by small sample sizes and bias caused by possible overrepresentation of full siblings (Hansen et al. 1997). Sampling should include multiple sample locations from each key waterway to evaluate longitudinal differences. Understanding longitudinal genetic diversity in a stream population can reveal allele distribution and help identify genetic barriers such as diversions or poor-quality habitat. The success of a habitat reconnection project can be assessed by measuring genetic diversity before and after project completion to evaluate population response (Moccetti et al. 2023). The current management plan for the Upper Missouri River does not make use of any genetic metrics for fisheries management. However, the plan stresses the need to be adaptive in management actions. The creation of the microsatellite multiplexes used in this study allows for future genetic analysis to be efficiently done in-state. The incorporation of genetic data would promote more informed

management of Brown Trout in the area. Unique alleles in divergent populations, such as Dry Creek, may arise from environmental extremes or isolation and could benefit the larger mainstem river population. Likewise, the Dry Creek population could benefit from incoming gene flow, reducing inbreeding depression. Brown Trout have a plastic life history strategy (Jonsson and Jonsson 2011), allowing them to be resilient to changing ecosystems if the alleles needed to adapt are present in the population. Therefore, the focus should be on increasing genetic diversity within and among populations through improved connection and habitat function (Wang et al. 2002). In cases where population reconnection is not feasible, genetic rescue through the introduction of individuals could also be a viable strategy as well.

Managers may want to consider translocation of spawning Brown Trout over Toston Dam, given that over half of the fish sampled below the dam (25 out of 40) originated from upstream locations. Evolutionarily, returning to natal waters is an effective strategy for juvenile production in salmonids (Keefer and Caudill 2014). Translocating spawning-capable fish over Toston Dam would enable them to reach proven, reproductively successful locations. However, translocation to enhance individual reproductive success does not guarantee a population-level increase in Brown Trout abundance below Toston Dam. Despite a range of dispersal mechanisms (Jonsson and Jonsson 2011; Birnie-Gauvin et al. 2019), the progeny of translocated fish are not guaranteed to emigrate downstream of the dam. This uncertainty makes it unclear to what extent the Brown Trout population below the dam is limited by the inability to migrate upstream to spawn.

The results of translocating Brown Trout above Toston Dam outside the spawning season are unknown, as our research did not examine upstream movements during such times. Most

upstream movements in potamodromous Brown Trout are related to spawning, but dams can also prevent trout from returning upstream to fulfil other ecological needs such as feeding, refuge, and exploration (García-Vega et al. 2022). When a large and productive water body such as Canyon Ferry Reservoir is available for feeding and refuge, returning upstream of Toston Dam may only be advantageous when spawning (Jonsson and Jonsson 2011). However, the presence of abundant piscivorous predators in Canyon Ferry Reservoir complicates this advantage by reducing the expected benefits to survival and growth of trout in a lacustrine environment. Even if juvenile recruitment to the Missouri River below the dam improves, other limiting factors remain, including interspecific competition, predation by piscivorous birds and fish, low flow regimes, and limited high-quality spawning habitat (FWP 2020). Therefore, any translocation efforts should be carefully weighed against effort spent directly improving habitat, with the priority given to habitat restoration.

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APPENDICES

APPENDIX A

TABLES

Table 1. Radio telemetry fixed station names, numbers, latitudes, longitudes, and years of operation.

Name	Site Number	Latitude	Longitude	Operation
Missouri River Trident	1	45.94778	-111.47871	2022 - 2024
Sixteenmile Creek Lower	2	46.11590	-111.37083	2022 - 2024
Sixteenmile Creek Upper	3a	46.13677	-111.29194	2022 - 2023
Gallatin River Mouth	3b	45.93593	-111.49193	2023 - 2024
Toston Dam	4	46.12029	-111.40699	2022 - 2024
Missouri River Lower	5	46.32674	-111.53898	2022 - 2024

Table 2. Locations where each Brown Trout genetic sample was obtained, sample sizes, distances to the Missouri River (Mo. R.) confluence in river kilometers (rkm), and whether the sampled populations were in tributaries or not (yes/no). The genetic diversity among populations is summarized by the average number of alleles (N_A), average allelic richness (A_R), average observed heterozygosity (H_O), average subpopulation expected heterozygosity (H_S), and average pairwise genetic differentiation (G_{ST}).

Sample Location	Latitude	Longitude	n	Distance to Mo. R. (rkm)	Tributary	N_A	A_R	H_o	H_S	G_{ST}
Mo. R. Below Toston	46.34885	-111.52235	28	-	No	8.533	8.311	0.676	0.700	0.067
Madison River	45.64010	-111.53091	30	38.3	No	8.867	8.528	0.684	0.688	0.075
Mo. R. Above Toston	45.99247	-111.43836	28	-	No	8.867	8.698	0.654	0.682	0.077
Gallatin River	45.88732	-111.41630	29	12.6	No	8.333	8.099	0.657	0.691	0.082
Sixteenmile Creek	46.12713	-111.26906	25	13.7	Yes	7.667	7.667	0.717	0.692	0.094
Jefferson River	45.79785	-111.70161	30	29.8	No	8.467	8.103	0.682	0.687	0.114
Marsh Creek	46.15546	-111.51068	27	3.1	Yes	6.533	6.431	0.642	0.659	0.132
Deep Creek	46.32670	-111.36863	25	15.1	Yes	7.267	7.267	0.672	0.677	0.146
Warm Springs Creek	46.13068	-111.51549	25	9.8	Yes	5.867	5.828	0.692	0.646	0.167
Dry Creek	46.26089	-111.34575	25	10.5	Yes	3.867	3.867	0.507	0.531	0.296

Table 3. Pairwise G_{ST} above diagonal and associated italicized *P*-values below diagonal between all Brown Trout samples. The Missouri River above and below Toston Dam are represented by Mo. R. AT and Mo. R. BT. Pairwise comparisons for G_{ST} were significantly different for all but 4 pairs, indicated in bold. Values were considered significant at $\alpha < 0.05$.

Sample Location	Deep	Dry	Gallatin	Jefferson	Madison	Marsh	Mo. R. AT	Mo. R. BT	Sixteenmile	Warm Springs	Average
Deep	--	0.272	0.083	0.170	0.097	0.169	0.091	0.091	0.106	0.235	0.146
Dry	*0.001*	--	0.278	0.310	0.256	0.356	0.268	0.266	0.281	0.38	0.296
Gallatin	*0.001*	*0.001*	--	0.045	0.025	0.100	0.025	**0.014**	0.031	0.133	0.082
Jefferson	*0.001*	*0.001*	*0.001*	--	0.044	0.130	0.047	0.045	0.072	0.166	0.114
Madison	*0.001*	*0.001*	*0.015*	*0.001*	--	0.079	0.019	**0.007**	0.028	0.121	0.075
Marsh	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	--	0.091	0.051	0.114	0.101	0.132
Mo. R. AT	*0.001*	*0.001*	*0.023*	*0.001*	*0.044*	*0.001*	--	**0.001**	**0.018**	0.129	0.077
Mo. R. BT	*0.001*	*0.001*	***0.096***	*0.001*	***0.232***	*0.001*	***0.427***	--	0.045	0.083	0.067
Sixteenmile	*0.001*	*0.001*	*0.006*	*0.001*	*0.011*	*0.001*	***0.066***	*0.001*	--	0.151	0.094
Warm S.	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	--	0.166

Table 4. Water $^{87}Sr:^{86}Sr$ and Sr:Ca ratios at 16 locations in the study area. Streams are in geographical order from upstream to downstream.

Stream	Latitude	Longitude	$^{87}Sr:^{86}Sr$	Sr:Ca mmol/mol
Gallatin River at Logan	45.88637	-111.44198	0.71158	2.03
Madison River at Interstate-90	45.90094	-111.52590	0.71512	1.91
Jefferson River at Interstate-90	45.91027	-111.54714	0.71224	3.32
Mo. R. Trident	45.95012	-111.47385	0.71212	2.26
Sixteenmile Creek (rkm 12.6)	46.13446	-111.27698	0.70872	3.91
Sixteenmile Creek mouth	46.10760	-111.39562	0.70881	3.87
Mo. R. below Sixteenmile	46.10777	-111.41960	0.71180	2.74
Mo. R. above Big Springs	46.12354	-111.39501	0.71231	2.67
Big Springs	46.12479	-111.39367	0.71082	3.14
Marsh Creek (rkm 3.1)	46.15442	-111.51429	0.70819	3.66
Warm Springs mouth	46.18508	-111.48026	0.70979	4.03
Crow Creek mouth	45.19255	-111.48891	0.70781	2.42
Dry Creek (rkm 10.5)	46.28421	-111.48026	0.71593	1.38
Deep Creek mouth	46.28421	-111.51247	0.71122	3.71
Deep Creek (rkm 15.3)	46.32670	-111.36863	0.71111	3.71
Mo. R. at Indian Campground	46.33542	-111.52965	0.71226	2.72

Table 5. Water sample locations with the number of fish assigned to each (n), average Euclidean distance, and the range of Euclidean distances. All locations on the Missouri River (Mo. R.) are grouped as "Mo. R. All".

Location (n)	Mean ± SD	Range
Mo. R. above Big Springs (2)	0.176 ± 0.015	0.166 - 0.187
Mo. R. Trident (4)	0.239 ± 0.053	0.169 - 0.297
Mo. R. below Sixteenmile (5)	0.304 ± 0.167	0.199 - 0.593
Big Springs (3)	0.413 ± 0.227	0.253 - 0.674
Marsh Creek (2)	0.424 ± 0.216	0.271 - 0.577
Gallatin River (37)	0.343 ± 0.191	0.027 - 0.697
Sixteenmile Creek lower (3)	0.493 ± 0.149	0.327 - 0.617
Crow Creek (3)	0.607 ± 0.043	0.579 - 0.657
Madison River (8)	0.498 ± 0.159	0.276 - 0.738
Dry Creek (3)	0.521 ± 0.163	0.348 - 0.673
Unknown Origin (10)	1.038 ± 0.355	0.783 - 1.706
Mo. R. All (11)	0.257 ± 0.121	0.166 - 0.593

Table 6. Missouri River (Mo. R.) fish assigned to each water sample location using otolith microchemistry, categorized by whether they were sampled from above or below Toston Dam.

Waterway	Above	Below	Total
Gallatin River	20	17	37
Madison River	6	2	8
Mo. R. Trident	2	2	4
Sixteenmile Creek lower	3	0	3
Mo. R. below Sixteenmile	3	2	5
Mo. R. above Big Springs	0	2	2
Big Springs	0	3	3
Marsh Creek	0	2	2
Crow Creek	0	3	3
Dry Creek	0	3	3
Unknown Origin	6	4	10

Table 7. Telemetered Brown Trout that ascended each major basin (male: M, female: F) by year, mean, maximum, and minimum values of the most upstream location by basin, and the total numbers of fish that returned to below Toston Dam, categorized by drainage. The Missouri River is abbreviated as "Mo. R."

Basin	2022	2023	Total	Mean (rkm)	Maximum (rkm)	Minimum (rkm)	Returned below Toston Dam
Gallatin	8 M, 8 F	9 M, 7 F	32	54.2	84.5	30.4	10 M, 7 F
Jefferson	4 M, 3 F	0 M, 3 F	10	85.2	124.2	58.4	1 M, 4 F
Madison	0 M, 2 F	3 M, 1 F	6	41.0	48.3	36.0	1 M, 3 F
Mo. R.	3[a]M, 2[a]F	3[a]M, 4[a]F	12[a]	25.5	30.1	0.0[b]	3 M, 4 F

[a] Values increased by the Trident fixed station.

[b] Released and then not seen again until returned to Toston Dam.

Table 8. Characteristics of Brown Trout telemetered in 2022 including, identification numbers, capture dates, sexes, total lengths, weights, major drainages ascended, farthest upstream locations and dates, distances from release point (river kilometers) above Toston Dam, and final known locations. The Missouri River is abbreviated as "Mo. R." and the Gallatin River above the confluence of the East Gallatin River is "AEG".

Fish ID	Tag Date	Sex	Length (mm)	Weight (g)	Drainage Ascended	Most upstream location	Date	Distance (rkm)	Final Known Location
13	10/26/2022	M	574	1619	Gallatin	Smith Creek	1/27/2023	72.9	Smith Creek
15[d]	10/26/2022	F	523	1252	Gallatin	E. Gallatin River	12/10/2022	63.4	Canyon Ferry Pond 2
18	10/26/2022	F	478	1801	Gallatin	Gallatin River (AEG)	12/10/2022	63.4	Mo. R. below Toston Dam
19[a]	10/26/2022	F	594	2028	Gallatin	Baker Creek	9/6/2023	55.7	Baker Creek
20	10/26/2022	F	427	771	Gallatin	Gallatin River	11/10/2022	31.2	Mo. R. Trident
24	10/28/2022	F	401	680	Gallatin	E. Gallatin River	12/10/2022	73.2	Mo. R. Trident
25[a]	10/28/2022	M	488	1048	Gallatin	Rey Creek	12/10/2022	40.2	Rey Creek
26	10/28/2022	M	615	2350	Gallatin	Gallatin River (AEG)	12/10/2022	55.2	Gallatin River (AER)
27	10/28/2022	M	531	1139	Gallatin	Gallatin River	12/10/2022	44.1	Mo. R. below Toston Dam
28[e]	10/28/2022	F	559	1565	Gallatin	E. Gallatin River	12/10/2022	84.5	Mo. R. below Toston Dam
31	10/28/2022	M	579	1769	Gallatin	Gallatin River	11/7/2022	30.7	Mo. R. below Toston Dam
32[b]	10/28/2022	F	465	862	Gallatin	Gallatin River (AEG)	12/10/2022	57.5	Mo. R. above Toston Dam
34	10/28/2022	F	521	1143	Gallatin	Gallatin River	12/10/2022	32.5	Mo. R. below Toston Dam
36[e]	10/31/2022	M	584	1588	Gallatin	Gallatin River (AEG)	12/10/2022	63.6	Mo. R. below Toston Dam
38	10/31/2022	M	564	1361	Gallatin	Smith Creek	1/5/2023	72.3	Smith Creek
40	10/31/2022	M	640	2821	Gallatin	Gallatin River	12/10/2022	32.2	Gallatin River

[a] Inactive at most upstream location
[b] Recapture, moved twice
[c] Confirmed mortality
[d] Predated by pelican
[e] Inactive tag

Table 8 Continued.

Fish ID	Tag Date	Sex	Length (mm)	Weight (g)	Drainage Ascended	Most upstream location	Date	Distance (rkm)	Final Known Location
11	10/26/2022	F	498	1152	Jefferson	Jefferson River	12/10/2022	64.2	Mo. R. below Toston Dam
12	10/26/2022	F	513	1238	Jefferson	Jefferson River	12/30/2022	116.0	Jefferson River
14	10/26/2022	F	394	626	Jefferson	Jefferson River	12/10/2022	73.2	Mo. R. below Toston Dam
21	10/26/2022	M	587	1837	Jefferson	Jefferson River	12/30/2022	124.2	Mo. R. below Toston Dam
35[a]	10/28/2022	M	582	2009	Jefferson	Jefferson River	9/6/2023	63.6	Jefferson River
37	10/31/2022	M	658	2608	Jefferson	Jefferson River	12/30/2022	113.5	Jefferson River
39	10/31/2022	M	551	1733	Jefferson	Jefferson River	12/10/2022	58.4	Jefferson River
22	10/26/2022	F	391	531	Madison	Madison River	12/10/2022	38.5	Mo. R. below Toston Dam
23	10/28/2022	F	538	1370	Madison	Madison River	12/10/2022	43.8	Mo. R. below Toston Dam
16	10/26/2022	M	706	4119	Mo. R.	Mo. R.	10/28/2022	28.6	Mo. R. Trident
17	10/26/2022	M	762	4536	Mo. R.	Mo. R.	11/30/2022	19.5	Mo. R. above Toston Dam
29	10/28/2022	M	602	1969	Mo. R.	Mo. R.	1/13/2023	29.8	Mo. R. below Toston Dam
30	10/28/2022	F	447	925	Mo. R.	Mo. R.	1/6/2023	28.6	Mo. R. below Toston Dam
33[a]	10/28/2022	F	460	1007	Mo. R.	Mo. R.	11/8/2022	28.6	Mo. R. Trident

[a] Inactive at most upstream location
[b] Recapture, moved twice
[c] Confirmed mortality
[d] Predated by pelican
[e] Inactive tag

Table 9. Characteristics of Brown Trout telemetered in 2023 including, identification numbers, capture dates, sexes, total lengths, weights, major drainages ascended, farthest upstream locations and dates, distances from release point (river kilometers) above Toston Dam, and final known locations. The Missouri River is abbreviated as "Mo. R." and the Gallatin River above the confluence of the East Gallatin River is "AEG".

Fish ID	Tag Date	Sex	Length (mm)	Weight (g)	Drainage Ascended	Most upstream location	Date	Distance (rkm)	Final Known Location
41	10/13/2023	F	543.56	1343	Gallatin	Gallatin River (AER)	12/13/2023	56.5	Mo. R. below Toston Dam
42	10/13/2023	M	675.64	2590	Gallatin	Smith Creek	11/17/2023	74.2	Mo. R. below Toston Dam
43	10/13/2023	F	497.84	894	Gallatin	Gallatin River	12/13/2023	49.4	Gallatin River
48	10/17/2023	M	568.96	1606	Gallatin	Gallatin River	12/5/2023	30.4	Mo. R. above Toston Dam
49[e]	10/19/2023	M	723.9	4232	Gallatin	Gallatin River	11/14/2023	30.4	Mo. R. below Toston Dam
51	10/19/2023	M	711.2	3951	Gallatin	Gallatin River	12/4/2023	30.4	Mo. R. below Toston Dam
52	10/19/2023	M	650.24	2903	Gallatin	Gallatin River (AER)	12/13/2023	60.5	Mo. R. below Toston Dam
53[ac]	10/19/2023	F	497.84	962	Gallatin	Camp Creek	12/4/2023	55.8	Camp Creek
55	10/19/2023	F	530.86	1361	Gallatin	E. Gallatin River	12/20/2023	70.3	Mo. R. Below Toston Dam
58	10/20/2023	M	563.88	2028	Gallatin	Dry Creek	12/20/2023	66.6	Mo. R. below Toston Dam
61	10/23/2023	M	591.82	2336	Gallatin	Thompson Creek	1/5/2024	80.8	Mo. R. below Toston Dam
62	10/23/2023	F	505.46	1238	Gallatin	Gallatin River (AER)	12/20/2023	69.4	Mo. R. below Toston Dam
64	10/23/2023	M	668.02	2372	Gallatin	Gallatin River	11/3/2023	30.4	Mo. R. Trident
65[a]	10/23/2023	F	429.26	699	Gallatin	E. Gallatin River	1/31/2024	65.5	E. Gallatin River
68	11/1/2023	M	513.08	1098	Gallatin	Gallatin River (AER)	12/20/2023	51.8	Mo. R. below Toston Dam
69	11/1/2023	F	538.48	1134	Gallatin	Gallatin River	4/10/2024	40.2	Mo. R. below Toston Dam

[a] Inactive at most upstream location
[b] Recapture, moved twice
[c] Confirmed mortality
[d] Predated by pelican
[e] Inactive tag

Table 9 Continued.

Fish ID	Tag Date	Sex	Length (mm)	Weight (g)	Drainage Ascended	Most upstream location	Date	Distance (rkm)	Final Known Location
57	10/20/2023	F	485.14	826	Jefferson	Jefferson River	11/30/2023	78.2	Mo. R. below Toston Dam
63	10/23/2023	F	403.86	671	Jefferson	N. Boulder River	12/12/2023	97.2	Mo. R. Trident
70	11/1/2023	F	469.9	1021	Jefferson	Jefferson River	12/20/2023	63.9	Mo. R. below Toston Dam
44[a]	10/13/2023	M	525.78	1266	Madison	Madison River	12/20/2023	42.2	Madison River
45	10/13/2023	M	591.82	2077	Madison	Madison River	12/20/2023	36.0	Mo. R. below Toston Dam
54	10/19/2023	M	421.64	717	Madison	Madison River	12/20/2023	37.2	Madison River
67	10/23/2023	F	464.82	889	Madison	Madison River	12/20/2023	48.3	Mo. R. below Toston Dam
46	10/17/2023	F	441.96	785	Mo. R.	Mo. R.	10/17/2023	0.0	Mo. R. below Toston Dam
47	10/17/2023	F	497.84	1021	Mo. R.	Mo. R.	11/16/2023	30.1	Mo. R. below Toston Dam
50	10/19/2023	F	414.02	735	Mo. R.	Mo. R.	10/21/2023	28.6	Mo. R. below Toston Dam
56	10/19/2023	M	449.58	816	Mo. R.	Mo. R.	10/21/2023	28.6	Mo. R. Trident
59	10/20/2023	M	596.9	2268	Mo. R.	Mo. R.	10/21/2023	28.6	Mo. R. below Toston Dam
60	10/23/2023	M	541.02	1588	Mo. R.	Mo. R.	10/26/2023	29.8	Mo. R. below Toston Dam
66[ac]	10/23/2023	F	520.7	1347	Mo. R.	Sixteenmile Creek	12/9/2023	24.5	Sixteenmile Creek

[a] Inactive at most upstream location
[b] Recapture, moved twice
[c] Confirmed mortality
[d] Predated by pelican
[e] Inactive tag

APPENDIX B

FIGURES

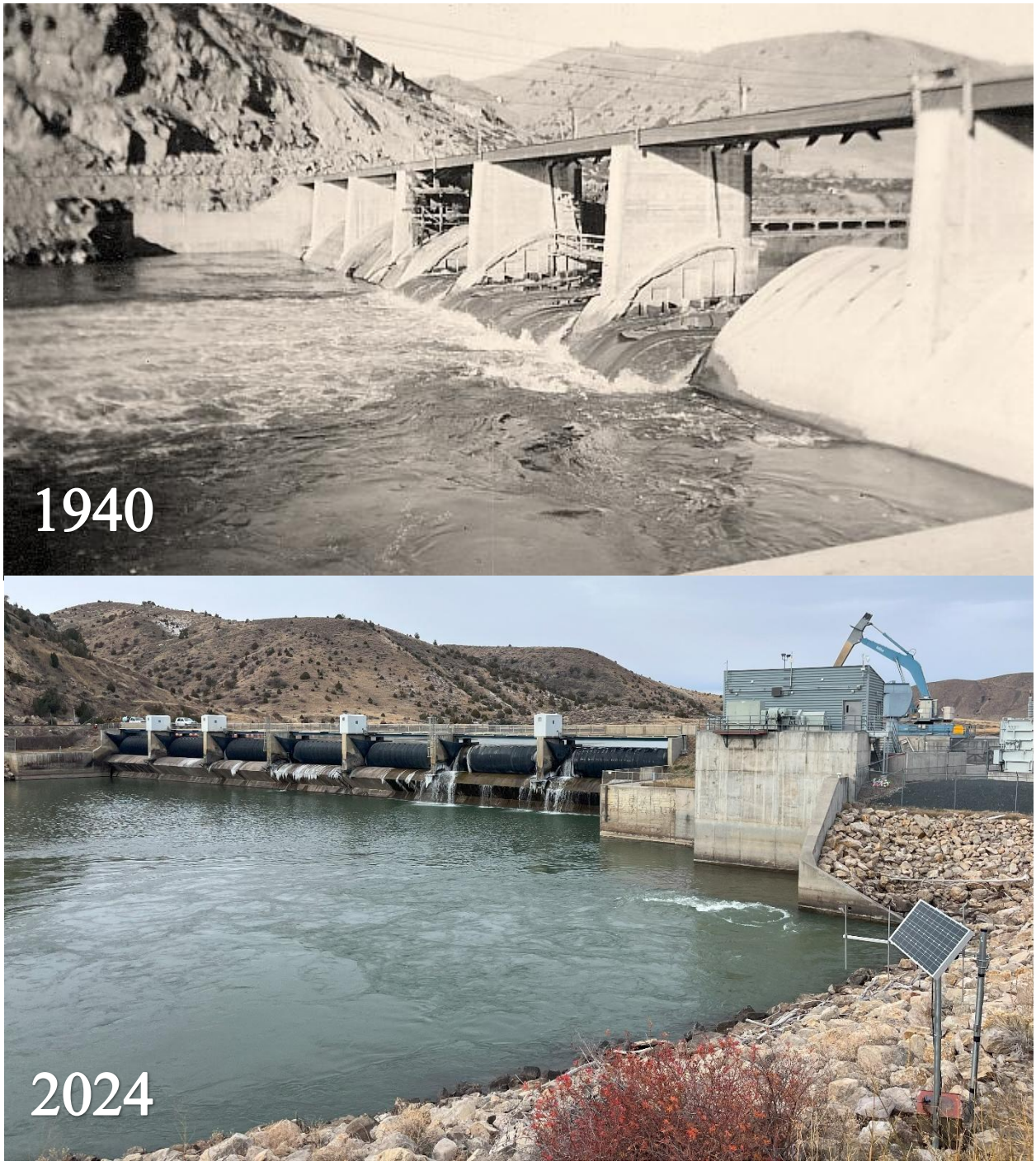

Figure 1. Toston Dam on the upper Missouri River during base flow. (Top) Toston Dam in its early years of operation as an irrigation diversion dam. (Bottom) The modern dam, with the hydropower facility visible on the right and a fixed-station radio receiver at the bottom right.

Figure 2. Upper Missouri River sub-basin including the mainstem Missouri River and select Brown Trout spawning tributaries.

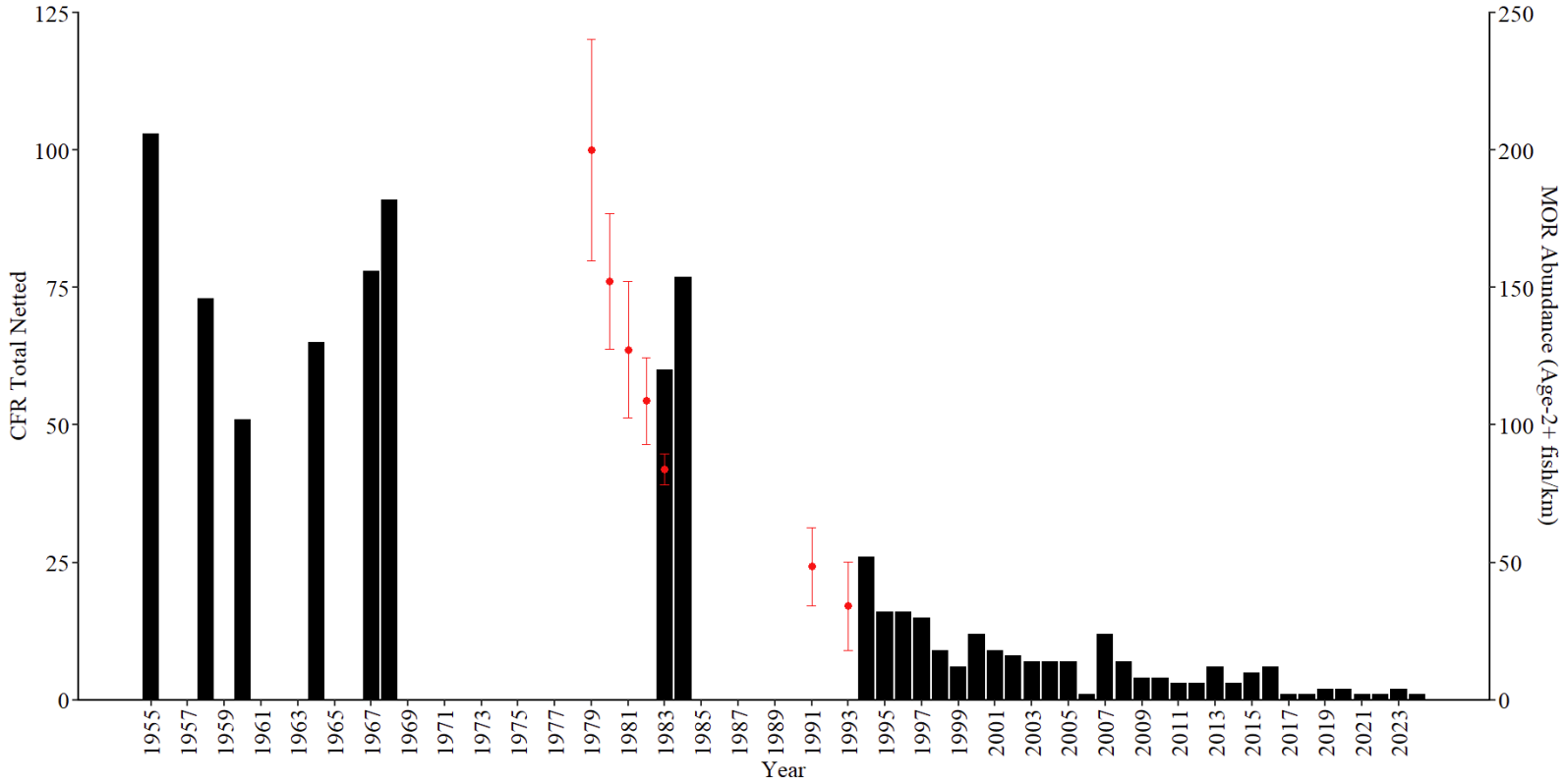

Figure 3. Total numbers of Brown Trout captured during FWP summer sinking gill net surveys in Canyon Ferry Reservoir (CFR), paired with seven springtime abundance estimates of age-2+ fish per km in the Missouri River (MOR) downstream of Toston Dam (FWP, unpublished data).

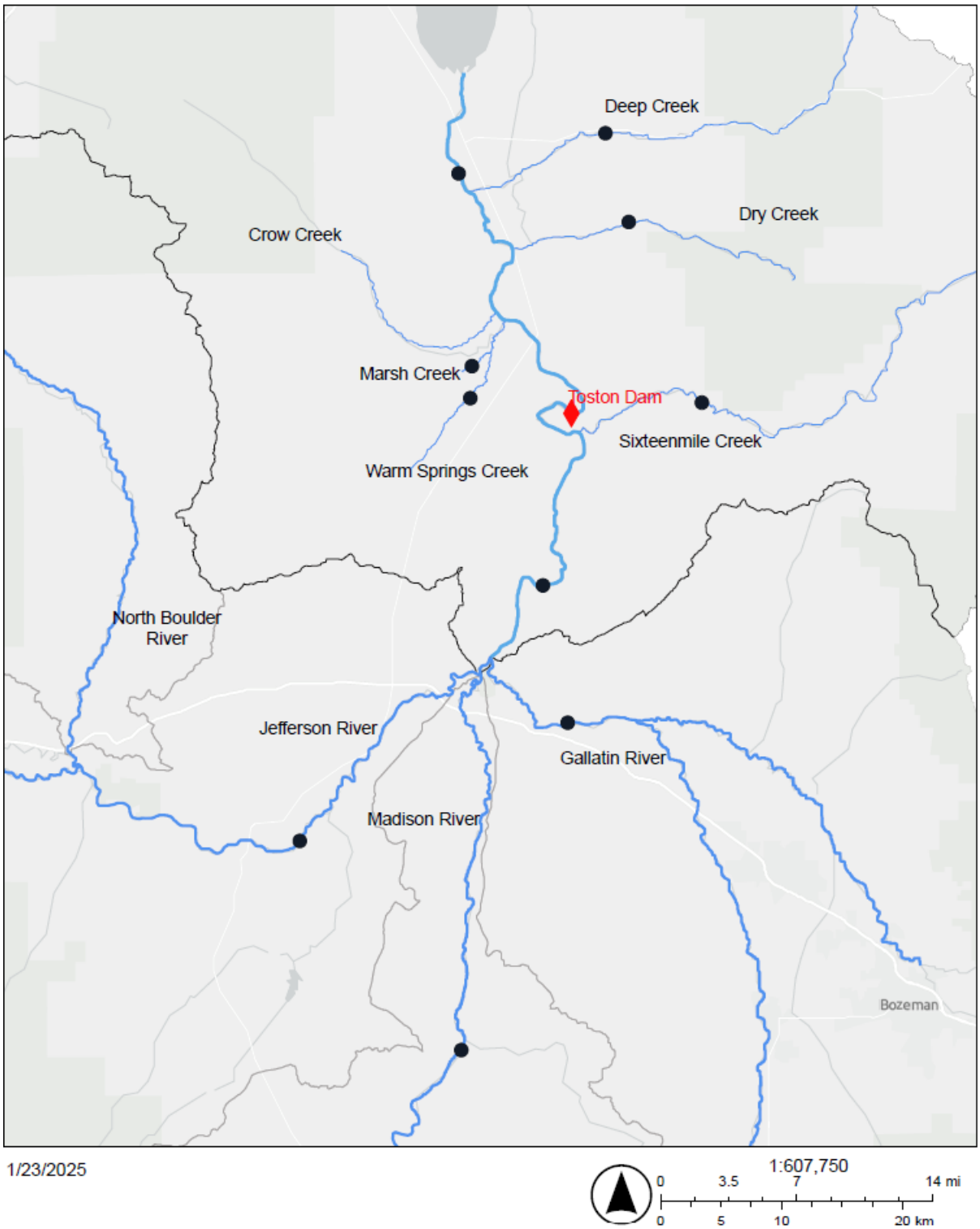

Figure 4. Genetic sampling locations (circles) for Brown Trout. Toston Dam is indicated by the diamond. The black line delineates the Upper Missouri River sub-basin boundary.

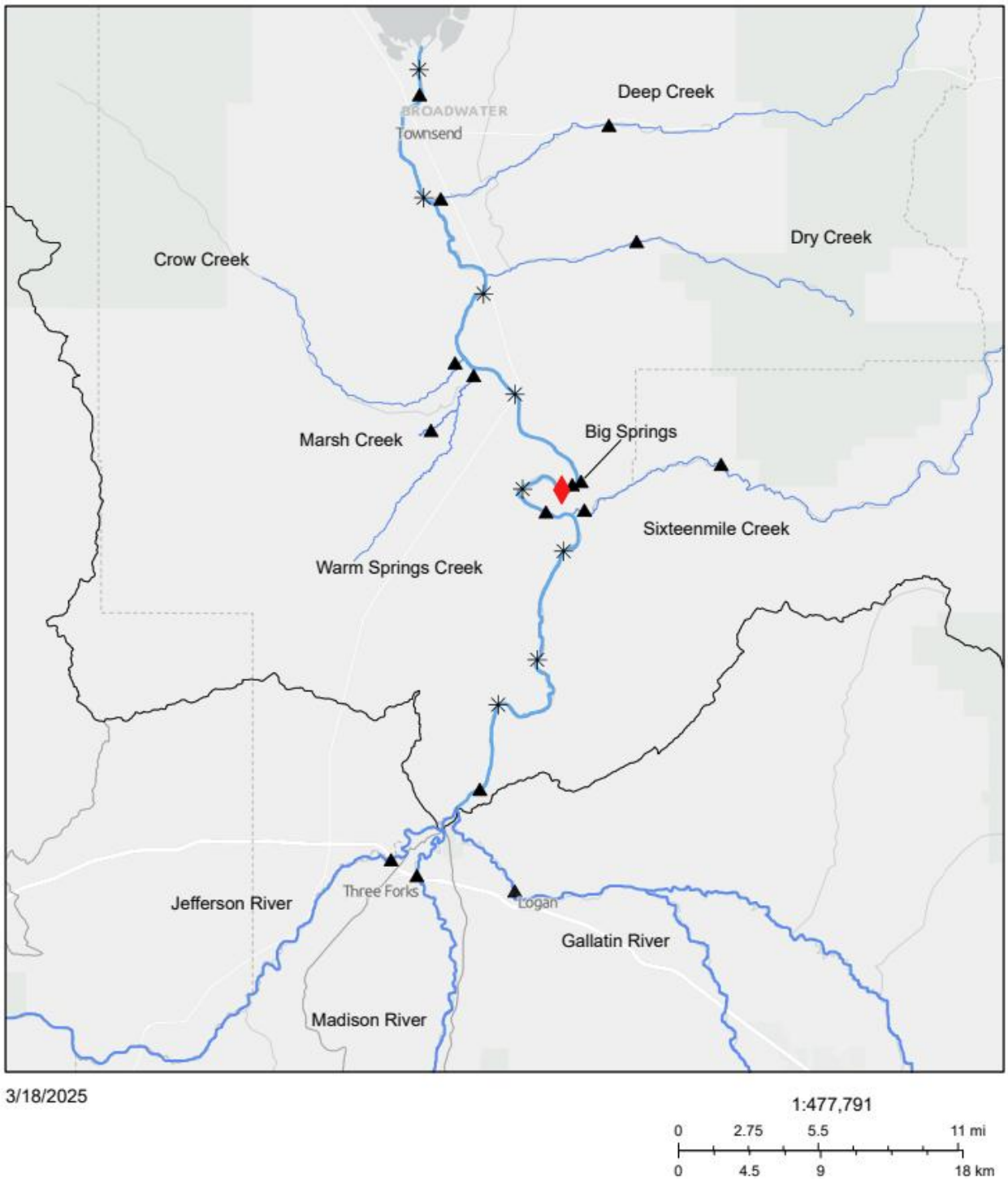

Figure 5. Water chemistry (triangles) and otolith (asterisks) sample locations. Sampling reaches were 8.3 km long above Toston Dam, and 9.1 km long below the dam. Toston Dam is represented by the red diamond. The black line delineates the Upper Missouri River sub-basin boundary.

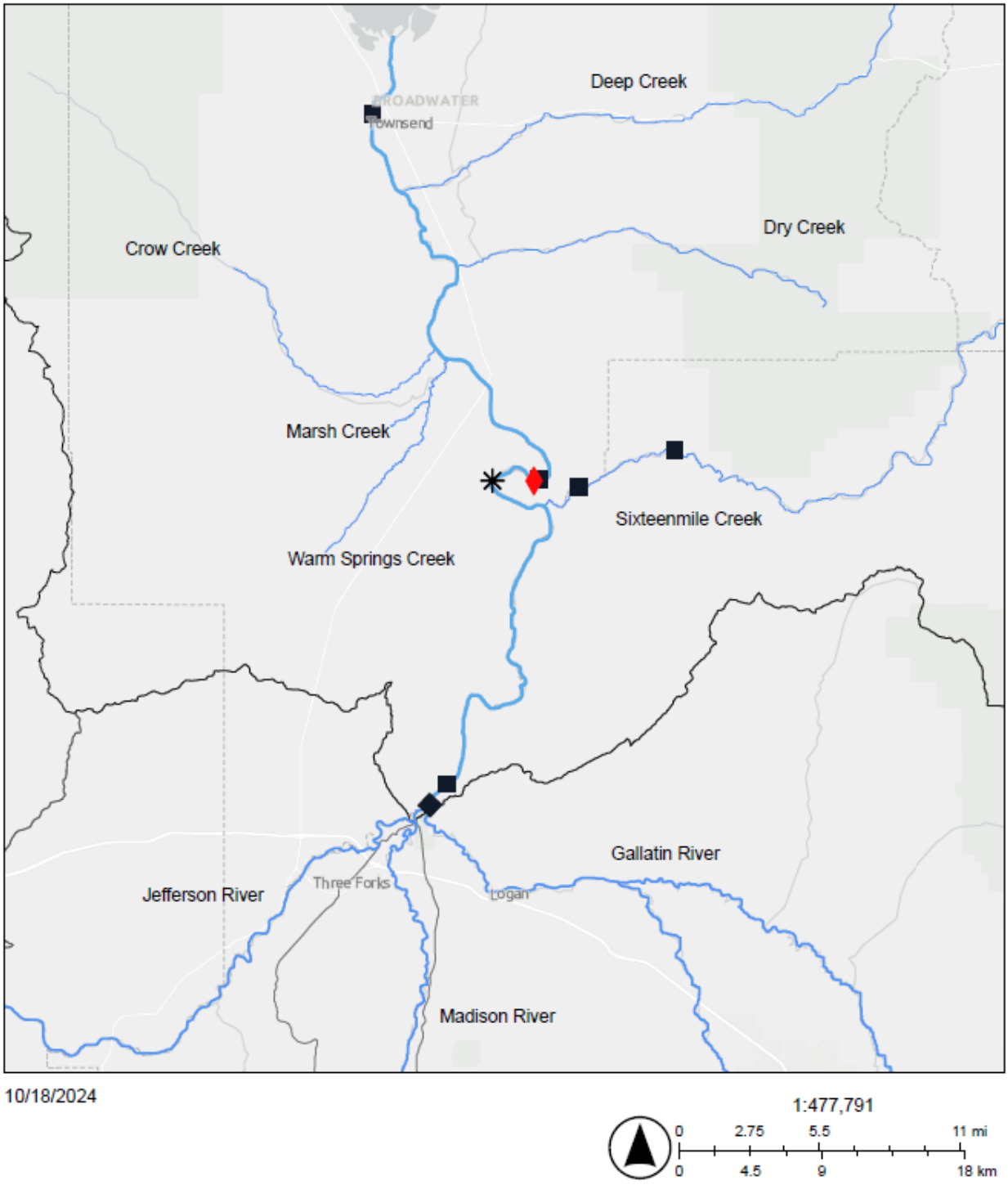

Figure 6. Locations (squares) of radio telemetry fixed stations in 2022, in relation to Toston Dam (red diamond) and the fish release point (asterisk). The upstream Sixteenmile Creek site was relocated to the mouth of the Gallatin River in 2023 (black diamond). The black line delineates the Upper Missouri River sub-basin boundary.

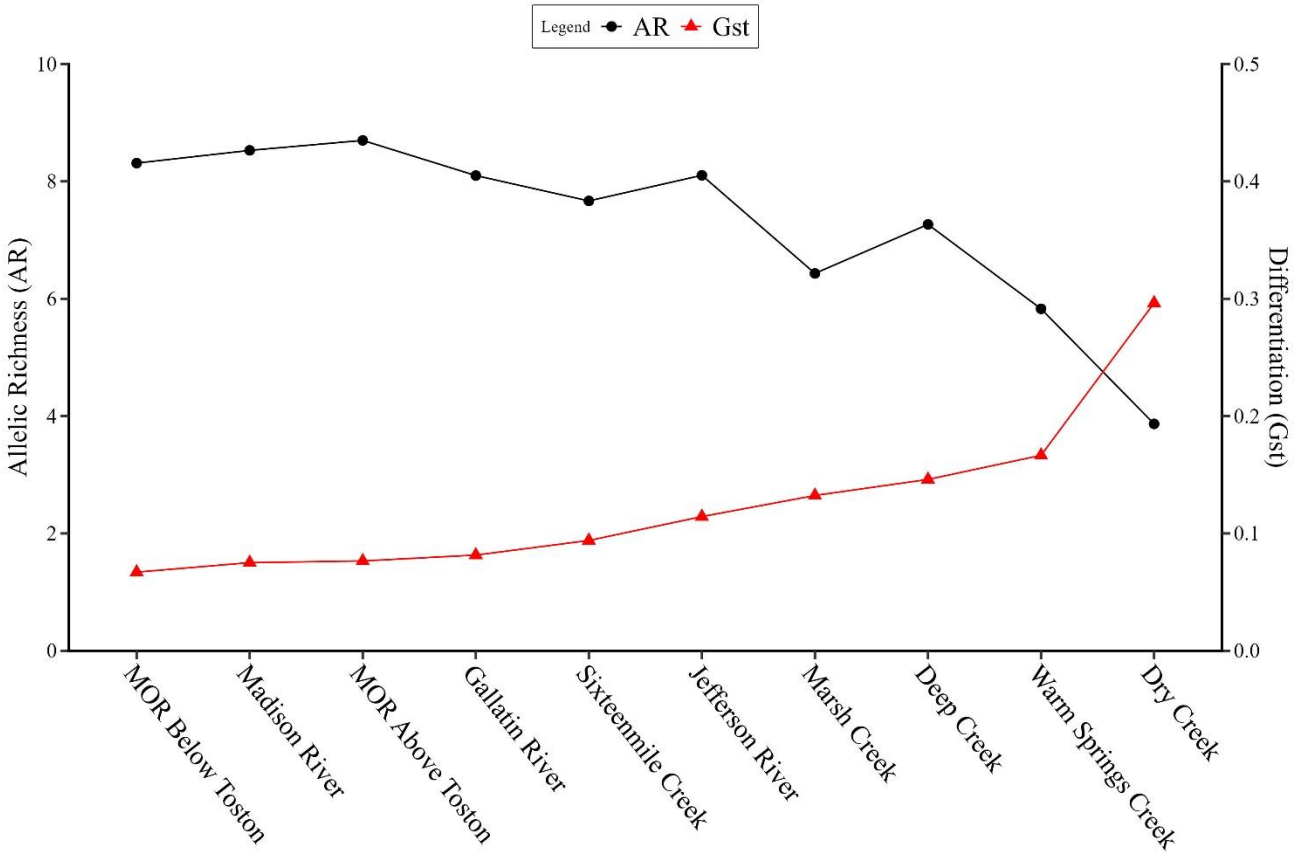

Figure 7. Average allelic richness (A_R) and pairwise genetic differentiation (G_{ST}) among all Brown Trout samples. Values are sorted by G_{ST}. Points are connected for visual emphasis only; no linear or sequential relationship between adjacent populations is implied.

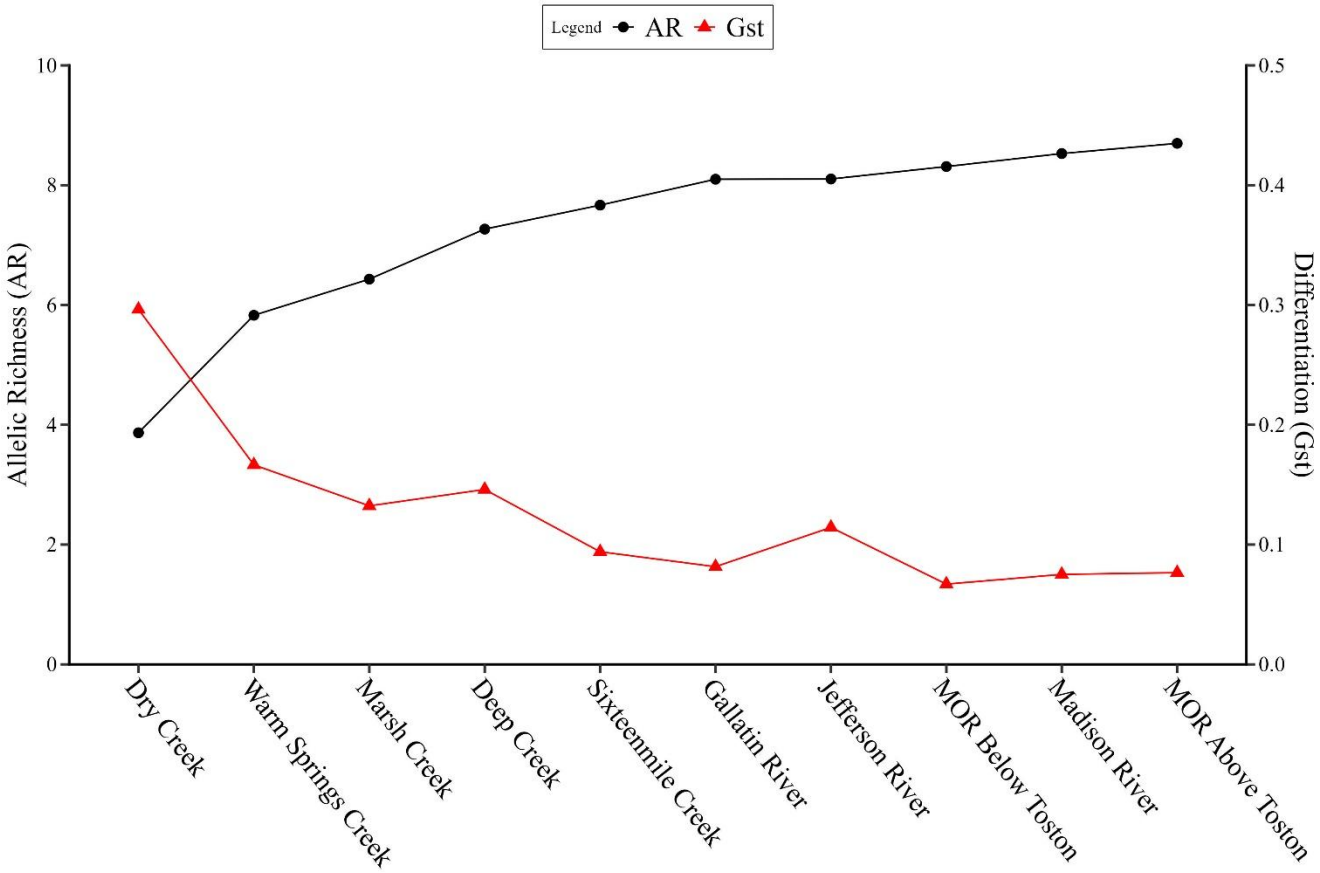

Figure 8. Average allelic richness (A_R) and pairwise genetic differentiation (G_{ST}) among all Brown Trout samples. Values are sorted by A_R. Points are connected for visual emphasis only; no linear or sequential relationship between adjacent populations is implied.

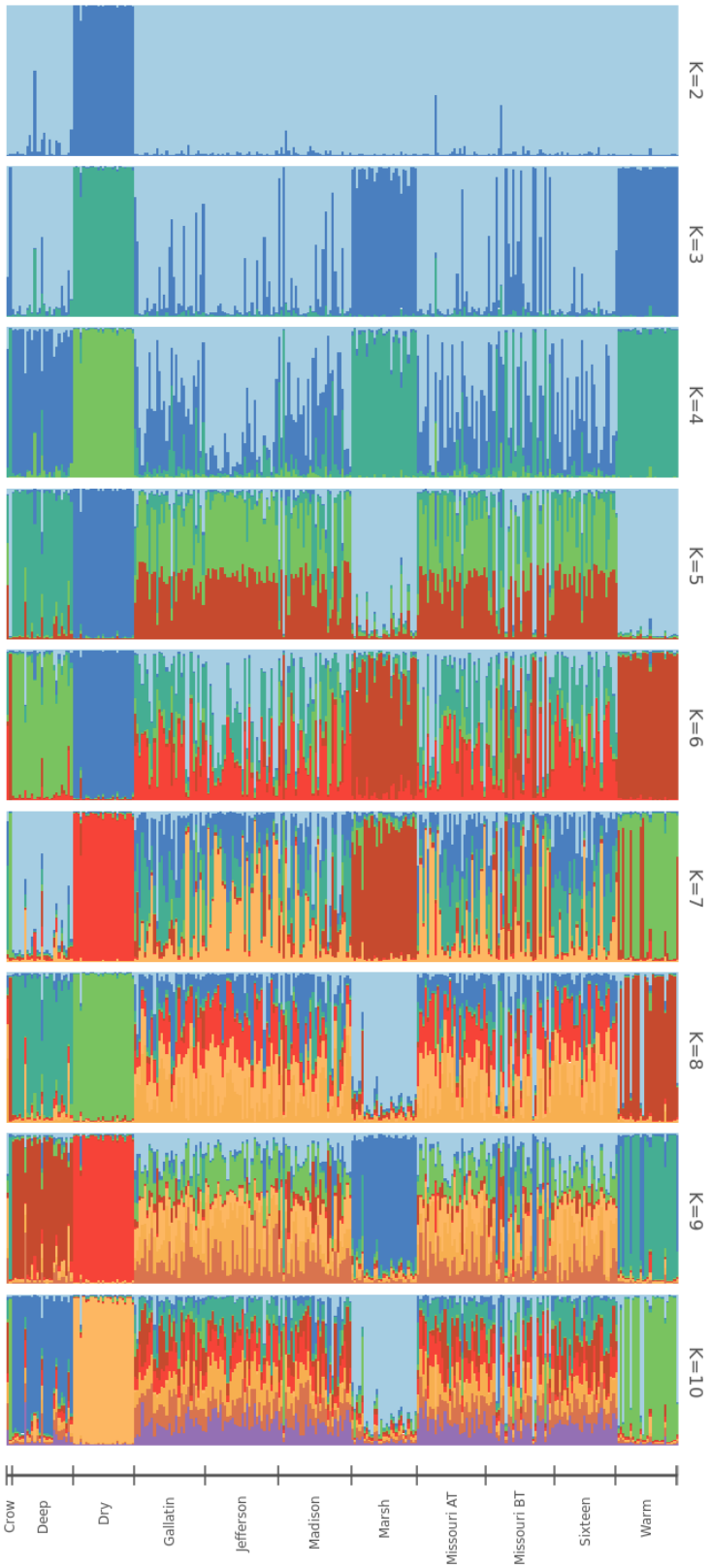

Figure 9. Allele frequency differences detected by the program STRUCTURE, and assignment of individuals to sub-populations based on the assumption of K populations. In each scenario, a color represents one population, and the genetics of each individual is represented by a stacked bar.

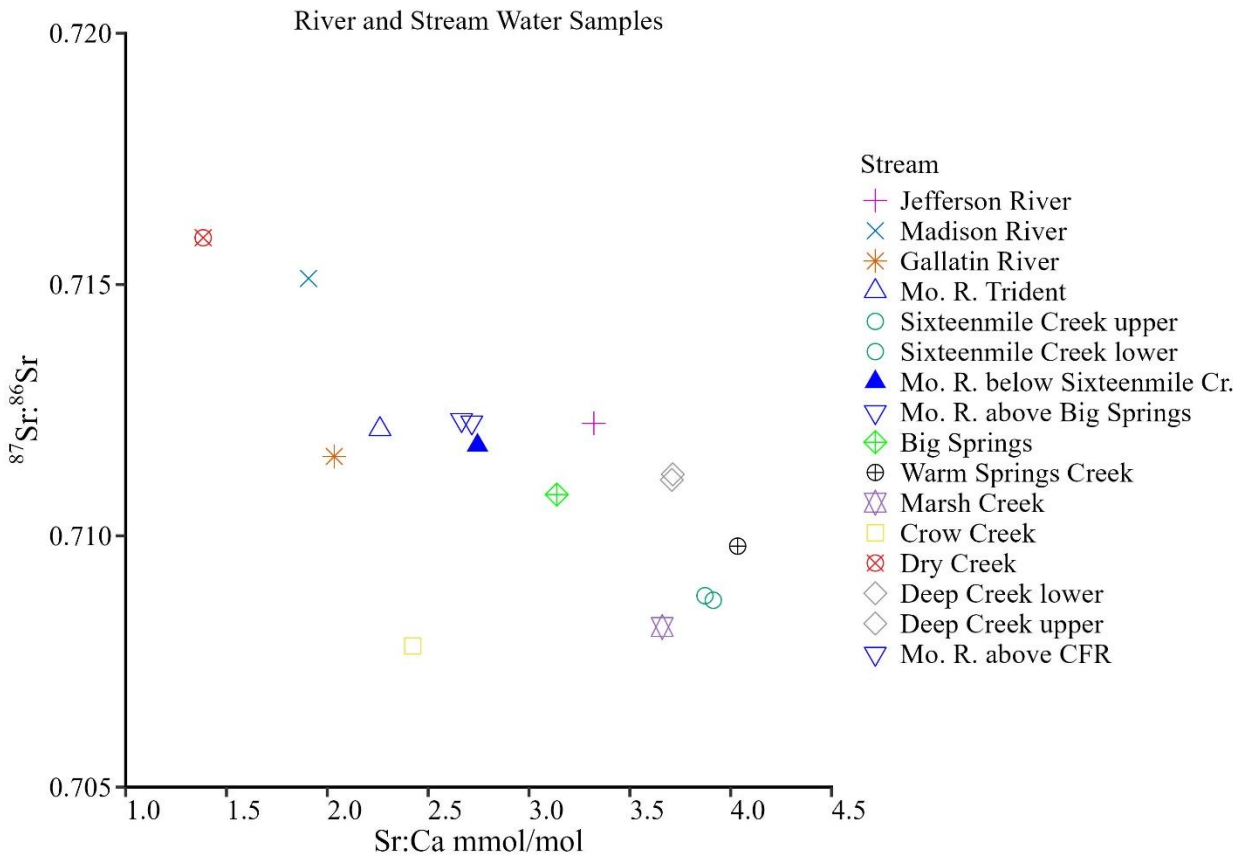

Figure 10. Sr:Ca and $^{87}Sr:^{86}Sr$ ratios of water samples collected from potential Brown Trout recruitment sources.

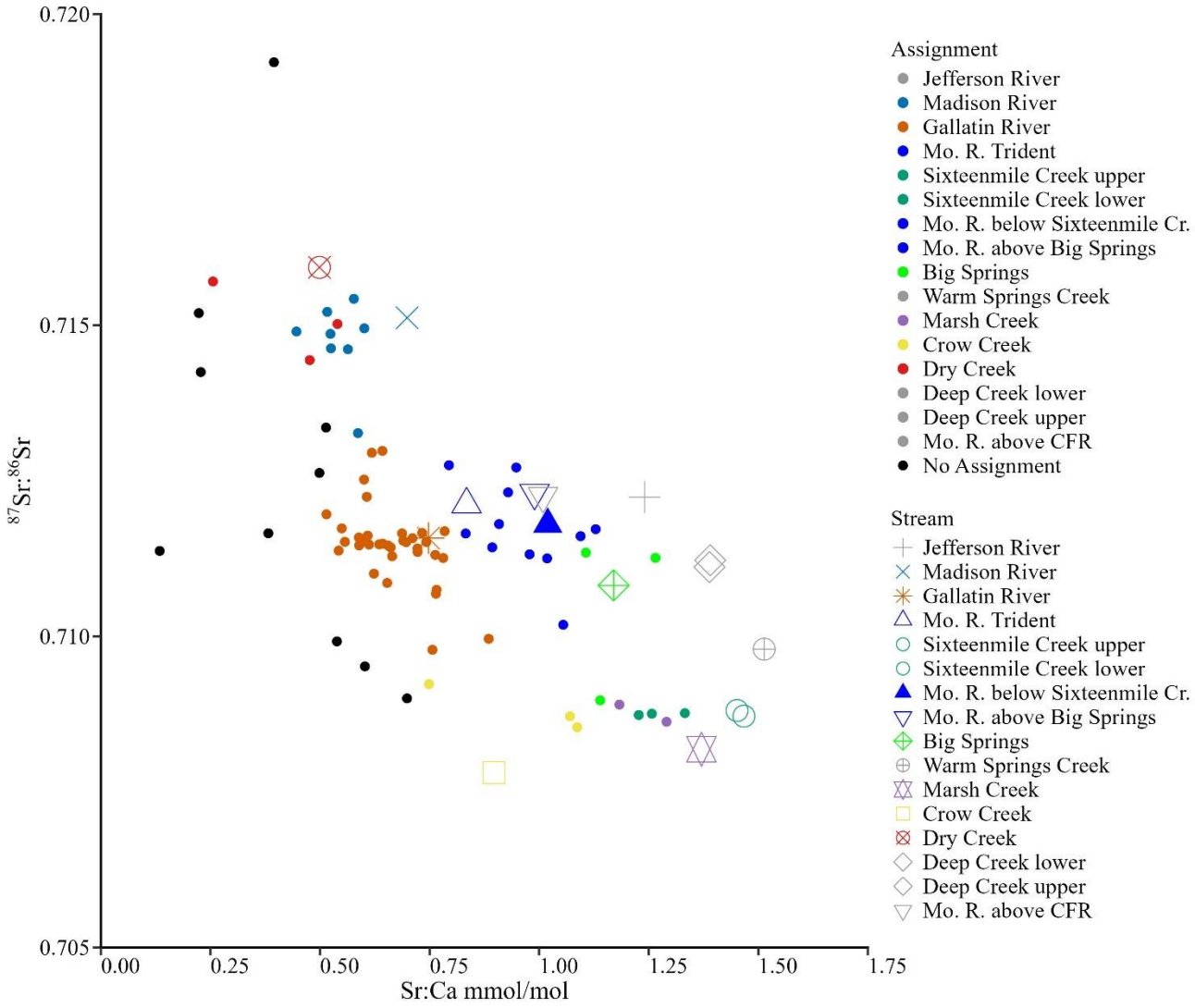

Figure 11. Adjusted Sr:Ca (mmol/mol) = 0.3829x – 0.0314, and corresponding $^{87}Sr:^{86}Sr$ ratios. Otolith elemental signatures are overlaid, with assignments to specific water sources indicated by matching colors. Waterways with no fish assigned to them are colored gray.

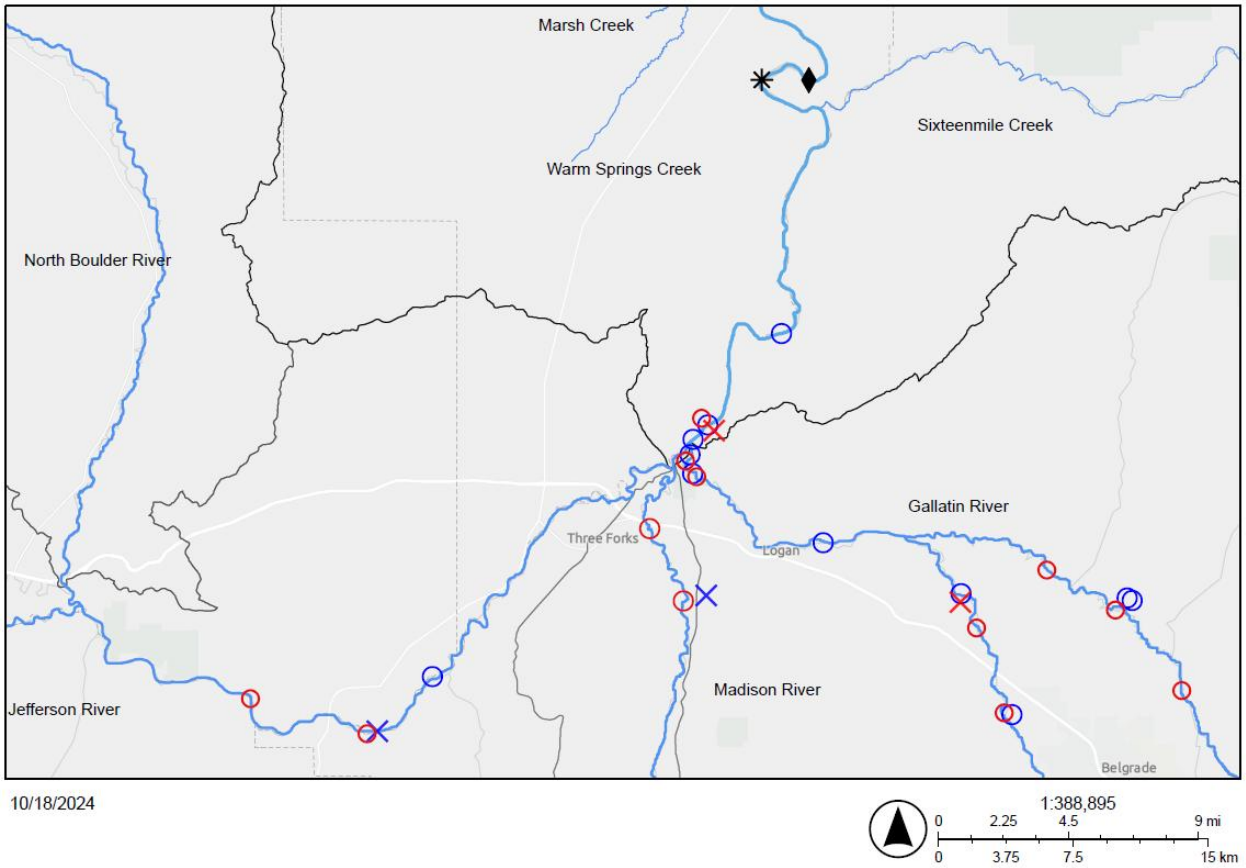

Figure 12. Farthest upstream locations (circles) of radio-tagged Brown Trout documented after transport above Toston Dam (diamond) in 2022. Female fish are indicated by red marks and male fish by blue. Inactive tags are indicated with a cross and the translocation site is marked by a black asterisk.

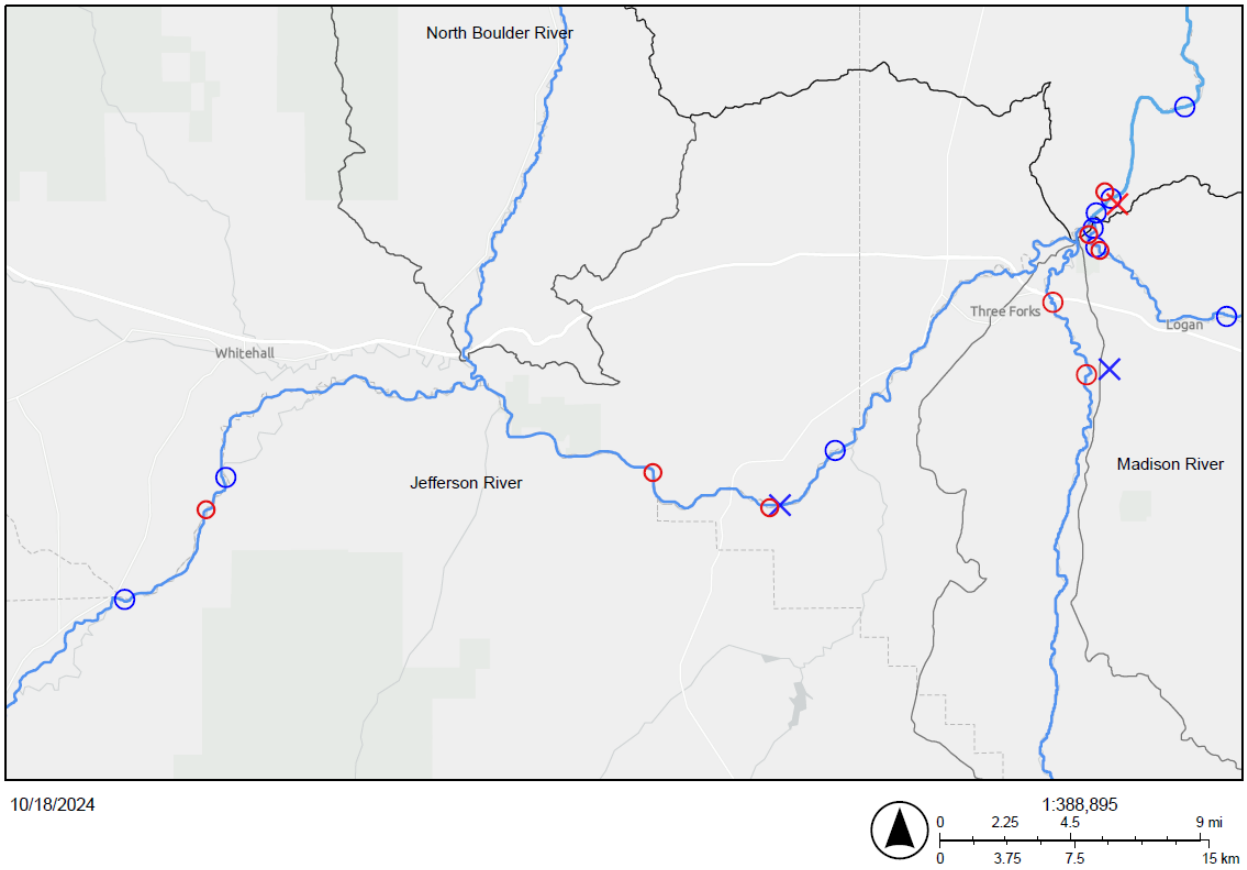

Figure 13. Farthest upstream locations (circles) of radio-tagged Brown Trout documented in the Jefferson River in 2022. Female fish are indicated by red marks and male fish by blue. Inactive tags are indicated by a cross.

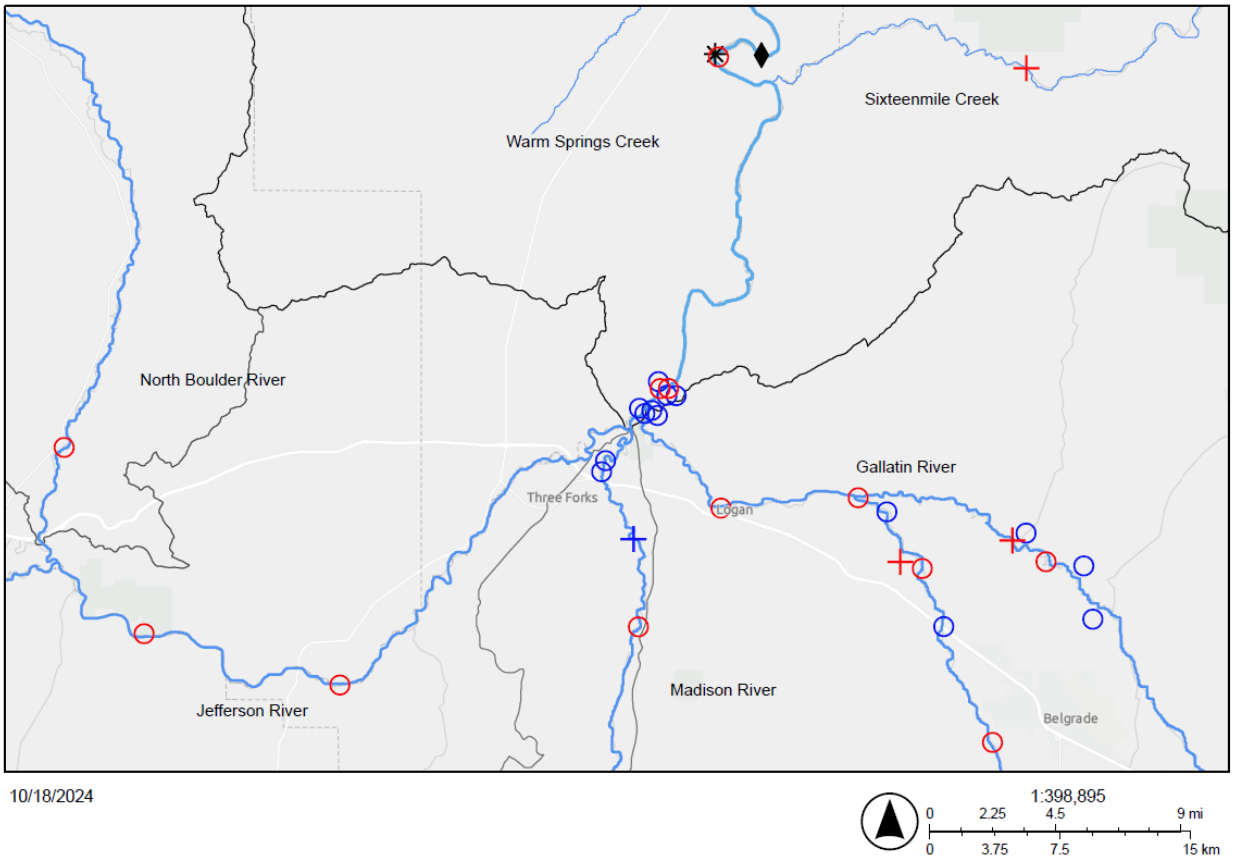

Figure 14. Farthest upstream locations (circles) of radio-tagged Brown Trout documented after transport above Toston Dam (diamond) in 2023. Female fish are indicated by red marks and male fish by blue. Inactive tags are indicated with a cross and the translocation site is marked by a black asterisk.