SUCCESS OF WESTSLOPE CUTTHROAT TROUT AND ARCTIC GRAYLING CONSERVATION TRANSLOCATIONS IN YELLOWSTONE NATIONAL PARK, MONTANA AND WYOMING, USA

by

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ABSTRACT

Restoration of native Westslope Cutthroat Trout *Oncorhynchus clarkii lewisi* and fluvial Arctic Grayling *Thymallus arcticus* in Yellowstone National Park is a high priority for fishery managers. Restoration of the East Fork Specimen Creek and Grayling Creek watersheds included construction of fish barriers to isolate watersheds, application of rotenone to eliminate nonnative and hybridized fish, and translocations of native fish. We sampled these watersheds in 2018 and 2019 to evaluate the success of restoration efforts by 1) estimating the stage of recovery achieved by translocated populations, 2) determining contributions of Westslope Cutthroat Trout donor sources to the translocated populations in the East Fork Specimen Creek watershed by investigating their genetic ancestries, and 3) making comparisons of recovery between the East Fork Specimen and Grayling Creek watersheds. Recovery of Westslope Cutthroat Trout in both watersheds is progressing, with expected differences in stage of recovery between the two watersheds attributable to a 5-year difference in restoration timelines. Conversely, recovery of Arctic Grayling in Grayling Creek appears improbable without management intervention, but the surprising documentation of naturally reproduced individuals engenders a hopeful future for continued Arctic Grayling recovery efforts. Interspecific hybrid introgression discovered in Westslope Cutthroat Trout populations in East Fork Specimen and Grayling creeks probably resulted from barrier failure or incomplete eradication of hybrid fish during rotenone treatments. Whereas all Westslope Cutthroat Trout donor sources contributed to the recovering population in East Fork Specimen Creek, contributions were disproportionate to numbers translocated, indicating potential fitness differences among donor sources. Findings from this study have already helped Yellowstone National Park fishery managers make adaptive management decisions and will help inform future native fish conservation translocations.

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Introduction

The notable losses of native species worldwide, in what is considered the sixth mass-extinction event (Ceballos et al. 2015), affect ecological functions and the socioeconomic benefits they provide (Pimentel et al. 1997, 2005; Valiente-Banuet et al. 2015). The most-identified threats to native species are habitat loss, habitat degradation, and nonnative species (Wilcove et al. 1998). Consequently, restoring native species to suitable habitats that can sustain them in the future and that are free of nonnative species is a task managers and researchers are undertaking. One strategy commonly used is conservation translocation.

Conservation translocation projects can be used to expand ranges of imperiled species within historical ranges (i.e., reintroductions) or establish new conservation populations outside historical ranges (i.e., introductions; IUCN SSC 2013). Several notable conservation translocations have been successful following the extirpation or extinction of a species in the wild (e.g., California Condors *Gymnogyps californianus*, Walters et al. 2010; gray wolves *Canus lupis*, Smith et al. 2016; black-footed ferrets *Mustela nigripes*, Dobson and Lyles 2000; and Kihansi spray toads *Nectophrynoides asperginis*, Nahonyo et al. 2017). Moreover, auspicious research to implement conservation translocation projects is progressing for imperiled species across numerous taxa (e.g., plants, Godefroid et al. 2011; freshwater mussels, Haag and Williams 2014; amphibians and reptiles, Germano et al. 2014; and macroinvertebrates, Jourdan et al. 2019).

Because nonnative species are a major threat to native species (Wilcove et al. 1998), conservation translocations are often coupled with eradication or suppression of nonnative species. For example, giant tortoise *Chelonoidis hoodensis* reintroductions in the Galapagos Islands, Ecuador, coincided with feral goat *Capra hircus* eradications (Gibbs et al. 2014). Island fox *Urocyon littoralis* reintroductions in the Channel Islands, California, were coupled with live removals of Golden Eagles *Aquila chrysaetos* and eradications of feral pigs *Sus scrofa* (Coonan et al. 2005). The absence of Bald Eagles *Haliaeetus leucocephalus* and the presence of feral pigs increased the Golden Eagle population, causing higher predation upon foxes in this ecologically complex example (Roemer et al. 2001). Declines of native Banded Kokopu *Galaxias fasciatus* across New Zealand have been attributed to nonnative salmonids (McDowall 2006). Therefore, researchers captured Banded Kokopu, held them in refuge waters, and reintroduced them following nonnative Brown Trout *Salmo trutta* eradications in two streams in Karori, Wellington (Pham et al. 2013). Native fish conservation translocation projects are underrepresented compared to those of mammals and birds (Seddon et al. 2005), but have been increasingly used to restore native freshwater salmonids (Minckley 1995; Harig et al. 2000; Liermann 2001; Al-Chokhachy et al. 2009; Dunham et al. 2011; Soorae 2011, 2013; Cochran-Biederman et al. 2015; Hayes and Banish 2017; Wilson 2017; LeChaminat 2019; Clancey et al. 2019; Payne et al. 2019; Michigan Arctic Grayling Recovery Initiative, migrayling.org).

Native fish translocations generally occur in target waters that are isolated (by natural waterfalls) or can be isolated (by construction of fish barriers) from nonnative fishes that pose threats to native fishes through hybridization (Allendorf and Leary 1988), competition (Shepard 2010), or predation (Koel et al. 2019b). However, because about half of fish-bearing streams in the western United States contain nonnative fishes (Schade and Bonar 2005), project managers must often consider conservation translocations into recipient waters that are historically fishless or contain nonnative fishes, or both. Whereas translocations of fishes outside their historical ranges had lower probabilities of success compared to those within (Harig et al. 2000), translocations into waters containing nonnative fishes may fail to fully address the threats they pose (Al-Chokhachy et al. 2009). Therefore, many fish translocation projects include a nonnative fish eradication phase, usually achieved by mechanical (electrofishing, angling, netting) or chemical (piscicides) removal techniques.

Native fish translocation projects are expensive and time consuming, especially if barrier construction and nonnative fish removal are required. Therefore, monitoring to determine project outcomes is vital to assess whether project goals were met. For example, the native fish restoration project in Cherry Creek, Montana, was estimated to cost US$2.2 million (Clancey et al. 2019). The 20-year project included barrier construction, multiple piscicide treatments to remove nonnative fishes, conservation translocations of genetically pure fish, and several years of post-project monitoring (Andrews et al. 2013, 2016; Clancey et al. 2019). Post-project monitoring confirmed that eradication of nonnative fishes was achieved and a robust Westslope Cutthroat Trout *Oncorhynchus clarkii lewisi* population persists, making it a valuable example in native fish translocation case studies.

Assessing Conservation Translocations

Outcomes of conservation translocations in the published literature are variable or often unknown. A review of 116 multi-taxon reintroductions reported 27% of those projects failed, 26% were successful, and 47% had unknown outcomes (Fischer and Lindenmayer 2000). A similar review of 260 freshwater fish reintroductions reported 42% of those projects failed and 58% were successful (Cochran-Biederman et al. 2015). The success rates of reintroductions in the published literature are probably biased toward those that were labeled successful (Fischer and Lindemayer 2000; Miller et al. 2014), indicating many unsuccessful projects go unreported. Most conservation translocation projects are pursued with the overall goal to establish a "a self-sustaining population" that can persist through time without additional management (Seddon 1999), but determining whether a project was successful can be difficult when clear definitions for translocation success are not universal (Minckley 1995).

Population Recovery Stages. Successful population recovery to the desired status of a self-sustaining population is generally outlined by the progression of three stages: 1) survival and establishment of translocated individuals, 2) population growth through reproduction of translocated individuals and their offspring, and 3) population persistence and regulation after carrying capacity is reached; i.e., "a self-sustaining population" (Seddon 1999; Sarrazin 2007; Robert et al. 2015). However, the expected stages of population recovery in translocation projects vary by author, are sometimes vaguely defined, and can be named ambiguously (e.g., "established"; Kocovsky et al. 2018), perhaps, to broadly apply across multiple taxa. The stages of population recovery will inherently vary depending upon project characteristics (species, number and age of individuals translocated, number of translocation events, available project resources, etc.), so project leaders should clearly define expected stages of population recovery that are tailored to project goals, and implement thorough post-project monitoring to determine which stage of population recovery was achieved.

Published case studies of freshwater fish translocation projects seemingly focus more on early stages of population recovery (survival, spawning, recruitment; Cochran-Biederman et al. 2015) and less on late stages of population recovery (regulation, persistence), probably because most post-project monitoring occurs within a few of years after translocation events when early stages of population recovery occur. A translocated population might take several years to reach a regulation stage, so determination thereof may require several years of monitoring effort.

Comparisons of pre- and post-restoration population metrics are a useful way for researchers to evaluate outcomes of restoration projects (Roni et al. 2013). Pre-restoration data can provide guidelines for the stage of recovery expected to be achieved (or exceeded) by the post-restoration population. For example, native fish translocations were considered successful in Cherry Creek, Montana, because post-restoration population densities equaled or exceeded those of the pre-restoration non-native fish population (Clancey et al. 2019). If pre-restoration population data are lacking, comparisons of population metrics between target and reference streams could also be used to gauge project success (Roni et al. 2013).

Clearly defined population recovery stages that are tailored to project goals and thorough post-project monitoring will help managers to better evaluate translocation project outcomes. Post-project monitoring is instrumental to implement adaptive management strategies as needed (Nichols and Armstrong 2012), which should lead to favorable project outcomes. The knowledge gained about the specific recovery stage that a translocated population failed or partially failed to achieve is imperative to pinpointing “why” it failed. Publishing pertinent knowledge gained from post-project monitoring, especially for projects that failed or partially failed, will in turn improve outcomes of future conservation translocations.

Donor Source Influences. Many variables influence the success of freshwater fish translocation projects (Harig et al. 2000; George et al. 2009; Cochran-Biederman et al. 2015; IUCN SCC 2013), including the donor sources used for translocations, which is an important component of project success (Houde et al. 2015; Andrews et al. 2016; LeCheminant 2019). Options for potential donor sources will vary depending on species status (listed as threatened or not under the Endangered Species Act of 1973; 16 U.S.C. 1531 – 1544, 87 Stat. 884), project goals (to create a genetically diverse or distinctive lineage), target-area type (lentic or lotic environment), desired life history strategy (fluvial, adfluvial, or lacustrine), and project idiosyncrasies (time, monetary, and accessibility considerations). Deliberations about which donor source to use may be necessary when multiple sources are available (Andrews et al. 2016). Houde et al. (2015) outlined two strategies for selecting donors. The pre-existing adaptation strategy involves selecting appropriately "matched" donor sources (either environmentally to the target environment or genetically to the extirpated population). The adaptive potential strategy involves selecting donor sources that will produce genetically diverse populations that have a high probability of adapting to their new environment (either by translocating one genetically diverse donor source or multiple genetically distinct donor sources). Knowledge gaps exist in both strategies, especially for projects where the adaptive potential strategy was implemented by translocating multiple donor sources (only 3 of 23 case studies in Houde et al. 2015). The lack of multi-source case studies possibly stems from a lack of multiple donor sources to use, and from uncertainties regarding outbreeding depression (Huff et al. 2010; Weeks et al. 2011; Jamieson and Lacy 2012).

Few freshwater fish translocations that implemented the multi-source adaptive potential strategy monitored donor source contributions to the recovering population (exceptions are Wilson et al. 2007; Huff et al. 2010; Andrews et al. 2013, 2016; Campbell et al. 2017) and fewer monitored donor source contributions in populations that had reached later stages of population recovery. Population performance metrics for early recovery stages, e.g., embryonic and juvenile survival of native trout, have been shown to vary by donor source (Drinan et al. 2012; Andrews et al. 2013, 2016). However, evaluation of translocated populations that have reached later stages of recovery (after reproduction occurs) would help researchers and managers to better understand donor source influences, particularly donor source fitness. Population genetic ancestry (genetic structure), in relation to the different donor sources used, provides a useful index to apparent donor source fitness (Huff et al. 2010). Results from case studies where population genetic ancestry was investigated in multi-source translocations were variable such that some donor sources outperformed the others (Walleye *Sander vitreus*, Wilson et al. 2007; Slimy Sculpin *Cottus cognatus*, Huff et al. 2010), some donor sources were unrepresented (Peregrine Falcons *Falco peregrinus*, Tordoff and Redig 2001), admixture between donor sources was common (large-flowered sandwort *Arenaria grandiflora*, Zavodna et al. 2015), and mixed variations of the aforementioned (Coho Salmon *O. kisutch*, Campbell et al. 2017; fishers *Pekania pennant*, Hapeman et al. 2017). Therefore, information gained from estimating relative donor source contributions to translocated populations, especially populations that have reached later stages of recovery, will improve understanding of longer-term donor source influences and possible outcomes of projects that used multi-source selections.

Project Comparisons. Comparisons among conservation translocation projects can help contribute general knowledge to the relatively new field of conservation translocation biology (Minckley 1995), especially for native freshwater fishes. Components of translocation projects such as the stages of population recovery achieved, timelines of recovery, fish densities, size structures, body conditions, donor source contributions, and habitat characteristics can be compared among projects to help better explain project-to-project variation and predict project outcomes. Knowledge gained from these comparisons will help to better inform future translocation projects and increase the number of successful projects. Increased numbers of successful conservation translocations will in turn increase the potential to help imperiled species by decreasing their extinction risk.

Westslope Cutthroat Trout and Arctic Grayling

Westslope Cutthroat Trout *Oncorhynchus clarkii lewisi* (WCT) and Arctic Grayling *Thymallus arcticus* are two imperiled species in Yellowstone National Park, Montana and Wyoming, USA, that would benefit from successful conservation translocations (Koel et al. 2010). Genetically pure WCT are estimated to occupy about 22% of their historical range in the western United States (Shepard et al. 2005) but only 5% of their historical range in the upper Missouri River watershed (Shepard et al. 1997). Additionally, genetically pure WCT were nearly extirpated from Yellowstone National Park by the 1930s (Varley and Schullery 1998). The only known remaining aboriginal population within the park was discovered in 2005 in Last Chance Creek—an isolated, 2-km long tributary to Grayling Creek in the Madison River drainage (Arnold et al. 2017). Declines of WCT are mostly attributed to hybridization with Rainbow Trout *O. mykiss* and other subspecies of Cutthroat Trout *O. clarkii* spp. and displacement by Brown Trout *Salmo trutta* and Brook Trout *Salvelinus fontinalis* (Allendorf and Leary 1988; U.S. Office of the Federal Register 2000; Behnke 2002; Quist and Hubert 2004; Shepard et al. 2005; Shepard 2010).

Arctic Grayling in the contiguous United States historically existed in two disjunct populations—one in Michigan and the other in the upper Missouri River watershed in Montana and Wyoming (Vincent 1962; U.S. Office of the Federal Register 2014). The Michigan population was extirpated in the 1930s from the combination of overfishing, logging, and nonnative fishes (Vincent 1962). The upper Missouri River population also experienced drastic declines, especially among fluvial populations that were estimated to occupy 4 – 5% of their historical range (Kaya 1992). Fluvial grayling were extirpated from streams within Yellowstone National Park and from all but one stream within their native range in Montana—the Big Hole River (Kaya 1992; Steed et al. 2010; Arnold et al. 2017). Arctic Grayling declines are attributed to nonnative fish introductions, fluvial habitat fragmentation and loss such as dam construction (Hebgen Reservoir), and fishing pressure (Vincent 1962; Varley 1981; Kaya 1992, 2000). Past stocking efforts in lakes of the upper Missouri River drainage established 20 introduced populations in addition to six native adfluvial populations (U.S. Office of the Federal Register 2014). Some efforts to reintroduce fluvial grayling to streams were successful (Liermann 2001; Cayer and McCullough 2014), but most reintroductions failed or resulted in relatively few individuals captured during post-project assessments (Kaya 1992; Kaya 2000; Cayer et al. 2014, 2015, 2016; Payne et al. 2019).

Both WCT and Arctic Grayling have been candidates for listing under the Endangered Species Act (WCT, U.S. Office of the Federal Register 2000, 2003; Arctic Grayling, U.S. Office of the Federal Register 2014, 2020) and both are considered species of concern by the state of Montana (WCT, S2; Arctic Grayling, S1; Montana Natural Heritage Program and Montana Fish, Wildlife & Parks 2020). Both species also have high conservation priorities in Yellowstone National Park (Koel et al. 2010; Arnold et al. 2017).

Yellowstone National Park fishery managers completed two large-scale native fish conservation translocation projects in the East Fork Specimen Creek and Grayling Creek watersheds whereby barriers were built to isolate headwater streams, piscicide treatments were conducted to remove nonnative fishes, and conservation translocations of WCT and fluvial Arctic Grayling were completed (Arnold et al. 2017). Translocations occurred for three consecutive years in all restoration areas. Whereas some age-1 and older fish were translocated by vehicle or helicopter, most translocations were completed using remote-site incubators (hereafter RSIs; Rupert et al. 2007), which facilitated late-stage embryo development within the restoration area (Arnold et al. 2017).

Assessing the outcomes of WCT and Arctic Grayling conservation translocation efforts in the East Fork Specimen Creek and Grayling Creek watersheds is important to National Park Service fishery managers to determine if additional management intervention is needed in those watersheds, and to help inform future native fish translocations in other watersheds. Perhaps the most important component in evaluating these conservation translocation projects is to determine which stages of population recovery, if any, were achieved by the translocated populations. Additionally, WCT in the East Fork Specimen Creek watershed were translocated using four different donor sources, which presented an opportunity to investigate the outcome of a multi-source translocated population that has had 7 – 12 years to advance through the stages of population recovery. Because native fish conservation translocations in East Fork Specimen and Grayling creeks occurred about five years apart, an opportunity existed to compare similarities and differences between these two watersheds and contribute general knowledge to the field of translocation biology of native freshwater fishes. Although some pre- and post-restoration data were collected by National Park Service personnel prior to this study (Koel and York 2006; Arnold et al. 2017), formal comparisons and thorough evaluations of project outcomes had not been made heretofore.

Study Objectives

The overall goal of this study was to evaluate the success of conservation translocations of WCT and Arctic Grayling in the East Fork Specimen Creek and Grayling Creek watersheds in Yellowstone National Park, Montana and Wyoming, USA, and to provide guidance for future translocations. We investigated three specific objectives to evaluate translocation efforts. Our first objective was to define stages of population recovery that are specific to native fish translocations whereby most individuals were translocated as embryos, outline biological metrics for each stage of recovery to use as inference for whether that stage of recovery was achieved, and determine which stage of population recovery was achieved by WCT and Arctic Grayling populations in each watershed. Our second objective was to investigate the genetic ancestry of the translocated WCT population in the East Fork Specimen Creek watershed 7 – 12 years post-translocations to determine whether relative contributions differed among the four donor sources that were used; genetic ancestry of the WCT population in Grayling Creek was not investigated because only two donor sources were used for translocations there and the population had had little time for natural reproduction to occur. Lastly, our third objective was to compare components of these translocation projects such as stages of recovery achieved, fish densities, size structures, body conditions, and general habitat characteristics between the East Fork Specimen Creek and Grayling Creek watersheds to determine how they differ given the 5-year difference in restoration timelines between them.

Study Areas and Past Restoration Activities

East Fork Specimen Creek Watershed

The headwaters of East Fork Specimen Creek originate at High Lake in the Gallatin River drainage in northwestern Yellowstone National Park, Montana (Figure 1). High Lake has a surface area of about 2.9 ha, a maximum depth of about 6 m, and an elevation of 2,675 m. High Lake is fed by two inlet streams, several small seep springs, snowmelt, and precipitation. August water temperatures were 16.5°C at the surface and 8.3°C at the bottom in 2005 (Koel and York 2006). Although High Lake was historically fishless because a natural waterfall (about 0.4 km downstream from the lake's outlet) precluded fish colonization, nonnative Yellowstone Cutthroat Trout *O. c. bouvieri* were stocked into High Lake in 1937 (Varley 1981) and persisted for several decades. Gill net sampling in High Lake in 1970 and 2005 resulted in the captures of 58 and 64 Yellowstone Cutthroat Trout, respectively (Koel and York 2006).

The main stem and tributaries in the East Fork Specimen Creek restoration area total to 39.5 km. The main stem varies in elevation from 2,231 – 2,675 m from the lower-most barrier to the High Lake outlet. Tributaries originate at high elevations in the mountains and most are intermittent with flows that fluctuate with snowmelt. East Fork Specimen Creek water sources include the High Lake outlet, snowmelt, rainfall, and several seeps and springs (Koel and York 2006). East Fork Specimen Creek is an exceptionally cold stream with monthly mean water temperatures that did not exceeded 7°C during the summer months in 2018 and 2019. Nonnative Yellowstone Cutthroat Trout from High Lake and nonnative Rainbow Trout stocked in the Gallatin River invaded East Fork Specimen Creek and compromised the genetics of the native WCT population there. Estimated abundance of the hybridized WCT population was 2,485 fish in main-stem East Fork Specimen Creek in 2004 and 2005 (Koel and York 2006).

The National Park Service implemented a WCT translocation project in the East Fork Specimen Creek watershed from 2006 to 2012 (Figure 2; Arnold et al. 2017). Restoration activities included 1) applying rotenone to High Lake in 2006 to remove the nonnative Yellowstone Cutthroat Trout population, 2) translocating genetically pure WCT into High Lake from 2007 to 2009, 3) constructing a temporary log barrier on East Fork Specimen Creek in 2008 to isolate about 11.2 main-stem km and 28.3 tributary km, 4) applying rotenone to East Fork Specimen Creek in 2008 and 2009 to remove the hybridized WCT population, and 5) translocating genetically pure WCT to East Fork Specimen Creek from 2010 to 2012.

Donor Sources of Westslope Cutthroat Trout. Donor sources of WCT that were used in the East Fork Specimen Creek watershed comprised three wild sources and one conservation hatchery source. Donor sources used in High Lake were from WCT populations in the Geode Creek/Oxbow Creek complex (hereafter Geode Creek), Last Chance Creek, and the Sun Ranch Hatchery (Table 1). Donors used in main-stem East Fork Specimen Creek were from Geode Creek and Muskrat Creek.

Only genetically pure WCT populations that had a 99% probability of containing < 1% non-WCT genetic material (based on genetic testing) were used as donor sources for translocations. Two genetically pure WCT populations exist in Yellowstone National Park. Genetically pure WCT were discovered in 2005 in Geode Creek, which is an isolated tributary to the Yellowstone River outside of the native range of the subspecies (Koel et al. 2007). Historical records indicate Geode Creek was stocked with "cutthroat trout" in the 1920s (Varley 1981) but contain no documentation regarding their origins. Genetic analyses determined that the Geode Creek cutthroat were genetically pure WCT (Koel et al. 2007) but were inconclusive in identifying the original donor source (T. Koel, Yellowstone National Park, personal communication). Regardless, the newly discovered genetically pure WCT population provided Yellowstone National Park fishery managers with a conveniently proximate donor source. Genetically pure WCT were also discovered in 2005 in Last Chance Creek, which is an isolated tributary to Grayling Creek (Koel et al. 2006). Last Chance Creek WCT are the last known aboriginal WCT population remaining within their historical range in the Park.

Other genetically pure WCT populations extant to tributaries of the upper Missouri River basin were also used as donors. Genetically pure WCT were discovered in Muskrat Creek, which is an isolated tributary to the Boulder River in the Elkhorn Mountains, Montana. A fish barrier was constructed, and nonnative Brook Trout eradication was completed to help conserve this genetically pure WCT population (Shepard and Nelson 2004; Shepard 2010). The Sun Ranch Hatchery WCT brood source was developed at the privately owned Sun Ranch in the Madison River Valley, Montana. This brood comprises several wild WCT donor sources from tributaries in the upper Missouri River drainage of Montana, including Last Chance, Muskrat, Ray, and White's creeks (Clancey and Lohrenz 2015). Ray and White's creeks are both isolated tributaries to the upper Missouri River drainage in the Big Belt Mountains, Montana.

Grayling Creek Watershed

Grayling Creek, in western Yellowstone National Park, Montana and Wyoming (Figure 1), was a tributary to the Madison River prior to the construction of Hebgen Reservoir in 1915. The main stem and tributaries in the Grayling Creek restoration area total to 78.7 km. The main stem varies in elevation from 2,131 – 2,400 m from the lower-most barrier to the North and South forks. Tributaries originate at high elevations in the mountains and several are intermittent with flows that fluctuate with snowmelt. Grayling Creek water sources include snowmelt, rainfall, and several seeps and springs. Monthly mean water temperatures in Grayling Creek did not exceeded 10.2°C in the summer months in 2018 and 2019. Westslope Cutthroat Trout and fluvial Arctic Grayling are native to Grayling Creek, but nonnative Rainbow Trout, Yellowstone Cutthroat Trout, and Brown Trout compromised WCT genetics or competed with WCT, and ostensibly contributed to the extirpation of grayling (Kaya 2000).

The National Park Service implemented a native fish translocation project from 2012 to 2017 in Grayling Creek (Figure 2; Arnold et al. 2017). Restoration activities included 1) modifying a waterfall to create a permanent barrier in 2012 to isolate about 17.1 main-stem km and 61.6 tributary km and prevent upstream invasion of nonnative fishes, 2) applying piscicide (rotenone) to the isolated watershed in 2013 and 2014 to remove the hybridized and nonnative fish assemblage, and 3) translocating genetically pure WCT and fluvial Arctic Grayling from 2015 to 2017. Westslope Cutthroat Trout were translocated from Geode Creek and the Sun Ranch Hatchery (Table 1). Fluvial grayling were translocated from the Axolotl Chain of Lakes, which is a fluvial grayling brood source developed by Montana Fish, Wildlife & Parks that replicates the genetics of fluvial grayling in the Big Hole River, Montana (Magee and McCullough 2008; Table 1). The fluvial life-history form is native to Yellowstone National Park. Age-0 grayling were used instead of embryos in 2017 because conditions in Grayling Creek were unsuitable (deep snow and 0°C water temperatures) when embryos were ready for transportation.

Methods

Determining Stages of Population Recovery

Definitions and Biological Metrics. Herein, we provide definitions for the stages of population recovery expected from a native freshwater salmonid conservation translocation project whereby most fish were translocated as embryos using RSIs (East Fork Specimen Creek and Grayling Creek watersheds; Arnold et al. 2017). We also describe biological metrics that we measured to indicate a population had reached that specific stage. Theoretically, population recruitment models such as the Beverton-Holt recruitment model (Beverton and Holt 1957; Maceina and Pereira 2007) could be used to help estimate which stage of recovery a population achieved (see Figure 3 for a conceptual model).

Survival (Stage 1a): translocated individuals remained within the target restoration area and survived to maturity (Figure 3). Documenting presence or absence of translocated individuals throughout the restoration area is probably the simplest method to document survival, but we also estimated population abundance, density, and size structure and documented evidence of maturity (ripe eggs, milt) to more extensively investigate survival of translocated individuals. Because most translocations in the East Fork Specimen Creek and Grayling Creek watersheds occurred by translocating embryos using RSIs, age or size at maturity of the target species is an important variable to consider because natural reproduction will not occur until translocated individuals become mature. Freshwater salmonids reach maturity in 2 – 6 years in cold, headwater streams, and males usually mature earlier than females (Downs et al. 1997; U.S. Office of the Federal Register 2014).

Reproduction (Stage 1b): translocated individuals successfully reproduced, and their offspring also survived to maturity (Figure 3). We used the same metrics outlined in the survival stage to estimate reproduction of translocated individuals by sampling in the years following translocations when at least some natural reproduction possibly occurred. Captures of fish in small size groups would indicate naturally reproduced individuals were present. Depending on age at maturity, population growth may not be realized for several years despite occurrence of small rates of natural reproduction because numbers of births might not offset deaths until the population reproduces consistently.

Population Growth (Stage 2): consistent natural reproduction of translocated individuals and their offspring resulted in population growth (Figure 3). Evidence that population growth was achieved was inferred by calculating a growth rate using two or more population abundance estimates ($\widehat{N}_{5\ years\ post\text{-}translocation} < \widehat{N}_{10\ years\ post\text{-}translocation}$). Additionally, population size-structure metrics were investigated for evidence of recruitment among year classes to use as an index to population growth.

Population Regulation (Stage 3): the population reached carrying capacity and is therefore regulated by environmental variables and inter- and intra-specific competition, i.e., density-dependent variables (Figure 3); the population persists with minimal to no management intervention. We investigated fish body conditions of the translocated populations to infer relative competition levels experienced by individuals. Theoretically, populations that experience lower levels of competition (lower population density and less resource limitation) will have higher mean body conditions whereas populations that experience higher levels of competition (higher density and more resource limitation) will have lower mean body conditions (Johnson et al. 1992; Blackwell et al. 2000). Additionally, some pre-restoration data were collected in East

Fork Specimen (abundance, density, and body conditions) and Grayling creeks (body conditions) on fish populations that presumably had reached the population regulation stage because those populations had existed there for several decades. Therefore, we also compared pre- and post-restoration population metrics of abundances, densities, and body conditions to deduce whether the translocated populations were similar to the pre-restoration populations. Comparisons to reference streams could also be used if pre-restoration data were lacking.

Notably, one population could exhibit multiple stages of population recovery within a watershed. For example, the population at one site within the watershed could have reached the population regulation stage whereas it was still at the population growth stage at a different site. Several variables such as rates of population dispersal, time of year that the population is assessed, and habitat characteristics could influence the stages of recovery achieved and are important to consider when assessing population recovery.

Sampling Methods

East Fork Specimen Creek. Crews planned to sample fish in East Fork Specimen Creek in the autumn of 2018 and 2019 using multi-pass-depletion methods; however, a wildfire near Bacon Rind Creek precluded crews from safely entering the watershed in 2018 so East Fork Specimen Creek was sampled only August 19 – 24, 2019. Crews multi-pass-depletion sampled (3 – 4 passes) 10 random sites, 3 historical sites, and one tributary site (Figure 4). The 10 random sites were generated (one site about every one kilometer) using ArcGIS (Desktop: Release 10.6; Environmental Systems Research Institute, Redlands, California). Three historical sites where pre-restoration data were collected in 2004 and 2005 (Koel and York 2006) were resampled in 2019. Because no fish were captured in tributaries during exploratory sampling, only the largest tributary was sampled. All multi-pass-depletion sites were 100 – 200-m long. Block nets were placed at the downstream and upstream extents of sites, but sometimes a stream feature (e.g., waterfall, shallow riffle) was used on the upstream extent of a site in lieu of a block net. Three to four passes were made in an upstream or downstream direction depending on water velocities (higher water velocities made it difficult to safely walk upstream). Only one pass was conducted if no fish were captured in the first pass. Fish were stunned using one or two Smith-Root (Vancouver, Washington) LR-24B backpack electrofishers and retrieved with dip nets. Crews used pulsed DC with 30-Hz frequency and 12% duty cycle settings (equivalent to a 4-ms pulse width), and the "quick setup" feature was used to set voltage (typically 300 – 400 V). Fish were counted and held in modified minnow traps after each pass. Insufficient depletion sometimes occurred when fish captures were low (e.g., when 6, 2, and 4 fish were captured on passes 1, 2, and 3, respectively). Therefore, some sites were extended from 100 m to 200 m if between 5 and 11 fish were captured during the first pass to try to improve catch vectors and attain sufficient depletion. All fish were released after measurements were recorded and passes were completed.

High Lake. Crews conducted two-event, mark-recapture abundance estimation sampling for WCT using gill nets set from inflatable rafts in High Lake July 16 – 19, 2018, but not in 2019. Six random sites for gill-net sets were generated using ArcGIS by dividing the lake shoreline into six equal sections and generating random starting locations within each section (Figure 5). Gill nets were 2.4 m tall, 90 m long, and had six monofilament panels that varied in mesh size (bar) from 19 – 51 mm by 6-mm increments (Syslo et al. 2014); gill nets were weighted with 2.3-kg weights. Gill-net sets started near the shoreline at a depth of 1.5 m and continued in a straight line towards the center of the lake. Crews alternated placement of small- and large-meshed net ends closest to shore. Gill nets were checked at 3-h intervals during daylight hours and removed from the water after 9 h. Captured fish were held in modified minnow traps until all gill nets were removed from the water. The recapture event started 24 h after the marking event.

Crews also conducted multi-pass-depletion fish abundance estimation in the inlets and outlet to High Lake on July 18, 2018, and July 9, 2019. Crews multi-pass-depletion sampled three random sites—one site in each inlet and the outlet to High Lake (Figure 5). Random sites were generated using ArcGIS. Sites were 100 – 200 m long. Whereas the outlet site was sampled in both 2018 and 2019, the two inlet sites were only sampled in 2019 because low flows precluded sampling in 2018. The same multi-pass depletion sampling methods that were used in East Fork Specimen Creek were used at High Lake. Additionally, one expert-skilled angler sampled along the N and NE shores for about 1.5 h using fly fishing gear in both 2018 and 2019 (Figure 5). Fish captured by angling were not included in abundance estimates.

Grayling Creek. Crews conducted two-event, mark-recapture abundance estimation sampling of WCT and Arctic Grayling in main-stem Grayling Creek July 30 – August 14, 2018, and July 22 – August 8, 2019. Crews planned to sample the entire main stem in a downstream direction starting at the confluence of the North Fork and South Fork tributaries. However, time constraints caused crews to sample only random sites that were generated within each main-stem kilometer (n = 14) using ArcGIS. Therefore, the first 3 km were continuously sampled whereas shorter lengths (0.1 – 0.5 km) were sampled farther downstream. Electrofishing mechanical issues caused sites in the lower portions of the watershed to be skipped (kilometers 10, 12 – 16 in 2018 and kilometers 10 and 12 in 2019). Crews electrofished 30% (5.1 km) of main-stem Grayling Creek in 2018 and 45% (7.6 km) in 2019 (Figure 6). Crews captured fish using a Smith-Root (Vancouver, Washington) VVP-15B electrofisher operated out of a polyethylene

Coleman crawdad boat. Continuous direct-current (100% duty cycle) and 400-V settings were used, but voltage was adjusted as necessary to maximize fish captures and preclude fish injuries. Recapture events occurred 5 – 7 days after marking events.

Crews also conducted multi-pass-depletion fish abundance estimation sampling in tributaries to Grayling Creek July 30 – August 14, 2018, and July 22 – August 8, 2019. Crews multi-pass-depletion sampled 20 random sites in 2018 and 10 random sites in 2019 (Figure 6). Random sites were generated using ArcGIS within the first 500 m upstream of a tributary's confluence because no fish were captured > 500 m upstream in tributaries during exploratory sampling. The same multi-pass-depletion sampling methods that were used in East Fork Specimen Creek were also used in Grayling Creek. However, substantial effort was spent sampling sites that had few or no fish in 2018 and we estimated fish recruitment to those sites in 2019 was low because most fish were still immature in 2018 (age-3 and younger). Therefore, sites that had no fish in 2018 were not revisited in 2019, and only one pass was conducted if fewer than five fish were captured in the first pass in 2019. Insufficient depletion often occurred in 2018 when fish captures were low. Therefore, some sites were extended from 100 m to 200 m in 2019 if between 5 and 11 fish were captured during the first pass to try to improve catch vectors and attain sufficient depletion.

Fish Handling Methods, Measurements, and Observations. All fish captured in both the East Fork Specimen and Grayling Creek watersheds were handled similarly. Fish were anesthetized by immersion in a solution of AQUI-S 20E (AQUI-S New Zealand Ltd, Lower Hutt, New Zealand) and stream water, titrated to efficacy (15 – 30 mg/L; NEON 2017). Fish were identified to species, measured (total length, TL; mm), weighed (g), and scanned for an existing passive integrated transponder (PIT) tag (Biomark, Boise, Idaho; FS-2001 ISO proximity reader); PIT tags were implanted into fish lacking tags. Fish ≥ 80 mm TL were abdominally implanted with either a 12-mm (fish 80 – 120 mm TL) or 23-mm (fish >120 mm TL) long half-duplex PIT tag (Oregon RFID, Portland, Oregon; 12 mm HDX, 23 mm HDX). Fish < 80 mm TL were not tagged because they were more likely to succumb to stress-induced mortality (B. Shepard, B.B. Shepard and Associates, personal communication). A 4-mm incision was made with a #11 scalpel blade about 15 – 20 mm anterior to the left pelvic fin origin and lateral to the midline and the tag was gently inserted into the abdominal cavity (Roussel et al. 2000); sutures were not used. Several scales were collected from fish by scraping with a dull blade above the lateral line and anterior to the adipose fin to determine fish ages. A small anal or caudal fin clip was collected using scissors and stored in ethyl alcohol (absolute, 200 proof) for genetic analysis to determine fish genetic ancestry. Fish were released within 200 m of their capture location after recovery from anesthesia. Rocky Mountain Sculpin *Cottus bondi* (previously recorded as Mottled Sculpin *Cottus bairdii* in Yellowstone National Park) were netted and counted but not tagged nor measured. Furthermore, observations such as presence or absence of age-0 fish, spawning behavior of adults, and evidence of maturity (milt or egg excretion) were recorded during field sampling.

Data Analyses

All data analyses were performed in R (v. 3.6.1; R Core Team 2019) or Program MARK (v.6.2; White and Burnham 1999).

Multi-Pass-Depletion Abundance Estimation. We used multi-pass-depletion models to estimate abundance in East Fork Specimen Creek in both pre- and post-restoration time periods, and in tributaries to Grayling Creek that were sampled in 2018 and 2019. We assessed five assumptions associated with these models.

Assumption 1: *the population was closed to births, deaths, immigration, and emigration*. Crews conducted removal sampling in a single visit to ensure the population was closed to births, deaths, and recruitment of individuals to sampled sizes. Block nets were placed on the downstream and upstream extents of sites to prevent fish movement, but sometimes a stream feature (e.g., waterfall, shallow riffle) was used on the upstream extent of a site instead. Block-net failures sometimes occurred but the influences thereof on abundance estimates were probably minimal (Young and Schmetterling 2004).

Assumption 2: *sampling effort was constant across passes*. Crews thoroughly sampled all habitat in each pass and recorded shock times. Shock times often decreased with successive passes because fewer fish were present. However, we have minimal concern for an assumption violation because ensuring all habitat is thoroughly sampled in each pass is more important than attaining exactly constant sampling effort across passes (Riley and Fausch 1992; Meyer and High 2011).

Assumption 3: *all individuals had equal capture probabilities within a pass*. All personnel performed the same tasks throughout a site to reduce personnel-related influence on fish captures. Fish < 60 mm TL (*n* = 1, 2004 and 2005; *n* = 5, 2018 and 2019) were excluded from abundance estimates because captures thereof occurred rarely (Figure 7); electrofishing is selective towards larger fish (Neumann and Allen 2007). Standardized sampling procedures and exclusion of small fish with low capture probabilities minimized violation of this assumption.

Assumption 4: *capture probabilities were constant across passes or varying capture probabilities were appropriately modeled*. Decreasing capture probabilities over successive passes is common in removal sampling (Zippin 1958; Riley and Fausch 1992) because the fish easiest to capture are captured first. Therefore, we fit and assessed a multi-pass-depletion model that allowed capture probabilities to vary among passes when more than two passes were conducted. Standardized sampling procedures and the ability to test for varying capture probabilities minimized violation of this assumption.

Assumption 5: *the population was adequately depleted with successive passes*. Inadequate depletions sometimes occurred because fewer fish were captured on the first pass than on a subsequent pass, especially if the number of fish captured in the first was low (< 10). Whereas some sites were lengthened if < 10 fish were captured on the first pass to increase the likelihood of adequate depletion, others were inadequately depleted such that estimation failed. Therefore, we also estimated abundances using a Bayesian model (Carle and Strub 1978) that is more robust to inadequate depletion than traditional removal models.

We estimated abundance at multi-pass-depletion sites using maximum-likelihood estimation for WCT ≥ 60 mm TL. Individual capture histories were constructed using a “1” for the pass fish were captured in and “0” for all other passes. For example, individuals captured in the second pass of a three-pass-depletion event had capture histories of “010.” Both the behavioral (M_b) and time-varying capture probability behavioral (M_{tb}) models (Otis et al. 1978) were used as multi-pass-depletion models by setting $c = 0$. Model M_{tb} differs from Model M_b because it allows p to vary with each pass; Models M_b and M_{tb}were compared using AIC$_c$ in Program MARK v. 6.2 (White and Burnham 1999).

We also estimated abundance using a Bayesian maximum-weighted likelihood estimation model (Carle and Strub 1978; function “removal,” package “FSA”; Ogle et al. 2019) to compare with maximum-likelihood estimates and to obtain reliable abundance estimates when estimation failed with maximum-likelihood models. Carle-Strub abundance estimates were calculated as the smallest integer greater than or equal to the total number of unique individuals captured that satisfied the likelihood (Carle and Strub 1978). The constant capture probability assumption applied because the Carle-Strub estimator lacks the ability to test for time-varying p.

We used generalized linear regression to predict abundance at one-pass sites in Grayling Creek (n = 6). A quasi-*Poisson* model (instead of a *Poisson* model to account for overdispersion) was used to evaluate the relationship between estimated abundance (Carle-Strub estimates) and number of fish captured in the first pass at multi-pass sites in Grayling Creek (n = 8). Model residuals were assessed (function “allEffects,” package “effects”; Fox 2003, Fox and Weisberg 2018, 2019) and the predictor variable was natural-log transformed to account for residual variation. Therefore, the final quasi-*Poisson* model (dispersion parameter = 2.75) was:

$$\log(\mu\{Carle\text{-}Strub\ Abundance\ Estimates|\log\ (\#\ fish\ captured\ on\ pass\ 1\}) =$$

$$\beta_0 + \ \beta_1 * \#\ fish\ captured\ on\ pass\ 1,$$

with abundance estimates following a Poisson distribution:

$$Carle\text{-}Strub\ Abundance\ Estimates \sim Poisson\ (\mu).$$

The positive linear relationship between estimated abundance and natural-logged number of fish captured on the first pass ($F_{1,6}$ = 20.2, P = 0.004; function “Anova,” package “car”; Fox and Weisberg 2019) was used to predict abundance at one-pass sites; 99% confidence intervals (instead of 95% prediction intervals) were reported with predicted abundance estimates because methods to calculate prediction intervals for quasi-Poisson models are not readily available (M. Greenwood, Montana State University Department of Mathematical Sciences, personal communication).

All abundance and density estimates represent numbers of fish prior to the first sampling event. Fish densities (fish/km) were estimated in sampled sections by dividing Carle-Strub-estimated and quasi-Poisson-predicted site abundances by site lengths. Densities were extrapolated to strata. We defined strata as the representative stream lengths that sites were randomly chosen from; strata were defined a priori. Stream densities were mapped using ArcGIS. We estimated overall mean densities and 95% confidence intervals using Cochran's methods (Pollock et al. 1994) and used this overall density estimate (fish/km) to calculate overall abundance (fish/km multiplied by total stream km).

We compared pre- and post-restoration estimated densities using the Carle-Strub estimator in East Fork Specimen Creek at the three sites where pre-restoration data were collected (one upstream and one downstream site within the restoration area, and one control site downstream of the restoration area). Pre-restoration abundances and densities were not estimated in Grayling Creek such that comparisons could not be made for it.

Mark-Recapture Abundance Estimation. Closed-population mark-recapture models were used to estimate fish abundances in main-stem Grayling Creek. We assessed five assumptions associated with these models.

Assumption 1: *the population was closed to births, deaths, immigration, and emigration*. Crews conducted recapture sampling within seven days of initial sampling to better ensure that the population was closed to births, deaths, and recruitment of individuals to sampled sizes. Fish were released within 100 m of their capture location to decrease the likelihood of immigration and emigration, but fish movement was still possible. Use of block nets in main-stem Grayling Creek was impractical while tow-barge electrofishing because crews moved only downstream and over long distances such that substantial extra effort would have been required to maintain block nets on 17 stream km over a seven-day period. Whereas most recaptured WCT remained at or near their original capture location, 40% moved a mean distance of 0.54 km downstream in 2018 and 26% moved a mean of 0.06 km upstream in 2019 (Figure 8), indicating strong evidence for assumption violation in 2018, but little evidence thereof in 2019 (because distances moved in 2019 were shorter than the lengths of most sample sites). A positive linear relationship between distance moved and WCT TL was present in 2018 ($F_{1,\,23} = 7.16$, $P = 0.01$; Figure 9) but not in 2019 ($F_{1,\,56} = 1.12$, $P = 0.30$; Figure 9). Two outliers (Cook's Distance > 1) that moved the longest distances ($\text{WCT}_{\text{outlier1}}$ 237 mm TL, moved 5.37 km; $\text{WCT}_{\text{outlier2}}$ 297 mm, moved 3.01 km) drove the relationship. These two fish were possibly making post-spawning migrations. Violation of the movement assumption in closed-population mark-recapture models decreases precision of abundance estimates if movement is random and biases accuracy of abundance estimates if movement is nonrandom (Kendall 1999). Because we counted marked fish as newly captured fish if they were recaptured in a different section from which they were first marked, abundance estimates may be biased higher than true abundance. Therefore, we acknowledge this assumption violation and note that it may bias our 2018 abundance estimates for WCT in Grayling Creek to be higher than the true abundance. We have no concerns regarding violation of this assumption in 2019.

Assumption 2: *marked individuals behaved the same as unmarked individuals*. We had no reason to suspect fish handled during sampling behaved differently than unmarked WCT. However, we fit and assessed a mark-recapture model that allowed for a behavioral response to tagging (Otis et al. 1978), when applicable.

Assumption 3: *all individuals had homogeneous capture probabilities within a sampling event*. All sampling crews were made up of similarly skilled personnel performing the same tasks throughout a site to reduce personnel-related influence on fish capture probabilities. Fish < 80 mm TL were excluded from abundance estimates because captures thereof occurred rarely (Figure 7). Whereas capture probabilities can vary depending on fish sizes during electrofishing surveys (electrofishing is selective towards larger fish; Neumann and Allen 2007), closed-population mark-recapture models are generally robust to varying capture probabilities (Kendall 1999). Standardized sampling procedures and exclusion of small fish with low capture probabilities minimized violation of this assumption.

Assumption 4: *marks were not lost or overlooked*. The PIT-tag retention rates of salmonids are variable (≥ 98%, Prentice et al. 1990; 86% and 83%, Buzby and Deegan 1999; 84.8%, Roussel et al. 2000; ≥ 98.1%, Dare 2003; 97%, Bateman and Gresswell 2006; 62 – 80%, Bateman et al. 2009). Crews marked most fish by both implanting a PIT tag and clipping a distal portion of a fin to evaluate potential tag loss. Additionally, implantation scars of PIT-tagged fish were still evident seven days post-tagging, and one year later in some individuals. Although two tag losses were documented, they were not influential to abundance estimates. One WCT lost a PIT tag during anesthesia recovery in a holding bucket on the final sampling event in 2019; the tag was reinserted before the WCT was released. One WCT captured in 2019 appeared to have both an old implantation scar from 2018 and no PIT tag; the WCT was handled as if it were a newly captured fish. Because fish had up to three marks that were visible to crews (PIT tag, abdominal scar, and fin clip) and the two documented tag losses did not affect abundance estimates, our concerns of violation of this assumption are minimal.

Assumption 5: *marking individuals did not decrease survival*. Fish received either a 12-mm, 23-mm, or no PIT tag, depending on TL (see Sampling Methods), to minimize tag-to-body size ratios (Zale et al. 2005) and thereby decrease tagging-related mortalities. Only 6 of 773 fish

(< 1%) died during handling in both years of mark-recapture sampling and no dead fish were observed by crews that spent several hours hiking along the stream. Fish could have died after release (Dare 2003) or fish carcasses could have been missed or scavenged. However, we think minimal concern for a violation of this assumption exists because few fish deaths were directly observed.

We estimated abundance using the two-sample Lincoln-Petersen bias-adjusted (L-P) estimator:

$$\widehat{N} = \frac{(n_1 + 1)(n_2 + 1)}{m_2 + 1} - 1$$

(Laplace 1786, Petersen 1896, Lincoln 1930, Chapman 1951, cited by Williams et al. 2002), where n_1 and n_2 were the number of fish captured in the first and second sampling events, respectively, and m_2 was the number of marked fish captured in the second sampling event. Variance for the L-P estimator was calculated as:

$$\widehat{var} = \frac{(n_1 + 1)(n_2 + 1)(n_1 - m_2)(n_2 - m_2)}{(m_2 + 1)^2(m_2 + 2)}$$

(Seber 1982). Approximate 95% confidence intervals were calculated based on values for capture probability and m_2 (function “mrClosed,” package “FSA”; Ogle et al. 2019; Table 2 in Krebs 1989).

We also estimated abundance using maximum-likelihood estimation, when applicable, to compare with L-P estimates; comparing abundances derived using different models could increase or decrease confidence in those estimates. Individual capture histories were categorized as “11,” “10,” “01,” or “00,” where “1” is captured and “0” is not captured. For example, a fish with a capture history of “10” was captured in the first sampling event but not the second. Capture histories were used to estimate the number of individuals never captured. Abundance was estimated by adding the estimated number of individuals never captured and the number of unique fish captured (Lukacs 2019). Both the constant capture probability (M_0) and behavioral (M_b) models (Otis et al. 1978) were used to estimate abundance of WCT ≥ 80 mm TL in 2018 and 2019. Whereas recapture probability (c) was assumed equal to capture probability (p) in Model M_0, c could differ from p in Model M_b to incorporate a potential behavioral response to marking (Otis et al. 1978). Depletion of newly captured fish must occur over sampling events for Model M_b estimates to be reliable (Seber and Whale 1970). Models M_0 and M_b were compared using Akaike's Information Criteria corrected for small sample size (AIC_c) in Program MARK v. 6.2 (White and Burnham 1999).

All abundance and density estimates represent numbers of fish prior to the first sampling event. Fish that died during the first sampling event were excluded from calculations but added to abundance estimates whereas fish that died during the second sampling event were included in calculations. We reported numbers of unique individuals when catches were too low to estimate abundances. Fish densities (fish/km) were estimated in sampled sections by dividing L-P abundances by section lengths. We calculated lengths (km) of sampled and unsampled stream sections using GPS coordinates in ArcGIS. Densities were extrapolated to strata. We defined strata as the distances sampled plus half the distances upstream and downstream to the next-nearest sections; strata were defined ad hoc because stream lengths sampled varied among sites and years. Stream densities were mapped using ArcGIS.

We estimated overall mean densities and 95% confidence intervals using Cochran's overall estimation of population mean and standard error for a stratified random sampling design (Pollock et al. 1994) by extrapolating densities to strata and weighting strata by sizes (km). We used this overall density estimate (fish/km) to calculate overall abundance (fish/km multiplied by total stream km).

Size Structure Estimation. We investigated the size structures of fish in East Fork Specimen and Grayling creeks to infer which stage of recovery was achieved by the translocated populations. We fit three to four Gaussian curves (function “normalmixEM,” package “mixtools”; Benaglia et al. 2009) to TL frequency distributions (10-mm bins) of WCT captured in East Fork Specimen (2019) and Grayling (2018 and 2019) creeks. We determined the number of Gaussian curves to fit (n = 3 or 4) by visually assessing how well curves matched normal distributions within the multinomial-distributed TL data. Age groups were inferred for fish that were defined by a common Gaussian curve within each population. For example, fish whose TLs were defined by the first Gaussian curve within a population were grouped together as the most immature fish whereas fish whose TLs were defined by the second and third Gaussian curves represented the middle and oldest age groups, respectively. Gaussian-defined age groups were used to infer survival or recruitment among age groups. The presence and number of fish defined by the smallest age group and the number of years since the last translocation event was used to infer whether natural reproduction had occurred.

Arctic Grayling that were captured in Grayling Creek were also assigned to age groups. Scales that were collected from grayling in the field were mounted on glass microscope slides with a cover slip and photographed using a stereomicroscope (Leica Application Suite v4.1 M165C, Leica Microsystems Ltd., Wetzlar, Germany). Ages of grayling were determined by counting the number of transitions from warm to cold growing seasons observed in scale growth (Quist et al. 2012). Ages were estimated by two readers and no discrepancies in ages between readers existed.

Body Condition Estimation. We investigated body condition, or wellness, of WCT captured in East Fork Specimen and Grayling creeks to determine whether translocated populations exhibited body conditions that could be indicative of the regulation stage of population recovery (Stage 3). We compared pre- and post-restoration population body conditions to determine how translocated populations compared to the pre-restoration populations they replaced. Relative weight (W_r) was used as an index to estimate body condition of fish among years that both TL and weight data were collected in East Fork Specimen Creek (1994, 1998, 2004, 2005, 2015, 2016, and 2019) and Grayling Creek (2006, 2007, 2009, 2018, and 2019). Individual W_r was calculated as:

$$W_r = W/W_s * 100,$$

where W is fish weight (g) and W_s is a TL-specific standard calculated as:

$$log_{10}(W_s) = a' + b * log_{10}(TL),$$

where lotic cutthroat a' = -5.189 and b = 3.099 (Neumann et al. 2012). We excluded fish < 130 mm TL based on minimum-size guidelines and categorized W_r into two TL categories (130 – 200 mm and > 200 mm TL) based on standards for proportional size distribution models (Neuman et al. 2012). We visualized individual W_r (package "beanplot," Kampstra 2008) by year, TL category, and waterbody.

We statistically compared population mean W_r in pre- and post-restoration time periods by TL category using a mixed-effects ANOVA model (function "lmer," package "lmerTest"; Kuznetzova et al. 2017) with restoration period (pre- and post-restoration), TL category (130 – 200 mm and > 200 mm), and their interaction as fixed effects, and year as a random effect:

$$W_{r_{ij}} = \beta_0 + \beta_1 I_{Period=Post,i} + \beta_2 I_{TL=>200\,mm,i} + \beta_3 I_{Period=Post,i} I_{TL=>200\,mm,i} + Year_i + \varepsilon_{ij},$$

where I = i^{th} year and j = j^{th} W_r within i^{th} year,

$Year_i \sim N(\sigma, \sigma^2_{Year})$, and $\varepsilon_{i,j} \sim N(\sigma, \sigma^2_{\varepsilon})$,

$I_{Period=Post,i}$ = 1 if post-restoration period, 0 if pre-restoration period, and

$I_{TL=>200\ mm,i}$ = 1 if TL category = > 200 mm, 0 if 130 mm – 200 mm.

We interpreted term significance (α = 0.05) in mixed-effects ANOVA models (function "Anova" and specified the "F" test statistic, package "car"; Fox and Weisberg 2019) and refined models as needed. Because both WCT and several hybrid fish were captured in East Fork Specimen Creek in 2019, we also used a mixed-effects ANOVA model with species (WCT or hybrid) as a main effect and site (n = 13) as a random effect to estimate possible differences in mean W_r between WCT and hybrids. Few hybrid fish were captured in Grayling Creek, so differences in mean W_r between WCT and hybrid fish were not investigated there.

The assumptions of mixed-effects ANOVA models are 1) data were randomly sampled, 2) data were independent among years, 3) residuals and random effect residuals were normally distributed, and 4) residuals had equal variance. Assumptions 1 and 2 were possibly violated because sites were not randomly sampled prior to 2018 and 2019 and some sites were repeatedly sampled among consecutive years. We assessed the normality and equal variance assumptions using residual plots of the full interactive model for each waterbody (function "residplot," package "predictmeans"; Luo et al. 2020). Residuals appeared heavy-tailed and right-skewed in East Fork Specimen Creek (n = 349) and heavy-tailed and left-skewed in Grayling Creek (n = 757). However, we have minimal concern for assumption violation because sample sizes were large and no major outliers were identified (Cook's distance < 0.5). Random effect residuals appeared normal in East Fork Specimen Creek (n = 7) and moderately normal in Grayling Creek (n = 5) despite small sample sizes. Whereas residual variances appeared equal in East Fork

Specimen Creek, they varied in Grayling Creek. Therefore, we have moderate concern for a violation of the equal variance assumption in Grayling Creek; the large differences in residual variances are probably attributable to the years with small and large sample sizes having large and small variances, respectively (n = 12, 2007; n = 9, 2009; n = 334, 2018; n = 369, 2019). We acknowledge the potential violations thereof and limit inference to the sites that were sampled.

Genetic Methods and Data Analyses

Genetic ancestries were estimated for the WCT populations in the East Fork Specimen Creek watershed in relation to the different donor sources used, and interspecific hybrid introgression was investigated post hoc in both the East Fork Specimen Creek and Grayling Creek watersheds because alleles found in preliminary analyses suggested some fish were probably hybridized. All fish that were captured during sampling had a small anal or caudal fin clip collected from them to be genetically analyzed. Fin clips were collected using scissors and stored in ethyl alcohol (absolute, 200 proof). All genotyping and analyses were performed by the Idaho Department of Fish and Game (IDFG) fish genetics lab in Eagle, Idaho. Genomic DNA extractions were carried out using a Nexttec^{TM} DNA isolation kit according to the manufacturer's instructions (www.nexttec.biz). Diagnostic loci were genotyped using the genotyping-in-thousands sequencing methodology of amplicon sequencing (Campbell et al. 2015). Genetic data were summarized using the genetic software program *structure* (version 2.3.4; Pritchard et al. 2000).

Interspecific Hybrid Introgression. Genetic samples from all fish captured in main-stem East Fork Specimen Creek (n = 303, 2019) and High Lake (n = 12, 2018; n = 16, 2019) were investigated for hybrid introgression to identify hybridized fish and exclude them from the genetic ancestry analysis. We also investigated hybrid introgression in fish captured in Grayling Creek in 2018 (n = 137) and 2019 (n = 110) because surveys conducted by National Park Service fishery personnel resulted in the capture of one hybrid fish in the Dry Reach tributary to Grayling Creek in 2018 (T. Koel, National Park Service, personal communication; Montana Conservation Genetics Lab, unpublished data). Samples from all fish captured in lower Grayling Creek and all tributaries, and random samples from fish in three main-stem strata in upper Grayling Creek were tested. All samples were screened for interspecific hybrid introgression at 38 single nucleotide polymorphisms (SNPs) that exhibit diagnostic allele frequency differences between WCT, Yellowstone Cutthroat Trout, and Rainbow Trout (M. Campbell, IDFG, unpublished data). Westslope Cutthroat Trout, Yellowstone Cutthroat Trout, and Rainbow Trout admixture was quantified for individual fish using *structure*. Data from previous studies of WCT, Yellowstone Cutthroat Trout, Rainbow Trout, and first-generation hybrids (M. Campbell, IDFG, unpublished data) were specified as “knowns”; alleles of possible hybridized fish, “unknowns,” were then back-assigned as either WCT, Yellowstone Cutthroat Trout, or Rainbow Trout (k = 3 in *structure*). Individual fish were designated as genetically pure if alleles assigned ≥ 98.0% to one taxon and genetically hybridized if they assigned < 98.0%. Only fish identified as genetically pure WCT were included in the genetic ancestry analysis.

Genetic Ancestry. Genetic ancestries of the WCT populations in East Fork Specimen Creek in 2019 and High Lake in 2018 and 2019 were investigated to determine donor source contributions. Westslope Cutthroat Trout donor sources used in the East Fork Specimen Creek watershed were Geode Creek, Last Chance Creek, Muskrat Creek, and the Sun Ranch Hatchery (Table 2). The Sun Ranch Hatchery was made up of several primary donors and their admixtures, including Last Chance, Muskrat, Ray, and White’s creeks (Clancey and Lohrenz

2015; Figure 10). Because we did not have genetic samples from translocated fish (or their parents), determining whether Last Chance Creek and Muskrat Creek alleles had originated from primary donor sources or the Sun Ranch Hatchery was not possible within the scope of this study. Consequently, donor source analyses pertain to the five primary ancestral donor sources, which are Geode, Last Chance, Muskrat, Ray, and White's creeks (Figure 10).

Genetic samples were screened on a 373-SNP panel that was developed for a similar WCT ancestry study in Cherry Creek, Montana. The 373 SNPs were selected from a larger WCT restriction site associated DNA (RAD) capture panel (D. Drinan and K. Naish, University of Washington, unpublished data); one SNP is strongly associated with sex (Cassinellie et al. 2018) and the other 372 SNPs were selected for high heterozygosity within donors and high genetic differentiation (F_{ST}) between donors (B. Shepard, personal communication). We used the 373-SNP panel designed for Cherry Creek because three of the five primary donor sources were used in both translocation projects. Donor sources included in the 373-SNP panel were Muskrat, Ray, and White's creeks (each in 2007, 2008, and 2009) whereas Geode and Last Chance creeks were not. Therefore, additional genetic samples from WCT captured in Geode (2005 and 2019) and Last Chance (2005, 2008, and 2009) creeks were screened on the 373-SNP panel to determine if they could be differentiated from the other donor sources. We also estimated relative contributions of primary donor sources to the Sun Ranch Hatchery using data from previously genotyped Sun Ranch Hatchery samples (2007, 2008, and 2010; B. Shepard, personal communication). Because years when genetic samples were collected did not always correspond to the same years fish were translocated (Table 2), we assumed that samples collected for genotyping were genetically representative of the individuals that were translocated. For example, the WCT samples genotyped from Geode Creek in 2005 and 2019 were assumed to genetically represent the WCT that were translocated from Geode Creek into the East Fork Specimen watershed from 2007 – 2012.

The donor source ancestry analysis was completed for genetically pure WCT captured in High Lake and East Fork Specimen Creek using *structure* (USEPOPINFO model; Pritchard et al. 2000). Samples from the five known primary donor sources (Geode, Last Chance, Muskrat, Ray, and White's creeks) were pre-specified as "knowns" ($k = 5$ in *structure*) and were used to back-assign alleles of the WCT we wanted to investigate, the "unknowns" (Table 3). Preliminary analyses indicated that samples collected from White's Creek in 2007 were differentiated from 2008 and 2009 samples (M. Campbell, IDFG, unpublished data), so the 2007 White's Creek samples were also included as "unknowns." *Structure* was used to summarize genetic data for "knowns" and "unknowns" whereby the different groups (Table 3) were assigned to one or more clusters with associated percentages. Clusters represented distinct genetic groupings, and associated percentages represented proportions of alleles that assigned to the clusters. For example, we expected the unknown Sun Ranch Hatchery groups (2007, 2008, and 2010) to assign to four clusters because the Sun Ranch Hatchery donors included Last Chance, Muskrat, Ray, and White's creeks (assuming all four donors could be genetically differentiated using the 373-SNP panel). If all four donors equally contributed to the genetics of the Sun Ranch Hatchery population, we would expect alleles to assign 25% to each of the four clusters. We used results from *structure* to infer the proportion of alleles that the five ancestral donors contributed to the WCT population in High Lake in 2018 and 2019, East Fork Specimen Creek in 2019, and the Sun Ranch Hatchery in 2007, 2008, and 2010.

The proportion of fish translocated from donor sources should be proportional to the proportion of alleles represented by those donor sources in the WCT population, if all donor sources contributed to the population equally. Therefore, we wanted to investigate the null hypothesis:

$$H_0: proportion\ translocated_{donor\ 1};\ proportion\ translocated_{donor\ i} \propto proportion\ alleles_{donor\ 1};\ proportion\ alleles_{donor\ i}.$$

However, the proportions of fish translocated from Muskrat and Last Chance creeks are confounded because alleles originated from both primary donor sources and the Sun Ranch Hatchery; we cannot directly measure the proportion of Muskrat Creek and Last Chance Creek fish that originated from the Sun Ranch Hatchery versus those that were translocated directly within the scope of this study. Therefore, we investigated whether the proportion of fish translocated from Geode Creek was the same as the proportion of alleles represented by Geode Creek in the current WCT population:

$$H_0: proportion\ translocated_{Geode\ Creek} \propto proportion\ alleles_{Geode\ Creek}.$$

Survival and emergence data were not recorded for embryos translocated using RSIs. Therefore, total numbers of fish translocated per donor source were calculated as the sum of the number of embryos and number of age-1+ fish translocated with the assumption that all fish and embryos had equal survival. We predicted that the proportion of Geode Creek alleles would represent about half of the WCT population in High Lake (46%) and most of the WCT population in East Fork Specimen Creek (90%) based on proportions of fish translocated from the Geode Creek donor source (Table 2).

Expected heterozygosity (H_e), a measure of genetic diversity of a population, was reported for groups for which those data were available (M. Campbell, IDFG, unpublished data). Genetic diversity measurements could help explain differences observed in donor source contributions and help managers assess the genetic status or possible adaptive potential of translocated populations relative to other populations.

Project Comparisons

We summarized biological and abiotic data collected in the East Fork Specimen Creek and Grayling Creek watersheds to make compare comparisons of metrics between the two watersheds. Biological metrics that we compared included stages of recovery achieved by the translocated populations, densities, size structures, and relative weights, and the densities of fish and embryos that were translocated to the watersheds. Abiotic metrics that we compared included water temperatures, stream discharges, and elevations.

Size Structures. We compared size structures of fish assemblages among all years and waterbodies that TL data were collected in East Fork Specimen Creek (1994, 1997, 1998, 2004, 2005, 2015, 2016, and 2019), High Lake (2016, 2018, and 2019), and Grayling Creek (2006 – 2010, 2018, and 2019). Fish TLs (mm) were organized into groups by waterbody and year and visualized using relative-frequency histograms (10-mm bins). Groups sometimes included multiple species because fish assemblages were different depending on restoration period, year, and waterbody. We tested whether groups were different using Kruskal-Wallis multiple-comparisons because data were not normally distributed. Therefore, the null hypothesis was that TL rank medians were the same across groups ($\alpha = 0.05$). Follow-up pair-wise comparisons were used to identify which groups were statistically different (family-wise $\alpha = 0.05$; function "kruskalmc," package "pgirmess"; Giraudoux 2018); statistically similar groups were assigned the same letter whereas statistically different groups were assigned different letters. Assumptions for Kruskal-Wallis multiple-comparison tests are 1) data were randomly sampled, 2) data were independent, and 3) data had similar distributions. Assumptions 1 and 2 were possibly violated because sites were not randomly sampled prior to 2018 and 2019, and data possibly contained repeated measures at some sites that were sampled in consecutive years. These violations are acknowledged, and inference may be limited to only sites that were sampled during years data were collected.

Abiotic Measurements. Water temperatures and stream discharges were frequently recorded in East Fork Specimen and Grayling creeks to document temperature trends and flow regimes that had not been thoroughly measured heretofore, and to possibly help explain variation observed in fish data. Onset HOBOware data loggers (U22-001; Bourne, Massachusetts) were deployed to record hourly water temperatures (°C) throughout East Fork Specimen ($n = 8$, 2019) and Grayling ($n = 9$, 2018; $n = 11$, 2019) creeks. Stream discharges were frequently measured (Fisher et al. 2012) at easily accessible locations near the downstream portions of both Specimen (Figure 4) and Grayling (Figure 6) creeks. Because the lower-most barrier to East Fork Specimen Creek is inaccessible, stream discharge measurements were on Specimen Creek proper. Crews recorded 15 discharge measurements in Specimen Creek and 18 discharge measurements in Grayling Creek during this study. Onset HOBOware data loggers (U20L; Bourne, Massachusetts) were deployed in stilling wells (as described by Platt 2019) to record hourly water pressures (kPa) at the stream discharge sites. Simple linear regression of stream discharge measurements and mean daily water pressures was used to predict stream discharges on days when discharge measurements were not collected. Elevation measurements were collected using CalTopo Backcountry Mapping (CalTopo LLC., Truckee, California).

Results

Stage of Population Recovery East Fork Specimen Creek Watershed

Main-stem Westslope Cutthroat Trout

Abundance. Overall estimated mean density of fish ≥ 60 mm TL in main-stem East Fork Specimen Creek was 259.3 fish/km (95% CI, 205 – 314) in 2019 (Table 4). Extrapolation of this density yielded an overall abundance of 2,692 fish (2,125 – 3,260) in 10.38 km (Table 4). Model selection for M_b or M_{tb} models resulted in delta AIC_c scores that were less than two units in 11 of 13 sites (Table 5). Therefore, only Model M_b estimates were compared with Carle-Stub estimates for each site; almost all estimates were the same (Table 6). Hereafter, we only refer to Carle-Strub-estimated parameters for multi-pass-depletion abundance estimations in the East Fork Specimen Creek watershed. Estimated capture probabilities ($\hat{p}$) among sites varied from 0.41 – 0.62 in 2019 during multi-pass-depletion sampling (Table 6). Estimated fish densities were generally higher in the upper portion of the watershed compared to the lower portion of the watershed (Figure 11). Estimated density was highest at Site 5 (780.0 fish/km) near where most fish translocations occurred, and lowest at Site 10 (25.0 fish/km) directly upstream of the lower-most log barrier (Figure 11).

Estimated overall abundance of fish in main-stem East Fork Specimen Creek in 2019 (2,692 fish) surpassed pre-restoration overall estimated abundance in 2004 – 2005 (2,485 fish; Koel and York 2006). At the individual historical sites, the translocated population density surpassed pre-restoration density at the upstream site (H1) but not at the downstream site (H2; Figure 12). Estimated density at the control site (H3; downstream of the restoration area) was slightly higher than the 2005 pre-restoration estimate but lower than the 2004 pre-restoration estimate; however, error associated with the 2004 pre-restoration estimate is probably too large to allow valid inference.

Size Structure. The East Fork Specimen Creek WCT population was represented by four age groups in 2019 (Figure 13). Most individuals were in the two youngest age groups and numbers of captured individuals by age group generally decreased with increasing age—a pattern common for fish and other species that exhibit higher death rates earlier in life than later in life (type III survivorship curve). Most fish captured were probably naturally reproduced WCT because seven years had passed since the last translocation event in 2012. Relatively few (11%) WCT individuals are ≥ 8 years old in small montane streams (Janowicz et al. 2018).

Body Condition. Relative weights of WCT in East Fork Specimen Creek pre- to post-restoration appeared to differ depending on TL category (Figure 14). Whereas W_r of WCT 130 – 200 mm TL were generally larger in pre-restoration years than in post-restoration years, W_r of WCT > 200 mm TL were similar among all years, except in 2019 when mean W_r was the largest, which may indicate that smaller WCT experienced higher levels of competition post-restoration than pre-restoration and larger WCT experienced similar levels of competition pre- and post-restoration.

Weak evidence existed to suggest mean W_r of genetically pure WCT (96.1; 95% CI, 93.9 – 98.5) and hybrid WCT (98.0; 95.1 – 100.8) in East Fork Specimen Creek differed in 2019 (*mixed-effects ANOVA*: $F_{1,\ 53.6} = 1.0545$; $P = 0.2857$). Therefore, we pooled both WCT and hybrid data for the East Fork Specimen Creek W_r mixed-effects ANOVA analysis.

As suspected, strong evidence existed for an interaction between restoration period and TL category after accounting for restoration period and TL category as main effects, and year as a random effect (*mixed-effects ANOVA*: $F_{1,\ 340.6} = 32.2430$, $P < 0.001$). Therefore, we used the interaction model:

$$W_{r_{ij}} = \beta_0 + \beta_1 I_{Period=Post,i} + \beta_2 I_{TL=\ >200\ mm,i} + \beta_3 I_{Period=Post,i} I_{TL=\ >200\ mm,i} + Year_i + \varepsilon_{ij},$$

where $\beta_0 = 102.810$ ($SE = 2.89$), $\beta_1 = -13.692$ ($SE = 4.14$), $\beta_2 = -11.936$ ($SE = 2.56$), and $\beta_3 = 16.924$ ($SE = 2.98$). Mean W_r for each restoration period by TL category was:

1) 102.8 (95% CI, 97.3 – 108.3) among fish 130 – 200 mm TL pre-restoration,

2) 89.1 (83.4 – 94.8) among fish 130 – 200 mm TL post-restoration,

3) 90.9 (84.6 – 97.2) among fish > 200 mm TL pre-restoration, and

4) 94.1 (88.1 – 100.1) among fish > 200 mm TL post-restoration, after controlling for main, interaction, and random effects (Figure 14). Strong evidence existed of a difference in mean W_r pre- and post-restoration in East Fork Specimen Creek among small fish ($t = -3.304$, $df = 5.418$, $P = 0.019$) but not among large fish ($t = 0.701$, $df = 8.339$, $P = 0.502$). Whereas mean W_r for WCT 130 – 200 mm TL was greater pre-restoration (102.8, 97.3 – 108.3) than post-restoration (89.118, 83.4 – 94.8), mean W_r for WCT > 200 mm TL was similar pre- (90.9, 84.6 – 97.2) and post-restoration (94.1, 88.1 – 100.1; Figure 14). Therefore, we conclude that mean W_r in East Fork Specimen Creek was different pre- and post-restoration among WCT 130 – 200 mm TL but not among WCT > 200 mm TL, after accounting for restoration period, TL category, their interaction, and year as a random effect.

Stage of Population Recovery. Westslope Cutthroat Trout in main-stem East Fork Specimen Creek had reached the population growth stage (Stage 2) of population recovery and possibly the population regulation stage (Stage 3) at some upstream sites. Survival (Stage 1a) and consistent natural reproduction of translocated individuals and their offspring (Stage 1b) was apparent based on the number of fish recruited to four age groups in 2019, which was seven years after the last translocation events had occurred in 2012. Recruitment of naturally reproduced individuals to the WCT population in 2019 was robust; the youngest two age groups representing immature and young adult fish were the largest of the four age groups. Although we were unable to collect two years of data to confirm population growth using two abundance estimates, we are confident that the magnitude of recruitment to the youngest age group in 2019 indicated the population was stable or growing (Stage 2). Because fish densities were generally lower at downstream sites compared to upstream sites, the population had probably not reached carrying capacity (Stage 3) throughout the entire watershed in 2019. Individuals will probably continue to disperse downstream where population growth potential is high. Site-specific densities indicated that the lower portion of the watershed could hold higher densities of WCT than what was observed in 2019 because estimated densities at the downstream site were higher in pre-restoration years than in 2019. However, estimated overall abundance comparisons indicate that the translocated population in 2019 had higher abundance than the pre-restoration population (Koel and York 2006). Additionally, mean body condition of the translocated population was lower than or similar to (depending on TL category) the pre-restoration population, which possibly indicates that the translocated population experienced similar resource limitation and competition levels in 2019 and in pre-restoration years. Therefore, the translocated WCT population may have achieved the population regulation stage of recovery (Stage 3) at some sites, such as in the upper portion of the watershed where fish densities were high.

High Lake Westslope Cutthroat Trout. Captures and recaptures of WCT in High Lake were too low to calculate reliable abundance estimates; only 12 and 16 WCT were captured in 2018 and 2019, respectively (Figure 15). Twelve WCT were captured in the first gill-net sampling event in 2018; six were marked and released and the other six died. No WCT were captured in gill nets during the recapture sampling event. Fish captured in 2018 were mostly large (326 – 452 mm TL). Additionally, no WCT were observed in the outlet during removal sampling and none were observed feeding at the surface of High Lake in 2018.

Although only 16 WCT were captured by electrofishing ($n = 12$) and angling ($n = 4$) in 2019, we unexpectedly documented occurrence of natural reproduction after observing no evidence of natural reproduction in 2018. Very small fish (≤ 75 mm TL) were captured in the inlets to High Lake and all fish captured in 2019 were smaller (46 – 269 mm TL) than fish captured in 2018. Spawning activity was also observed in the outlet of High Lake and several fish were actively feeding at the surface of High Lake in 2019. Estimated abundance of WCT in the outlet site was 9 (95% CI, 3 – 15, $\hat{p} = 0.44$; Table 6). No fish captured in 2019 had PIT tags from the previous year.

Stage of Population Recovery Grayling Creek

Westslope Cutthroat Trout

Abundance. Estimated overall mean density of WCT ≥ 80 mm TL in main-stem Grayling Creek was 302.8 fish/km (95% CI, 123 – 482) in 2018 and 128.2 fish/km (111 – 146) in 2019 in the same 10.23 km of stream (Table 4), indicating estimated overall density declined from 2018 to 2019. However, confidence intervals of these estimates overlapped. The estimated overall mean density of WCT ≥ 80 mm TL in the entire 15.58 km that was sampled in 2019 was 109.8

fish/km (91 – 128). Extrapolation of these densities to the main stem yielded an overall abundance of 3,096 WCT ≥ 80 mm TL (1,262 – 4,930) in 10.23 km in 2018 and 1,310 WCT (1,131 – 1,490) in 2019 (Table 4). The estimated overall abundance of WCT ≥ 80 mm TL in the entire 15.58 km that was sampled in 2019 was 1,710 WCT (1,422 – 1,997; Table 4). Model selection for M_0 or M_b models always resulted in Model M_0 as the top model or with delta AIC$_c$ scores that were less than two units (Table 5). Abundance estimation sometimes failed using Model M_b because adequate removal of newly captured fish was not attained. Therefore, only Model M_0 estimates were compared with L-P estimates for each section to determine if models derived similar or different estimates. All estimates were similar, except in Section 3 in 2018 (Table 7); discrepancies between estimates by the two models in Section 3 in 2018 probably stem from differences in capture probability estimates, $\hat{p}$ ($\widehat{p_{L\text{-}P}} = 0.08$); $\widehat{p_{M_0}} = 0.12$). Hereafter, we only refer to L-P-estimated parameters for mark-recapture abundance estimations in main-stem Grayling Creek. Estimated capture probabilities among sites were lower in 2018 ($\hat{p}$ = 0.08 – 0.12) than in 2019 ($\hat{p}$ = 0.18 – 0.53) during mark-recapture sampling (Table 7), which probably contributed to higher error associated with abundance estimates in 2018 than in 2019. Estimated fish densities were generally higher in the upper portion of the watershed than in the lower portion of the watershed (Figure 16). Estimated densities were highest in the main stem near Camp Creek in both 2018 (Section 4) and 2019 (Section 3–4), and lowest in the main stem between Camp Creek and Dry Reach Creek in 2018 (Section 5) and near Red Grass in 2019 (Section 6, Figure 16). Estimated overall mean density of WCT ≥ 60 mm TL in tributaries to Grayling Creek was not calculated in 2018 because large error was associated with some site density estimates (sites b and c; Table 6) but it was calculated in 2019 as 70.9 fish/km (59 – 83) in 7.47 km (Table 4). Extrapolation of the 2019 tributary density yielded an overall abundance of

530 WCT ≥ 60 mm TL (444 – 616) in 7.47 km in 2019 (Table 4). Model selection for M_b or M_{tb} models resulted in delta AIC$_c$ scores that were less than two units except at two sites (Table 5) but both model estimates were similar at these sites. Therefore, only Model M_b estimates were compared with Carle-Stub estimates for each site; all estimates were similar, except at Site c in 2018 (Table 6). Hereafter, we only refer to Carle-Strub-estimated parameters for multi-pass-depletion abundance estimations in Grayling Creek tributaries. Estimated capture probabilities ($\hat{p}$) among sites varied from 0.23 – 0.70 in 2018 and 2019 during multi-pass-depletion sampling (Table 6). Estimated densities varied from 50.0 to 360.0 fish/km in 2018 and 33.3 to 120.0 fish/km in 2019 among sites where at least one WCT was captured (Table 6). Estimated densities were highest in the farthest upstream North Fork site in both 2018 and 2019 (Site c; Figure 16). No WCT were captured in the first and only pass at 11 sites in 2018 and at 2 sites in 2019 such that densities were estimated to be zero fish/km there (Figure 16).

Size Structure. The Grayling Creek WCT population was represented by three age groups in 2018 and four age groups in 2019 (Figure 17). The youngest, middle, and oldest WCT age groups in 2018 were probably recruited from the 2017, 2016, and 2015 RSI translocations, respectively. All age groups were well represented in 2018 but the middle age group was the largest, suggesting WCT from the 2016 RSI translocations had relatively high survival. Whereas naturally reproduced WCT could have been present in the youngest age group in 2018, most of them were probably recruited from the 2017 RSI translocations.

A fourth age group of WCT was present in Grayling Creek in 2019, indicating that individuals in the youngest group were recruited from natural reproduction. The other three age groups were probably from the 2017, 2016, and 2015 RSI translocations. Most WCT captured in

2019 were in the two middle age groups, suggesting WCT from the 2017 and 2016 RSI translocations had relatively high survival. More WCT from the 2017 RSI translocations were captured in 2019 than in 2018, which is probably attributable to lower capture efficiency of those fish in 2018 when they were smaller. Far fewer WCT were in the youngest age group representing immature fish in 2019 than in 2018. However, we are confident that WCT in the youngest age group in 2019 were naturally reproduced individuals because two years had passed since the last translocation event in 2017. These immature fish provide the first documentation of natural reproduction of WCT in Grayling Creek since restoration efforts began.

Body Condition. Relative weights of WCT in Grayling Creek appeared lower in pre-restoration years (2006, 2007, and 2009) than in post-restoration years (2018 and 2019; Figure 18), possibly indicating that the post-restoration WCT population had not experienced similar competition levels to the pre-restoration population. No evidence existed of an interaction between restoration period and TL category after accounting for restoration period and TL category as main effects, and year as a random effect (*mixed-effects ANOVA*: $F_{1,\,308.4} = 0.0071$, $P = 0.9327$). Therefore, we removed the interaction term and fit an additive model:

$$W_{r_{ij}} = \beta_0 + \beta_1 I_{Period=Post,i} + \beta_2 I_{TL= >200\ mm,i} + Year_i + \varepsilon_{ij}$$

where $\beta_0 = 84.5139$ ($SE = 2.90$), $\beta_1 = 6.6788$ ($SE = 4.31$), and $\beta_2 = 4.7715$ ($SE = 0.69$). Mean W_r for each restoration period by TL category was:

1) 84.5 (95% CI, 79.4 – 89.8) among fish 130 – 200 mm TL pre-restoration,

2) 91.2 (85.3 – 97.0) among fish 130 – 200 mm TL post-restoration,

3) 89.3 (84.1 – 94.6) among fish > 200 mm TL pre-restoration, and

4) 96.0 (90.1 – 101.9) among fish > 200 mm TL post-restoration, after controlling for main and random effects (Figure 18). Although mean W_r was smaller pre-restoration than post-restoration in both TL categories (Figure 18), weak evidence existed to suggest the difference in mean W_r between restoration periods was significant (*mixed-effects ANOVA*: $F_{1,\,2.7} = 2.2990$, $P = 0.2394$). Therefore, we conclude that mean W_r of WCT in Grayling Creek was not different pre- and post-restoration, after accounting for the additive effect of TL category and the random effect of year. However, we caution that inference of this result is limited because sample sizes were small in pre-restoration years and only five years of data were collected.

Stage of Population Recovery. Westslope Cutthroat Trout in Grayling Creek had reached the initial phases of the reproduction stage of population recovery (Stage 1b). Survival of translocated individuals (Stage 1a) was apparent based on the numbers of fish recruited to three age groups in 2018, which represented translocations that occurred in 2015, 2016, and 2017. Newly recruited immature fish in the youngest group in 2019 indicated that natural reproduction had occurred (Stage 1b). Furthermore, several male WCT captured in both 2018 and 2019 were identified as mature fish because they were excreting milt during processing. Although we documented natural reproduction (Stage 1b), the magnitude of recruitment had not yet resulted in population growth (Stage 2) from 2018 to 2019, based on a decline in estimated abundance from 2018 to 2019. However, a decline in population abundance was not necessarily an unexpected result because most translocated fish were probably still immature in 2018 and 2019 (Figure 3). The oldest RSI-translocated fish, which were translocated in 2015, would have been three years old in 2018 and four years old in 2019. Mean body condition of the translocated population was higher than among the pre-restoration population, indicating the translocated population did not experience similar resource limitation and competition levels as did the pre-restoration population. Therefore, we infer that the translocated WCT population did not achieve the population regulation stage of recovery (Stage 3).

Arctic Grayling. Captures and recaptures of Arctic Grayling in Grayling Creek in 2018 (*n* = 22) and 2019 (*n* = 7) were too low to calculate reliable abundance estimates (Figure 19). Seven and 14 grayling were captured in the first and second sampling events, respectively, in 2018; two had marks in the second sampling event. Additionally, three grayling were captured in the South Fork during removal sampling in 2018. Two and five grayling were captured in the first and second sampling events, respectively, in 2019; none had marks. No grayling were captured in removal sampling in 2019. Estimated ages of captured grayling indicated that most were from the 2017 fry translocations in both years (Figure 20). Only two grayling captured in 2018 and only one captured in 2019 were from the 2016 RSI translocations and none were from the 2015 RSI translocations. However, a group of 10 – 15 age-0 fish was observed by crews during the second sampling event in Section 1 in 2019 (Figure 19), but the small size of these individuals precluded species identification in the field. Therefore, we preserved one 25-mm age-0 fish from this group; this fish was later identified as an age-0 grayling using microsatellite genetic analysis (A. Whitely, Montana Conservation Genetics Conservation Lab, unpublished data) and is the first documentation of natural reproduction of fluvial grayling in Yellowstone National Park in decades.

Interspecific Hybrid Introgression

East Fork Specimen Creek Watershed. Hybridized fish were detected in samples from East Fork Specimen Creek, but not in High Lake (Figure 21). We successfully genotyped 290 of the 303 individuals captured in East Fork Specimen Creek in 2019 and 25 of the 28 individuals captured in High Lake or its tributaries (2018 and 2019 combined). We identified 44 hybrid fish in East Fork Specimen Creek that had combinations of WCT alleles with Yellowstone Cutthroat Trout or Rainbow Trout alleles, or both. Expectedly, all fish captured at the site below the lower-most log barrier on East Fork Specimen Creek were identified as hybrids (*n* = 12). However, the other 32 hybrids were unexpectedly within the restoration area (Figure 21) indicating that 11.5% of fish were hybrids in 2019. Although hybrid fish were captured as far upstream as Site E3 in East Fork Specimen Creek, most hybrids were captured in the farthest downstream sites (E8 – E10 and H3; Figure 21). Therefore, we suspect that hybrid fish invaded into the East Fork Specimen Creek restoration area by breaching the lower-most log barrier. Hybrid fish were not detected in High Lake. The natural waterfall barrier probably inhibits upstream fish movement, but small sample sizes could have precluded hybrid detection in High Lake. Although improbable, hybridized fish could have survived piscicide treatments in East Fork Specimen Creek, could have been accidentally stocked during translocation efforts, or could have been illegally introduced above the barrier.

Grayling Creek. We identified seven hybrid fish in lower Grayling Creek in 2018 and four hybrid fish in 2019 that had combinations of WCT alleles with Yellowstone Cutthroat Trout or Rainbow Trout alleles, or both (Figure 22); 90 of 137 samples from 2018 and 96 of 110 from 2019 successfully genotyped. Therefore, 7.8% and 4.2% of fish were hybrids in 2018 and 2019, respectively. Some hybrids captured in 2018 were located in the lower portion of the watershed near the Dry Reach tributary where National Park Service crews captured a hybrid fish earlier in 2018, but others were found as far upstream as the South Fork (Figure 22). Hybrid fish captured in 2019 were found at all sites that were sampled in the lower main stem, albeit in low numbers. We suspect hybrid fish survived piscicide treatments in the Dry Reach tributary; the tributary presents a complex watershed where subterranean, marshy, and intermittent stream characteristics may have provided fish refugia from piscicides. Although improbable, hybrid fish could have breached the fish barrier, could have been accidentally stocked during translocation efforts, or could have been illegally introduced above the barrier.

Genetic Ancestry

Screening samples on the 373-SNP panel was mostly effective for differentiating between donor sources; therefore, we infer that the WCT populations in High Lake (2018 and 2019) and East Fork Specimen Creek (2019) exhibited admixed ancestries that represented *at least* four of the five primary ancestral donors. Donor sources assigned to five genetically distinct clusters (Table 8). All donor sources assigned to their own unique cluster(s) except Last Chance and White's creeks, which both assigned to Cluster 2. Consequently, alleles for unknown groups that assigned to Cluster 2 could represent alleles from Last Chance Creek or White's Creek, or both. Additionally, Muskrat Creek assigned to two clusters, such that alleles for unknown groups that assigned to Cluster 1 or Cluster 3 represent alleles from Muskrat Creek. Geode Creek and Ray Creek strongly and singly assigned to clusters 4 and 5, respectively.

Westslope Cutthroat Trout proportional donor source ancestries derived from one of the five genetic clusters in High Lake in 2018 and 2019 were unexpectedly different among years (Table 8). Muskrat Creek (Cluster 1, 41% and Cluster 3, 17%) and Ray Creek (Cluster 5, 20%) represented most of the proportional ancestry in High Lake in 2018. Interestingly, Geode Creek represented only 14%, despite the fact that nearly half (46%) of the fish translocated into High Lake were from the Geode Creek donor source; 8% of the proportional ancestry was from either Last Chance or White's creeks, or both (Cluster 2). Conversely, Geode Creek (Cluster 4, 50%) represented half of the proportional ancestry in High Lake in 2019 whereas Muskrat Creek

(Cluster 1, 28% and Cluster 3, 14%) and Ray Creek (Cluster 5, 5%) represented less than half; 4% of proportional ancestry was from either Last Chance or White's creeks, or both (Cluster 2). Muskrat, Ray, and White's creeks ancestries could have originated only from the Sun Ranch Hatchery fish that were translocated into High Lake. Therefore, Sun Ranch Hatchery represented the majority of proportional ancestry in High Lake in 2018 (by Muskrat and Ray creeks) whereas Geode Creek represented the majority in 2019.

The differences observed in WCT proportional donor source ancestries in High Lake from 2018 to 2019 could largely originate from small sample sizes, but the different methods used capture fish could have also influenced observed ancestries. We used gillnets to capture fish in 2018, which targeted larger fish. We switched to electrofishing and angling gear in both the Lake and its tributaries in 2019, which targeted smaller fish than in 2018. Fish that exhibited mostly Sun Ranch Hatchery ancestry could have had a higher probability of capture in 2018 because they grew larger and were therefore more susceptible to gill nets compared to the smaller fish that exhibited mostly Geode Creek ancestry that were captured in 2019.

Proportional donor source ancestries of WCT in East Fork Specimen Creek in 2019 were moderately mixed (Table 8). Geode Creek (Cluster 4) represented 59% of the proportional ancestry, Muskrat Creek (clusters 1 and 3) represented 22%, Ray Creek (Cluster 5) represented 13%, and Last Chance or White's creeks, or both (Cluster 2) represented 6%. Of the fish translocated into East Fork Specimen Creek, 90% were from Geode Creek donors and 10% were from Muskrat Creek donors (Table 2). Therefore, we expected Geode Creek to represent the most ancestry and Muskrat Creek to represent less. However, the proportional ancestry represented by Muskrat Creek was more than double (22%) what was expected based on proportion of embryos translocated from Muskrat Creek (10%). Additionally, the presence of

Ray, Last Chance, and White's creeks ancestries indicated that Sun Ranch Hatchery or both Sun Ranch Hatchery and Last Chance Creek fish contributed to the population in East Fork Specimen Creek. Because such fish were only translocated into High Lake, they moved downstream over the waterfall barrier and established in main-stem East Fork Specimen Creek.

The Sun Ranch Hatchery groups from 2007, 2008, and 2010 exhibited admixed ancestry of the four primary ancestral donor sources we investigated (Table 8). Most proportional ancestry assigned to Ray and Muskrat creeks and less assigned to Last Chance or White's creeks, or both. The Sun Ranch Hatchery 2007 group mostly assigned to Ray Creek (Cluster 4, 63%) whereas the 2008 and 2010 groups mostly assigned to Muskrat Creek (clusters 1 and 3). The 2007, 2008, and 2010 groups only assigned 18%, 9%, and 4%, respectively to Last Chance or White's creeks, or both (Cluster 2), despite both donor creeks contributing to the Sun Ranch Hatchery. Therefore, the Ray and Muskrat creeks primary donors contributed much more genetically to the Sun Ranch Hatchery fish than the Last Chance and White's creeks donors.

Whereas expected heterozygosity varied among donor sources (0.03 – 0.29), it was similar among recovering populations (0.25 – 0.29) in the East Fork Specimen Creek watershed (Table 8). The Sun Ranch Hatchery and Muskrat Creek donor sources had higher measures of expected heterozygosity than the Geode Creek or Last Chance Creek donor sources. Furthermore, several individuals in the translocated populations had admixed ancestries (M. Campbell, IDFG, personal communication), indicating that interbreeding among fish from different donor sources had occurred.

Project Comparisons

Westslope Cutthroat Trout in East Fork Specimen Creek had recovered to the population growth stage (Stage 2) seven years post-translocations whereas WCT in Grayling Creek had recovered to the reproduction stage (Stage 1b) two years post-translocations. Overall estimated mean density of WCT ≥ 60 mm TL in East Fork Specimen Creek was higher (259.3 fish/km) than density of WCT ≥ 80 mm TL in Grayling Creek in 2019 (109.8 fish/km), despite the fact that East Fork Specimen Creek had lower densities of fish and embryos translocated into it (10,300 in 40 km of stream or 258 fish/km) than did Grayling Creek (52,200 in 80 km of stream or 653 fish/km).

Size structures of fish assemblages in the East Fork Specimen Creek and Grayling Creek watersheds differed among waterbodies and years; TL rank medians differed among groups (Kruskal-Wallis multiple-comparison: χ^2 = 433.6, df = 17, $P < 0.001$). Although some TL groups were significantly different from each other, none were exclusively different from all other TL groups (pair-wise comparisons; Table 9). Total-length groups were often statistically similar among years both within and across waterbodies. High Lake TL groups were expectedly different from all other TL groups during the years that gill nets were used to capture fish (2016 and 2018) because they targeted larger fish.

Relative weights of WCT in East Fork Specimen and Grayling creeks were similar among restoration periods and TL categories after accounting for main and random effects, except among fish 130 – 200 mm TL pre-restoration. Westslope Cutthroat Trout 130 – 200 mm TL in East Fork Specimen Creek pre-restoration were larger than in Grayling Creek but the differences could be attributable to small sample sizes.

Elevations of the East Fork Specimen Creek and Grayling Creek watersheds were similar but water temperatures in East Fork Specimen Creek were colder than in Grayling Creek from May – September (Table 10). The East Fork Specimen Creek watershed ranges in elevation from 2,231 m at the lower-most barrier to 2,675 m at High Lake, and mean monthly water temperatures from May – September did not exceed 7.0°C in 2018 and 2019 (Table 10). Grayling Creek ranges in elevation from 2,131 m at the barrier to about 2,400 m to the North and South forks, and mean monthly water temperatures from May – September did not exceed 10.2°C in 2018 and 2019 (Table 10).

Expectedly, stream discharges were lower in Specimen Creek proper than in Grayling Creek (Figure 23). Predicted peak discharges in Specimen Creek proper (6.2 m^3/s) and Grayling Creek (about 7.7 m^3/s) occurred in late May to early June in 2018. Predicted peak discharges in Specimen Creek proper occurred slightly later (3.5 m^3/s in mid-June) than in Grayling Creek (7.7 m^3/s in early June) in 2019. However, one outlying measurement in Grayling Creek in 2019 (collected in mid-May) was as high as 9 m^3/s.

Discussion

Stage of Population Recovery

Recovery of WCT in both East Fork Specimen and Grayling creeks is progressing, with expected differences in stage of recovery between the two streams attributable to a 5-year difference in restoration timelines. Conversely, recovery of Arctic Grayling in Grayling Creek appears improbable without management intervention, but the surprising documentation of naturally reproduced individuals engenders a hopeful future for continued grayling recovery efforts.

Westslope Cutthroat Trout. As expected, the WCT population in East Fork Specimen Creek seven years post-translocations had reached a later stage in the progression of population recovery than the WCT population in Grayling Creek two years post-translocations. The WCT population in East Fork Specimen Creek had progressed to the population growth stage (Stage 2) of population recovery and the population regulation stage (Stage 3) at upstream sites based on high recruitment of WCT to small size classes, low estimated densities in the lower portion of the watershed, and comparisons of pre- and post-restoration density estimates, which indicated that the translocated population density surpassed the pre-restoration density at the upstream historical site but not at the downstream site. However, we were only able to collect post-restoration data there in 2019 and not in 2018. Therefore, estimating abundance again in the future and comparing that estimate to the 2019 estimate would help confirm if the population is growing, especially in the lower portion of the watershed where densities were lower than in the upper portion in 2019. Furthermore, pre-restoration abundances were estimated in only two years at only a few sites (Koel and York 2006), and precision of one estimate was too low to make valid inferences. Therefore, pre- and post-restoration comparisons possibly only reflect stochasticity within populations. Thoughtful monitoring plans should be implemented in future restoration projects to ensure enough years of precise data are collected in both pre- and post-restoration years to make informative comparisons (when pre-restoration populations are used to inform success of post-restoration populations) and balance the amount of resources spent collecting data with amount of high quality data collected.

The Grayling Creek WCT population had progressed to the reproduction stage (Stage 1b) of population recovery based on the first documentation of natural recruitment in 2019 but lack of population growth from 2018 to 2019. We expect population growth will occur within a few years if the WCT population in Grayling Creek progresses similarly to the population in East Fork Specimen Creek. In time, the size structure composition of the population should reflect one that is represented mostly by small fish, indicating that consistent, successful, natural reproduction has occurred.

Population recovery of WCT in the downstream portions of both the East Fork Specimen Creek and Grayling Creek restoration areas was limited. All WCT translocation sites were in the upper portion of the East Fork Specimen Creek watershed (Figure 11) and most were in the upper portion of Grayling Creek (Figure 16). Therefore, later stage population recovery at sites farther downstream could be spatiotemporally limited by dispersal of WCT from the upstream translocation sites. Conversely, downstream dispersal by juvenile WCT (age-1 and age-2) translocated to RSIs as embryos was common in Cherry Creek, Montana (Andrews et al. 2013), and was even more prevalent when those fish were adults (Clancey et al. 2019). Anglers have reported capturing WCT a few kilometers downstream from the Grayling Creek fish barrier (Yellowstone National Park volunteer angler reports, unpublished data; J. Carmack, personal communication), indicating some WCT dispersed downstream. The few WCT that were translocated into lower Grayling Creek were mostly juvenile and adult (age-1+) WCT from Geode Creek. Cutthroat Trout (hatchery-origin stocked at 120-mm lengths) have shown outmigration tendencies when stocked in other headwater streams that are isolated by barriers (Novinger and Rahel 2003). Therefore, fish that were translocated into the lower portions of Grayling Creek possibly emigrated downstream past the fish barrier whereas fish and embryos translocated in the upper portions of both Grayling and East Fork Specimen creeks possibly need more time to fully disperse and consistently populate the lower portions of restoration areas.

Habitat conditions possibly contributed to the lower estimated WCT densities in the lower portions of both watersheds compared to the upper portions. A wildland fire burned through the lower East Fork Specimen Creek watershed in 2007 (Owl Fire); wildland fires have been linked to degraded trout habitat and associated declines in WCT densities (Sestrich et al. 2011). Furthermore, U.S. Highway 191 is adjacent to the lower portion of Grayling Creek. Salmonids are negatively affected by chemicals that can leach into waterways from tire-rubber residue (Tian et al. 2020). The road is also subject to the application of traction sand to improve driving conditions during winter (Montana Department of Transportation, personal communication). Traction sand applied to U.S. Highway 191 was a major source of excess sedimentation in the Gallatin River watershed north of Grayling Creek (Dunn et al. 2014, 2018), and may also affect trout habitat in lower Grayling Creek.

The large differences we observed in the WCT population size structures in High Lake in 2018 and 2019 were probably attributable to small sample sizes (few fish captured), sampling bias, and seasonal temperature differences between years. No recent natural recruitment was evident in 2018 and no feeding activity was observed at the surface of High Lake during the 5-

day span crews were there, despite abundant adult caddisflies, mayflies, and mosquitos. Conversely, recent natural recruitment was evident in 2019 because crews captured small fish, and observed spawning activity in the outlet to High Lake and feeding activity at the lake surface. Different sampling gears used in the two years probably contributed to capture of only large fish in 2018 and only small fish in 2019. Seasonal temperature differences were apparent between years at High Lake despite sampling occurring only one week earlier in 2019 than in 2018; 2019 had more snowpack, higher inlet water discharges, and cooler air and water temperatures than 2018. Warmer weather conditions in 2018 possibly decreased WCT capture probabilities because water temperatures influence catchability of trout by gill nets (Ward et al. 2012).

Regardless of sampling bias, fewer WCT were captured at High Lake in both 2018 and 2019 than expected. The nonnative Yellowstone Cutthroat Trout population that existed in High Lake prior to rotenone treatment was robust (Koel and York 2006). River otters occupied the lake and preyed upon the Yellowstone Cutthroat Trout there, and hundreds of dead fish were collected following rotenone treatment in 2006 (T. Koel, National Park Service, personal communication). Therefore, the translocated WCT population in High Lake in 2018 and 2019 probably had not reached its full recovery potential but we are unsure why. The translocated WCT population in High Lake could have been affected by harsh winters, low dissolved oxygen concentrations, low survival from RSIs, low recruitment from low flows in the inlet tributaries, or emigration over the waterfall barrier. Genetic ancestry data indicated that some WCT emigrated over the barrier and resided in main-stem East Fork Specimen Creek. Dissolved oxygen was hypothesized to limit Arctic Grayling overwinter survival in a shallow, high-elevation lake (Warren et. al 2018; Gander et al. 2019). Future research into emigration over the waterfall barrier and the dynamics of dissolved oxygen concentrations in High Lake could help explain the reasons for the sparse WCT population in High Lake.

Arctic Grayling. Fluvial Arctic Grayling were scarce in Grayling Creek one- and two-years post-translocations, indicating that they struggled to achieve any stage of population recovery. Most captured grayling were from the 2017 fry translocations, only a few were from the 2016 RSI translocations, and none were from the 2015 RSI translocations. Survival of grayling embryos in RSIs was affected by fungal outbreaks in 2015 and 2016 (J. Arnold, National Park Service, personal communication). These fungal outbreaks during the 2015 and 2016 RSI translocations combined with very cold water temperatures may explain the lower recruitment observed from RSIs than from the fry translocations. However, variable rearing conditions among translocation years or compounding mortality acting on old fish may have caused this observed disparity. We cannot conclusively infer that translocation technique influenced survival because both RSI and fry translocations were never used in the same year. Moreover, methods to distinguish between RSI- and fry-translocated fish from the same year class would be needed if both translocation techniques were used in the same year. RSIs are hypothesized to facilitate imprinting of embryos to target streams (Kaeding and Boltz 2004; Kirkland 2012; Arnold et al. 2017; Wilson 2017), but this hypothesis has not been investigated among grayling embryos. Future research associated with grayling translocation efforts in Michigan aims to address this question (N. M. Watson, Michigan State University, personal communication). Use of RSIs in remote backcountry conditions can be expensive and time intensive. Helicopter transport of RSI materials and crews was required to and from remote sites in Grayling Creek. Remote-site incubators also needed daily maintenance to maintain sufficient flows and monitor fungal outbreaks. Future research to determine if survival, imprinting, and water preference differences exist among grayling that are translocated by different translocation techniques could influence future projects.

Downstream emigration may have caused the low overall numbers of Arctic Grayling captured in Grayling Creek, including those that originated from the 2017 fry translocations (i.e., fish not subject to fungal growth in RSIs). At least some grayling emigrated past the fish barrier and were caught by anglers downstream of the restoration area (J. Carmack; T. Koel, National Park Service; personal communications). Although the fish barrier is about 20 km downstream from most grayling translocation sites, fluvial grayling move as far as 97 km in the Big Hole River (Shepard and Oswald 1989) and seasonally migrate as far as 101 km between summer and winter habitats in Alaska (West et al. 1992). Outmigration of grayling was notable in other failed grayling translocation attempts in Montana and Michigan streams (Kaya 1992; Nuhfer 1992) but translocating grayling from fluvial genetic stocks instead of adfluvial stocks presumably addressed outmigration tendencies of young grayling (Kaya 1991). However, older fluvial grayling may need longer unfragmented habitat segments than currently available in Grayling Creek above the waterfall barrier. Future research using PIT technology could help determine if grayling translocations into headwater streams have failed because of outmigration; if so, managers may need to consider larger target watersheds for fluvial grayling restoration (e.g., the Madison River headwaters in Yellowstone National Park above Hebgen Reservoir, Montana, but nonnative fish removals would probably need to be considered there).

Genetic robustness of the fluvial Arctic Grayling brood in Axolotl Lake, which was used as the donor source for grayling in Grayling Creek, may limit its translocation success. Testing in 2019 revealed it has lower genetic diversity and allelic richness than the Big Hole River population it replicates (A. McCullough and M. Jaeger, Montana Fish, Wildlife & Parks, unpublished data). Although the Axolotl Lake brood has higher genetic diversity and allelic richness than all other known stocks of grayling in Montana (R. Kovach, Montana Fish, Wildlife & Parks, personal communication), it may lack some of the adaptive potential of wild fluvial grayling in the Big Hole River. Montana Fish, Wildlife & Parks is attempting to increase the genetic diversity of this brood by incorporating additional wild grayling from the Big Hole River into Axolotl Lake (R. Kovach, Montana Fish, Wildlife & Parks, personal communication). The National Park Service should use the best available donor source with the highest genetic adaptive potential for any future translocations of fluvial grayling into Grayling Creek because genetics of the donor source population are critical to any translocation project (Minckley 1995; Weeks et al. 2011; IUCN SCC 2013; Houde et al. 2015),

Whether habitat is suitable to support Arctic Grayling throughout the year in Grayling Creek is unclear in this headwater system—especially because they seasonally migrate (Shepard and Oswald 1989; West et al. 1992). Fluvial grayling historically occupied the lower portion of Grayling Creek near Hebgen Reservoir (Vincent 1962) but their presence in the upper portion of the watershed where restoration efforts occurred is unknown (Koel et al. 2010). Even if grayling never occupied the restoration area or only used it intermittently for summer refugia or spawning and rearing habitat, this area currently provides one of the largest interconnected watersheds in Montana and Yellowstone National Park with coldwater habitat within the historical range of fluvial grayling. This area is also free of nonnative species, making it one of the best current candidates for fluvial grayling restoration. Although grayling coexist in other watersheds with nonnative fishes (Kaya 1992; Byorth and Magee 1998), inference and preliminary research indicates grayling are negatively affected by nonnative fishes through predation and competition

(Kaya 1992, 2000; especially Brown Trout, N. M. Watson, Michigan State University, unpublished data).

The robust Rocky Mountain Sculpin population in Grayling Creek possibly contributed to decreased grayling survival by preying upon vulnerable fry exiting RSIs or fry that were translocated. The sculpin population persisted in Grayling Creek during rotenone treatments possibly because rotenone concentrations that kill trout do not always kill sculpin (Clancey and Lohrenz 2014) or interstitial spaces in cobble substrate (Arciszewski et al. 2015) were used to escape rotenone exposure. Rocky Mountain Sculpin sometimes prey upon fish eggs and fry (Bailey 1951; Katzman 1998) and were observed feeding upon small trout that had died during rotenone treatments in Grayling Creek (J. Williams, formerly National Park Service, personal communication). If sculpin preyed upon grayling, they probably also ate WCT fry, but survival of translocated WCT appears to exceed that of grayling.

Perhaps the order of recovery of native fish species influenced the success of Arctic Grayling recovery. Although both Arctic Grayling and WCT were translocated in the same years, grayling survival ostensibly was lower (probably from fungal outbreaks in RSIs in 2015 and 2016). Therefore, sculpin that survived rotenone treatments and WCT that were successfully translocated as embryos in 2015 and 2016 may have been able to outcompete vulnerable grayling fry that were translocated in 2017. Competitive interactions could have in turn compounded downstream emigration of grayling. Future research into these interactions between purportedly sympatric native fishes could help inform feasibility of grayling translocations into waters containing an already-established native fish assemblage. Although WCT, Rocky Mountain Sculpin, and grayling evolved in sympatry in Montana streams (Vincent 1962), compounding challenges such as genetic robustness, habitat fragmentation, and climate change could hinder the abilities of grayling populations to coexist with historically sympatric species (Kaya 1992).

Unexpectedly, we documented the first evidence of natural reproduction of fluvial Arctic Grayling in Yellowstone National Park in decades—a group of 10 – 15 age-0 fry observed in Grayling Creek on July 30, 2019. Arctic Grayling embryos incubate for about three weeks post-fertilization, spend 3 – 4 days within the gravel before emerging, and then school together for about three weeks (Wilson 2017). The grayling fry we observed were about 20 – 25 mm TL and were schooled together in late July. Therefore, we estimate their parents spawned in mid- to late June in 2019 (about 5 – 6.5 weeks prior to the day the fry were observed). Arctic Grayling can mature at 2 years of age (Barndt and Kaya 2000) but mature at older ages in coldwater streams in northern-latitude regions (Kruse 1959; Liknes and Gould 1987; Barndt and Kaya 2000; U.S. Office of the Federal Register 2014). Therefore, we cannot definitively determine which translocation event the adult grayling that successfully spawned in 2019 originated from, but we suspect they were from the RSI translocations because the RSI fish would have been older and larger and more likely to be mature in 2019 than the fry that were translocated in 2017.

Translocation dates for Arctic Grayling may have been poorly timed and possibly should have occurred later in the year to better match developmental ontogeny of naturally reproduced grayling; however, availability of a grayling donor source that spawns later in the year may constrain the ability to delay translocations to a later date. Whereas grayling were translocated in late May as eyed embryos or in early June as fry, naturally occurring fry probably hatched in early to mid-July. This one-month or more mismatch in development between naturally occurring grayling and the Axolotl Lake brood grayling may have resulted in unfavorable rearing conditions for RSI- or fry-translocated grayling leading to decreased survival.

Interspecific Hybrid Introgression

Interspecific hybrid introgression in the WCT populations in East Fork Specimen and Grayling creeks probably resulted from barrier failure or incomplete eradication of hybrid fish during rotenone treatments, or both. Most hybrid fish in East Fork Specimen Creek were located within two kilometers of the temporary fish barrier and we therefore suspect that the barrier failed to prevent upstream fish movement of hybrid fish known to occur downstream of it. Targeted chemical (piscicide) removal of all fish within the lower portion of the watershed combined with barrier modification may be an appropriate adaptive management response. However, hybrids have probably expanded farther upstream since their discovery in 2019. Therefore, translocations of genetically pure WCT could also be used to improve the genetic integrity of the population because complete removal of hybrid fish is improbable without treating the entire watershed. Hybrid introgression persisted in other WCT populations (10 – 20% hybrid fish) despite reducing inputs of hybrid sources (Bennet and Kershner 2009). Remediation efforts such as stopping hybrid invasion and augmenting the population with genetically pure WCT may have varying effects on introgression levels (Campbell et al. 2002). Moreover, the fish barrier on East Fork Specimen Creek was designed to be temporary because the National Park Service plans to construct a permanent barrier farther downstream on Specimen Creek proper, restore WCT to North Fork Specimen Creek, and re-connect the North and East forks to create a large, interconnected area containing genetically pure WCT (Arnold et al. 2017). If hybrid fish persist in East Fork Specimen Creek in the future (11.5% of individuals there were hybrids in 2019), they may pose possible contamination risks to a future genetically pure WCT population in North Fork Specimen Creek. Stopping further invasion of hybridized fish, targeted removal of hybrids, and augmentation of genetically pure WCT in East Fork

Specimen Creek would decrease levels of introgression there. However, complete re-treatment of East Fork Specimen Creek using piscicides, and subsequent translocations of genetically pure WCT to both the North and East forks of Specimen Creek after a permanent barrier is in place may be the best alternative to help preserve this species into the future.

We suspect rotenone treatments in Grayling Creek failed to completely eradicate hybrid fish from the restoration area. Hybrid fish were found in low numbers throughout the drainage in 2018; one cluster of three hybrids was near the Dry Reach tributary. Therefore, the Dry Reach tributary may represent a location where hybrid fish were not completely eradicated. Notably, the proportions of hybrid fish captured in Grayling Creek were low and declined from 2018 (7.8%) to 2019 (4.2%). These low proportions may indicate low fitness of early WCT-hybrid crosses (Muhlfeld et al. 2009), which can prevent hybrids from persisting in a population. Furthermore, the few hybrid alleles in the WCT population could become obsolete as WCT recovery progresses and more genetically pure fish are recruited to the population (Bennet and Kershner 2009). Continued genetic monitoring will help determine if adaptive management strategies should be implemented. For example, augmentation of genetically pure WCT by additional translocations could help genetically swamp hybrid alleles—particularly in the lower portion of the watershed, which contained more hybrids and fewer WCT than the upper portion of the watershed in 2019. Targeted removal (mechanical or chemical) of hybrid fish in and around this tributary during their spawning period may be effective. Genetically pure and hybrid Cutthroat Trout spawn at slightly different times (DeRito et al. 2010; Heim et al. 2019), providing an opportunity for selective removal.

Incomplete eradication of nonnative fishes and barrier failures are common reasons for native fish translocation project failures (Harig et al. 2000; Cochran-Biederman et al. 2015).

Therefore, monitoring plans should be incorporated into future native fish translocation projects. Environmental DNA (eDNA) sampling is an effective tool that can be used to determine if fish were eradicated (Dunker et al. 2016; Schumer et al. 2019; Carim et al. 2020). Extensive eDNA sampling during a gap year between rotenone treatment and native fish translocations could be used to ensure complete eradication was achieved. Furthermore, regular monitoring should be implemented after translocations occur to ensure any invasions of nonnative fishes or hybrids, or both, are detected early on. Although eDNA sampling cannot effectively detect whether an individual fish is hybridized (yet, Stewart and Taylor 2020), it can detect maternal lineages (mitochondrial DNA) of potentially hybridizing species (Herder et al. 2014). Therefore, eDNA could be used in conjunction with collection of tissue samples to monitor possible invasions of nonnative and hybridized fishes.

Because presumed barriers commonly fail to preclude upstream fish movement (Harig et al. 2000), managers should carefully consider potential barrier options, conduct tests to determine if pre-existing or constructed barriers preclude upstream fish movements at a range of flows, and have contingency plans if barrier failure is detected. Although naturally occurring barriers are better at impeding fish invasion than artificial barriers (Harig et al. 2000), natural barriers may be absent in otherwise prime candidate watersheds. Therefore, artificial barriers should be engineered using the best-available designs. Furthermore, multiple barriers, possibly of different types, used in combination may better prevent nonnative fish invasion (Carpenter and Terrell 2005).

Genetic Ancestry

Translocating WCT from multiple donor sources into the East Fork Specimen Creek watershed resulted in populations that exhibited admixed ancestries representing *at least* four of the five primary ancestral donors used. However apparent disproportionate contributions of donor sources indicated that differences in relative fitness existed among donors. The derived proportion of WCT genetic ancestry representing the Geode Creek donor source was disproportionately lower than expected in East Fork Specimen Creek in 2019 whereas the derived proportion representing Muskrat Creek was higher than expected. Therefore, we inferred that WCT with Muskrat Creek ancestry had higher relative fitness compared to WCT with Geode Creek ancestry. Genetic diversity estimates for donor source populations indicate that Geode Creek has relatively low genetic diversity (expected heterozygosity = 0.03) compared to Muskrat Creek WCT (0.27 – 0.29). Therefore, genetic diversity differences among different donor sources possibly influenced adaptive potential (Houde et al. 2015) and relative survival, resulting in disproportionate donor source contributions to genetic ancestry in East Fork Specimen Creek. Interestingly, genetic ancestries of WCT populations in East Fork Specimen and Cherry creeks (B. Shepard, personal communication) had parallel results. The Muskrat Creek donor source contributed more to the recovering populations than expected in both watersheds. Its higher relative fitness compared to other sources used in both projects could be attributable to its high genetic diversity. Genetic ancestries of WCT in both East Fork Specimen and Cherry creeks were largely admixed, and all (genetically differentiable) donor sources were represented in the recovering populations (B. Shepard, personal communication). Multi-source native fish conservation translocations in both East Fork Specimen and Cherry creeks resulted in relatively genetically diverse WCT populations that contain admixed individuals—possibly an indication that admixing has not resulted in outbreeding depression.

Probable differences in survival of WCT among RSI sites from the embryonic stage to the swim-up stage were not accounted for in this study. Therefore, observed differences in contributions among donor sources could have originated from variation in RSI success or variation in survival caused by translocation method. Managers of future translocation projects using RSIs should thoroughly document numbers of fish translocated from each donor source, locations where translocations occurred, and swim-up success from individual RSIs, if possible.

Genetic samples from translocated individuals or their parents should be collected to help address any future uncertainties regarding genetic stocks. For example, uncertainties about genetic variability or genetic purity of a donor source population that was used for translocations could be better investigated if genetic samples were collected from translocated individuals or their parents. Genetic technology is becoming increasingly available and affordable, genetic research regarding translocation projects is of increasing interest, and collection and storage of genetic samples comes at little expense to the fish and researchers involved.

Conservation translocation projects that use a single donor source are more common than projects that use multiple donor sources (Houde et al. 2015), possibly because uncertainties are associated with outbreeding depression when multiple genetically distinct donor sources can interbreed (Weeks et al. 2011; Jamieson and Lacy 2012). However, concerns of outbreeding depression may be overly inflated (Weeks et al. 2011). Therefore, the adaptive potential gained by increased genetic diversity from multi-source translocations may outweigh outbreeding depression risks. Future investigations into metrics such as relative-fitness comparisons between pure and backcrossed offspring over time could give further insight into potential risks of outbreeding depression in multi-source translocation projects.

Project Comparisons

The progression of population recovery of WCT in the East Fork Specimen Creek and Grayling Creek watersheds appeared similar, with differences in stage of recovery between the watersheds attributable to the 5-year difference in restoration timelines. Although density estimates of WCT in East Fork Specimen Creek were higher in 2019 than in Grayling Creek in 2019, we suspect WCT densities in Grayling Creek will increase as the WCT population there advances through the stages of population recovery. Whereas elevations of the East Fork Specimen Creek and Grayling Creek watersheds are similar, water temperatures are colder in East Fork Specimen Creek than in Grayling Creek (Table 10). Higher elevation and colder headwater streams limit age-0 Cutthroat Trout recruitment and growth more than in lower elevation and warmer headwater streams (Coleman and Fausch 2007). Therefore, observed differences in WCT densities between these two watersheds in the future may, in part, be attributable to differences in stream characteristics.

Interestingly, the WCT population in Cherry Creek, Montana, recovered to (or surpassed) pre-restoration densities more quickly and to a greater extent than in East Fork Specimen and Grayling creeks (Clancey et al. 2019). Estimated densities of WCT in Cherry Creek were orders of magnitude higher than in East Fork Specimen and Grayling creeks despite similar (or fewer) numbers of fish and embryos translocated there (39,000 fish and embryos translocated into 100 km in Cherry Creek; Clancey et al. 2019). Again, differences in stream characteristics probably contributed to differences in WCT recovery among the three watersheds (Coleman and Fausch 2007). The Cherry Creek restoration area ranges in elevation from 1,539 (at the lower-most barrier) to 2,652 m (at the headwaters of Cherry Lake; Clancey et al. 2019), but a large portion of the watershed is at less than 2,000 m elevation. In contrast, the East Fork Specimen Creek and

Grayling Creek restoration areas are entirely above 2,000 m elevation (Table 10). Furthermore, Cherry Creek mean monthly water temperatures from May – September (2008 – 2015; Clancey et al. 2019) were much warmer than in East Fork Specimen Creek and slightly warmer than in Grayling Creek (Table 10).

Observed differences in WCT densities among these three watersheds are probably attributable to differences in stream characteristics such as climate and habitat. Consequently, the high fish densities in Cherry Creek would probably not naturally occur in East Fork Specimen Creek. Project managers of future conservation translocation projects should carefully consider stream characteristics among candidate watersheds, and appropriately match them to population density goals. Reference streams that are used to infer conservation translocation project outcomes should also be carefully considered to ensure that comparable stream characteristics exist among reference and target watersheds so that measurable project goals are appropriately defined.

Conclusion

We defined stages of population recovery progression and outlined measurable biological metrics to use as a recovery guideline to assess the success of native fish conservation translocation projects whereby most translocations occurred using RSIs. We used this population recovery guideline to assess restoration efforts of WCT and Arctic Grayling in the East Fork Specimen Creek and Grayling Creek watersheds. We also investigated the genetic ancestries of the WCT populations in the East Fork Specimen Creek watershed to determine differences in donor source contributions. Finally, we made comparisons of WCT recovery between East Fork Specimen and Grayling creeks to document variable outcomes among similar studies, inform temporal and spatial differences in the progression of population recovery, and contribute to the general knowledge of translocation biology of native freshwater fishes.

We showed that translocations of WCT were progressing but recovery of genetically pure conservation populations in these two restoration areas has been threatened by the presence of hybrid fish. We unexpectedly documented the first evidence of natural reproduction of fluvial grayling in Yellowstone National Park in decades. However, we outlined several potentially limiting challenges to grayling recovery—including the challenge that relatively small and fragmented headwater streams may not be suitable to fluvial grayling recovery. Knowledge gained from these investigations has helped inform management decisions of WCT and fluvial Arctic Grayling in the East Fork Specimen Creek and Grayling Creek watersheds. Namely, addressing the presence of hybridized WCT and continuing efforts to recover fluvial grayling. The importance of assessing nonnative fish eradications and invasions presents a tough lesson learned in East Fork Specimen and Grayling creeks that should lead to additional preventive measures to monitor these challenges in the future—for example, implementing eDNA sampling as a monitoring tool for nonnative and hybrid fishes.

Arguably, native fish translocation projects with nonnative fish eradication occur rarely in waters that contain the best available habitat because of the socioeconomic values nonnative fisheries provide, creating a social-ecological mismatch (Beever et al. 2019). Native fish translocation projects may need to be considered in large and interconnected coldwater habitats that 1) are not degraded, 2) are free of nonnative species or from which nonnatives can be removed (especially Brown Trout), 3) have long-term resilience to climate change, and 4) are within or near the historical ranges of native fishes. The headwaters of the Madison River drainage upstream of Hebgen Reservoir represents one such watershed that meets most criteria.

Whereas a conservation translocation project is already underway to restore WCT and fluvial grayling in the headwaters of the Gibbon River, which is a headwater tributary to the Madison River (Koel et al. 2019a), expansion of restoration areas to include the entire headwaters of the Madison River would represent an important opportunity for large-scale native fish restoration.

The multi-source adaptive potential strategy to recover WCT to the East Fork Specimen Creek watershed appeared successful and conformed to outcomes at Cherry Creek (B. Shepard, personal communication). All donor sources used were represented, albeit not proportionally, and several individuals exhibited admixed ancestry. Therefore, concerns of outbreeding depression associated with multi-source translocations may be inconsequential in these systems, but future genetic studies to specifically address potential outbreeding concerns should be considered.

Knowledge and insights gained from this study should help to increase success rates of future native fish conservation translocation projects within Yellowstone National Park and elsewhere. Increasing our knowledge by assessing outcomes of native fish translocation projects will increase the likelihood of success for future projects. Knowledge gains should increase future conservation translocation efficacy and increased success rates will increase the potential to help imperiled species by decreasing their extinction risk.

Tables

Table 1. Numbers of Westslope Cutthroat Trout (WCT) and Arctic Grayling translocated from donor sources into the East Fork Specimen Creek and Grayling Creek watersheds. All Arctic Grayling translocated into Grayling Creek were from the Axolotl Lake brood, which has the fluvial life-history form that is native to Yellowstone National Park.

Year	Species	Donor source	Number and fish stage
		High Lake	
2007	WCT	Geode Creek	1,144 age 1+
	WCT	Sun Ranch Hatchery	1,200 embryos
	WCT	Last Chance Creek	177 embryos
2008	WCT	Geode Creek	890 age 1+
	WCT	Sun Ranch Hatchery	2,844 embryos
	WCT	Last Chance Creek	286 embryos
2009	WCT	Geode Creek	930 age 1+
	WCT	Geode Creek	838 embryos
		East Fork Specimen Creek	
2010	WCT	Geode Creek	4,503 embryos
2011	WCT	Geode Creek	< 1,300 embryos
	WCT	Muskrat Creek	> 1,000 embryos
2012	WCT	Geode Creek	3,550 embryos
		Grayling Creek	
2015	WCT	Geode Creek	680 age 1+
	WCT	Geode Creek	4,977 embryos
	WCT	Sun Ranch Hatchery	5,000 embryos
	Arctic Grayling	Axolotl Lake	110,000 embryos
2016	WCT	Geode Creek	263 age 1+
	WCT	Geode Creek	1,386 embryos
	WCT	Sun Ranch Hatchery	26,800 embryos
	Arctic Grayling	Axolotl Lake	50,000 embryos
2017	WCT	Sun Ranch Hatchery	13,100 embryos
	Arctic Grayling	Axolotl Lake	50,000 age 0

Table 2. Numbers and frequencies of Westslope Cutthroat Trout translocated into High Lake and East Fork Specimen Creek from 2007 – 2012 and years for which genetic data are available from those donor sources. Numbers of fish (both embryos and age-1+) translocated into High Lake and East Fork Specimen Creek are shown in parentheses.

		High Lake (*n* translocated)		East Fork Specimen Creek (*n* translocated)	
Donor source	Years genotyped	Years	Relative %	Years	Relative %
Geode Creek	2005 2019	2007 (1,144) 2008 (890) 2009 (1,768)	45.76% (3,802)	2010 (4,503) 2011 (1,300) 2012 (3,550)	90.34% (9,353)
Sun Ranch Hatchery[a]	2007 2008 2010	2007 (1,200) 2008 (2,844)	48.67% (4,044)	--	0% (0)
Last Chance Creek	2005 2008 2009	2007 (177) 2008 (286) *and* via Sun Ranch Hatchery	5.57% (463)	--	0% (0)
Muskrat Creek	2007 2008 2009	via Sun Ranch Hatchery	--	2011 (1,000)	9.66% (1,000)
Ray Creek	2007 2008 2009	via Sun Ranch Hatchery	--	--	0% (0)
White's Creek	2007 2008 2009	via Sun Ranch Hatchery	--	--	0% (0)

[a] The Sun Ranch Hatchery included the Last Chance, Muskrat, Ray, and White's creeks donor sources.

Table 3. Summary of Westslope Cutthroat Trout groups used in the genetic ancestry analysis in the East Fork Specimen Creek watershed. Groups were genotyped using a 373-SNP panel designed for the Cherry Creek translocation project (M. Campbell, IDFG, unpublished data) and analyzed in *structure* ($k = 5$). Groups were designated as either known or unknown in *structure* whereby known groups were used to back assign alleles of unknown groups.

Group	Year	Designation in *structure*	Description
		WCT donor sources	
Geode Creek	2005	known	archived IDFG
Geode Creek	2019	known	collected for this study
Sun Ranch Hatchery	2007	unknown	analyzed for Cherry Creek
Sun Ranch Hatchery	2008	unknown	analyzed for Cherry Creek
Sun Ranch Hatchery	2010	unknown	analyzed for Cherry Creek
Last Chance Creek	2005	known	archived IDFG
Last Chance Creek	2008	known	archived YNP
Last Chance Creek	2009	known	archived YNP
Muskrat Creek	2007	known	analyzed for Cherry Creek
Muskrat Creek	2008	known	analyzed for Cherry Creek
Muskrat Creek	2009	known	analyzed for Cherry Creek
Ray Creek	2007	known	analyzed for Cherry Creek
Ray Creek	2008	known	analyzed for Cherry Creek
Ray Creek	2009	known	analyzed for Cherry Creek
White’s Creek	2007	unknown	analyzed for Cherry Creek
White’s Creek	2008	known	analyzed for Cherry Creek
White’s Creek	2009	known	analyzed for Cherry Creek
		Translocated WCT populations	
High Lake	2018	unknown	collected to estimate donor ancestry
High Lake	2019	unknown	collected to estimate donor ancestry
EFSC	2019	unknown	collected to estimate donor ancestry

IDFG = Idaho Fish and Game; YNP = Yellowstone National Park; EFSC = East Fork Specimen Creek.

Table 4. Estimated overall mean densities, fish/km (95% CI), and overall abundances of Westslope Cutthroat Trout in East Fork Specimen Creek (2019) and Grayling Creek (2018 and 2019). Mean densities[a] were calculated using Cochran's overall estimation method for a stratified random sampling design (Pollock et al. 1994). Overall abundances were calculated by multiplying overall densities by stream lengths.

Year	TL (mm)	Overall mean density (fish/km)	Stream length (km)	Overall abundance
		East Fork Specimen Creek		
2019	≥ 60	259.3 (205 – 314)	10.38	2,692 (2,125 – 3,260)
		Grayling Creek main stem		
2018	≥ 80	302.8 (123 – 482)	10.23	3,096 (1,262 – 4,930)
2019	≥ 80	128.2 (111 – 146)	10.23 (same as 2018)	1,310 (1,131 – 1,490)
2019	≥ 80	109.8 (91 – 128)	15.58	1,710 (1,422 – 1,997)
		Grayling Creek tributaries		
2018		not calculated in 2018 (large error associated with density estimates at several sites)		
2019	≥ 60	70.9 (59 – 82)	7.47	530 (444 – 616)

[a] The Carle-Strub estimator with a quasi-Poisson generalized linear model was used to calculate site abundances in main-stem East Fork Specimen Creek and Grayling Creek tributaries; the Lincoln-Petersen bias-adjusted estimator was used to calculate section abundances in main-stem Grayling Creek.

Table 5. Model selection scores (ΔAIC$_c$) and parameter estimates for competing maximum-likelihood models for closed-population mark-recapture and removal abundance estimates of Westslope Cutthroat Trout at sites in the East Fork Specimen Creeks and Grayling Creek watersheds in 2018 and 2019.

Site	Model	ΔAIC_c	$\widehat{N}$ (SE)	$\widehat{p_1}$	$\widehat{p_2}$	$\widehat{p_3}$	$\hat{c}$
			East Fork Specimen Creek 2019				
1	M_b	0	23 (1.9)	0.51	--	--	0
	M_{tb}	0.1984	22 (0.0)	0.55	0.40	0.75	0
2	M_b	0	51 (4.8)	0.52	--	--	0
	M_{tb}	1.9616	50 (5.3)	0.53	0.58	--	0
3	M_b	0	45 (1.7)	0.68	--	--	0
	M_{tb}	1.8841	46 (4.2)	0.67	0.58	--	0
4	M_b	0	46 (3.4)	0.57	--	--	0
	M_{tb}	1.7769	50 (10.6)	0.54	0.44	--	0
5	M_b	0	78 (7.8)	0.47	--	--	0
	M_{tb}	1.9082	75 (8.9)	0.48	0.53	--	0
6	M_b	4.6101	27 (1.5)	0.67	--	--	0
	M_{tb}	0	27 (0.0)	0.59	0.92	--	0
7	M_b	0.1927	8 (0.0)	0.67	--	--	0
	M_{tb}	0	8 (0.0)	0.63	0.75	--	0
8	M_b	0.4501	11 (0.0)	0.68	--	--	0
	M_{tb}	0	11 (0.0)	0.64	0.80	--	0
9	M_b	3.0995	11 (1.7)	0.57	--	--	0
	M_{tb}	0	11 (0.0)	0.46	0.86	--	0
10	M_b	--	5 (0.0)	0.83	--	--	0
	M_{tb}	--	--	--	--	--	--
H1	M_b	0	23 (4.3)	0.37	--	--	0
	M_{tb}	3.2021	20 (2.2)	0.44	0.35	0.65	0
H2	M_b	0.1964	4 (0.0)	0.57	--	--	0
	M_{tb}	0	4 (0.0)	0.50	0.67	--	0
H3	M_b	0	13 (0.0)	0.68	--	--	0
	M_{tb}	1.3835	13 (0.0)	0.62	0.83	--	0
			High Lake 2019				
Outlet	M_b	5.5555	10 (3.0)	0.44	--	--	0
	M_{tb}	0	8 (0.0)	0.39	0.86	--	0
			Grayling Creek main stem 2018				
1	M_0	0	683 (170.9)	0.13	0.13	--	0.13
	M_b	--	*estimation error*	--	--	--	--
2	M_0	0	120 (73.9)	0.13	0.13	--	0.13
	M_b	2.0918	72 (75.5)	0.24	0.24	--	0.13
3	M_0	0	289 (127.5)	0.12	0.12	--	0.12
	M_b	--	*estimation error*	--	--	--	--

Table 5 continued.

4	M_0	0	352 (157.0)	0.11	0.11	--	0.11
	M_b	1.5734	167 (107.8)	0.24	0.24	--	0.10
5	--	--	--	--	--	--	--
			Grayling Creek main stem 2019				
1	M_0	0.8510	176 (30.6)	0.30	0.30	--	0.30
	M_b	0	120 (18.2)	0.51	0.51	--	0.27
2	M_0	0.3603	61 (22.3)	0.26	0.26	--	0.26
	M_b	0	33 (5.9)	0.60	0.60	--	0.21
3 – 4	M_0	0	457 (68.2)	0.24	0.24	--	0.24
	M_b	--	*estimation error*	--	--	--	--
5	M_0	0	61 (34.9)	0.19	0.19	--	0.19
	M_b	1.8957	33 (19.0)	0.39	0.39	--	0.17
6	M_0	0.8050	39 (7.3)	0.46	0.46	--	0.46
	M_b	0	30 (2.7)	0.73	0.73	--	0.38
7	--	--	--	--	--	--	--
			Grayling Creek tributaries 2018				
a	M_b	0.0932	6 (1.9)	0.51	--	--	0
	M_{tb}	0	6 (0.0)	0.50	0.60	--	0
b	M_b	0	39 (24.1)	0.36	--	--	0
	M_{tb}	--	*estimation error*	--	--	--	--
c	M_b	0	48 (38.4)	0.15	--	--	0
	M_{tb}	1.2315	32 (16.2)	0.28	0.13	0.35	0
d	M_b	1.0723	6 (0.0)	0.55	--	--	0
	M_{tb}	0	6 (0.0)	0.67	0.0	0.67	0
e	M_b	1.2885	10 (3.5)	0.35	--	--	0
	M_{tb}	0	9 (0.0)	0.22	0.71	0.50	0
f	M_b	2.6565	7 (0.0)	0.70	--	--	0
	M_{tb}	0	7 (0.0)	0.57	1.0	--	0
g	M_b	0.1169	7 (7.7)	0.23	--	--	0
	M_{tb}	0	5 (0.0)	0.20	0.25	0.75	0
			Grayling Creek tributaries 2019				
c	M_b	0	25 (3.6)	0.51	--	--	0
	M_{tb}	2.0627	23 (3.4)	0.51	0.60	--	0

Table 6. Estimated abundances ($\widehat{N}$), capture probabilities ($\hat{p}$), and densities of Westslope Cutthroat Trout ≥ 60 mm total length at sites in main-stem East Fork Specimen Creek, tributaries to High Lake, and tributaries to Grayling Creek in 2018 and 2019. Numbers of unique individuals captured at each site (n) are listed. Carle-Strub and M_b removal models were used to estimate abundances.

			$\widehat{N}$ (95% CI) capture probability ($\hat{p}$)		$\widehat{Density}$ (fish/km) (95% CI[a])	
Site	Site/stratum length (km)	n	Carle-Strub	M_b	Carle-Strub	M_b
			East Fork Specimen Creek 2019			
1	0.1/1.1	22	23	23	230.0	228.5
			(19 – 27)	(22 – 33)	(193 – 267)	(221 – 334)
			$\hat{p} = 0.50$	$\hat{p} = 0.51$		
2	0.2/1.1	46	51	51	255.0	256.8
			(42 – 60)	(47 – 70)	(210 – 300)	(236 – 351)
			$\hat{p} = 0.52$	$\hat{p} = 0.52$		
3	0.1/1.1	44	45	45	450.0	450.2
			(42 – 48)	(44 – 54)	(417 – 483)	(441 – 541)
			$\hat{p} = 0.68$	$\hat{p} = 0.68$		
4	0.1/1.1	43	46	46	460.0	463.0
			(40 – 52)	(44 -61)	(396 – 524)	(436 – 606)
			$\hat{p} = 0.57$	$\hat{p} = 0.57$		
5	0.1/1.1	67	78	78	780.0	783.2
			(63 – 93)	(70 -106)	(630 – 930)	(703 – 1,055)
			$\hat{p} = 0.47$	$\hat{p} = 0.47$		
6	0.2/1.1	27	28	27	140.0	137.5
			(25 – 31)	(27 -37)	(124 – 156)	(135 – 183)
			$\hat{p} = 0.64$	$\hat{p} = 0.67$		
7	0.2/1.1	8	8	8	40.0	40.0
			(6 – 10)	(8 – 8)	(32 – 48)	(40 – 40)
			$\hat{p} = 0.67$	$\hat{p} = 0.67$		
8	0.2/1.1	11	11	11	55.0	55.0
			(9 – 13)	(11 – 11)	(47 – 63)	(55 – 55)
			$\hat{p} = 0.69$	$\hat{p} = 0.69$		
9	0.2/1.1	11	11	11	55.0	56.8
			(9 – 13)	(11 – 23)	(43 – 67)	(55 – 114)
			$\hat{p} = 0.61$	$\hat{p} = 0.58$		
10	0.2/0.6	5	5	5	25.0	25.0
			(4 – 6)	(5 – 5)	(20 – 30)	(25 – 25)
			$\hat{p} = 0.83$	$\hat{p} = 0.83$		
H1	0.1/--	22	22	23	220.0	231.0
			(16 – 28)	(20 – 44)	(155 – 285)	(204 – 436)
			$\hat{p} = 0.41$	$\hat{p} = 0.37$		

Table 6 continued.

H2	0.1/--	4	4 (2 – 6) $\hat{p} = 0.57$	4 (4 – 4) $\hat{p} = 0.57$	40.0 (21 – 59)	40.0 (40 – 40)
H3	0.1/0.5	13	13 (11 – 15) $\hat{p} = 0.68$	13 (13 – 13) $\hat{p} = 0.68$	130.0 (113 – 147)	130.0 (130 – 130)
High Lake 2019						
O1	0.1/0.4	8	9 (3 – 15) $\hat{p} = 0.44$	10 (8 – 37) $\hat{p} = 0.39$	90.0 (30 – 150)	97.7 (81 – 367)
I1	0.2/0.5	2	--	--	--	--
I2	0.2/0.5	2	--	--	--	--
Grayling Creek tributaries 2018						
a	0.1/1.8	6	6 (3 – 9) $\hat{p} = 0.55$	6 (6 – 19) $\hat{p} = 0.51$	60.0 (33 – 87)	62.3 (60 – 192)
b	0.1/1.8	23	33 (5 – 61) $\hat{p} = 0.43$	39 (25 – 159) $\hat{p} = 0.36$	330.0 (51 – 609)	386.2 (248 – 1,587)
c	0.1/2.4	24	36 (5 – 67) $\hat{p} = 0.23$	48 (27 – 241) $\hat{p} = 0.15$	360.0 (52 – 668)	484.8 (268 – 2,414)
d	0.1/2.4	6	6 (5 – 7) $\hat{p} = 0.55$	6 (6 – 6) $\hat{p} = 0.55$	60.0 (46 – 74)	60.0 (60 – 60)
e	0.1/0.5	9	10 (5 – 15) $\hat{p} = 0.38$	10 (9 – 31) $\hat{p} = 0.35$	100.0 (46 – 154)	104.4 (91 – 313)
f	0.1/0.5	7	7 (6 – 8) $\hat{p} = 0.70$	7 (7 – 7) $\hat{p} = 0.70$	70.0 (59 – 81)	70.0 (70 – 70)
g	0.1/0.5	5	5 (1 – 9) $\hat{p} = 0.38$	7 (5 – 56) $\hat{p} = 0.23$	50.0 (14 – 86)	72.5 (51 – 561)

Table 6 continued.

Grayling Creek tributaries 2019

a	0.1/0.9	4	11 (7 – 19) one-pass site	--	113.8 (68 – 190[a])	--
b	0.1/0.9	4	11 (7 – 19) one-pass site	--	113.8 (68 – 190[a])	--
c	0.2/1.2	22	24 (18 – 30) $\hat{p} = 0.52$	26 (22 – 42) $\hat{p} = 0.51$	120.0 (89 – 151)	122.6 (112 – 209)
d	0.1/2.4	--	--	--	--	--
e	0.1/0.5	2	6 (3 – 14) one-pass site	--	61.5 (27 – 141[a])	--
f	0.1/0.5	4	11 (7 – 19) one-pass site	--	113.8 (68 – 190[a])	--
g	0.1/0.5	0	0	0	0	0
h	0.1/0.9	2	6 (3 – 14) one-pass site	--	61.5 (27 – 141[a])	--
i	0.1/1.2	1	3 (1 – 11) one-pass site	--	33.3 (10 – 109[a])	--

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[a] 99% confidence intervals instead of 95% prediction intervals were used for one-pass Carle-Strub quasi-Poisson predicted abundances.

Table 7. Estimated abundances ($\widehat{N}$), capture probabilities ($\hat{p}$), and densities of Westslope Cutthroat Trout ≥ 80 mm total length at sites in main-stem Grayling Creek in 2018 and 2019. Numbers of unique individuals captured at each site (n) are listed. Abundances were calculated using Lincoln-Petersen bias-adjusted and M_0 mark-recapture models.

Site	Site/stratum length (km)		$\widehat{N}$ (95% CI) capture probability ($\hat{p}$)		$\widehat{Density}$ (fish/km) (95% CI)	
		n	L-P	M_0	L-P	M_0
			2018			
1	2.7/3.5	170	642 (397 – 1,078) $\hat{p} = 0.12$	683 (442 – 1,139) $\hat{p} = 0.13$	236.1 (146 – 396)	251.1 (162 – 419)
2	0.8/2.3	30	95 (40 – 274) $\hat{p} = 0.13$	120 (52 – 397) $\hat{p} = 0.13$	126.6 (53 – 365)	160.6 (70 – 529)
3	1.0/2.0	65	197 (87 – 472) $\hat{p} = 0.08$	289 (145 – 698) $\hat{p} = 0.12$	195.9 (87 – 469)	287.8 (144 – 694)
4	0.3/1.3	75	313 (151 – 714) $\hat{p} = 0.11$	354 (174 – 854) $\hat{p} = 0.11$	1,155.6 (557 – 2,636)	1,307.0 (644 – 3,154)
5	0.3/1.2	8	23 (4 – 23) no recaptures	--	27.2 (12 – 70)	--
			2019			
1	2.7/3.5	91	171 (122 – 259) $\hat{p} = 0.34$	176 (134 – 260) $\hat{p} = 0.30$	62.9 (45 – 95)	64.7 (49 – 95)
2	0.8/2.3	28	55 (32 – 121) $\hat{p} = 0.31$	61 (38 – 138) $\hat{p} = 0.26$	73.3 (43 – 161)	80.6 (50 – 183)
3 – 4	1.7/3.3	193	436 (317 – 617) $\hat{p} = 0.20$	457 (354 – 628) $\hat{p} = 0.24$	258.6 (188 – 366)	271.4 (210 – 373)
5	0.5/2.1	21	51 (23 – 145) $\hat{p} = 0.18$	61 (30 – 196) $\hat{p} = 0.19$	104.5 (47 – 297)	124.8 (62 – 402)
6	0.8/2.5	28	38 (28 – 65) $\hat{p} = 0.53$	39 (31 – 64) $\hat{p} = 0.46$	45.8 (34 – 78)	46.7 (38 – 77)
7	1.1/1.9	16	80 (16 – 80) no recaptures	--	73.5 (15 – 74)	--

Table 8. Genetic ancestry and measures of expected heterozygosity (H_e) of donor sources and translocated Westslope Cutthroat Trout populations in High Lake and East Fork Specimen Creek (*structure* analysis, $k = 5$).

Group	H_e	Year	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
				WCT donor sources			
1° Geode	0.03	2005	0.00	0.00	0.00	**1.00**	0.00
1° Geode	0.03	2019	0.00	0.00	0.00	**1.00**	0.00
1° Last Chance	0.06	2005	0.00	**0.98**	0.00	0.00	0.02
1° Last Chance	0.07	2008	0.00	**0.99**	0.00	0.00	0.01
1° Last Chance	0.07	2009	0.00	**0.98**	0.00	0.00	0.02
1° Muskrat	0.27	2007	0.39	0.01	**0.60**	0.00	0.00
1° Muskrat	0.28	2008	**0.44**	0.00	**0.54**	0.00	0.01
1° Muskrat	0.29	2009	**0.61**	0.01	0.37	0.00	0.00
1° Ray	--	2007	0.00	0.02	0.00	0.00	**0.98**
1° Ray	--	2008	0.00	0.01	0.00	0.00	**0.99**
1° Ray	--	2009	0.00	0.01	0.00	0.00	**0.99**
1° White's	--	2007	0.15	**0.55**	0.30	0.00	0.00
1° White's	--	2008	0.00	**1.00**	0.00	0.00	0.00
1° White's	--	2009	0.00	**1.00**	0.00	0.00	0.00
Sun Ranch	0.18	2007	0.12	0.18	0.07	0.01	**0.63**
Sun Ranch	0.29	2008	**0.38**	0.09	0.25	0.01	0.28
Sun Ranch	0.29	2010	**0.39**	0.04	0.35	0.01	0.20
		Cluster majority:	**MC**	**LCC/WC**	**MC**	**GC**	**RC**
				Translocated WCT populations			
High Lake	0.29	2018	**0.41 (MC)**	0.08 (LCC/WC)	0.17 (MC)	0.14 (GC)	0.20 (RC)
High Lake	0.25	2019	0.28 (MC)	0.04 (LCC/WC)	0.14 (MC)	**0.50 (GC)**	0.05 (RC)
EFSC	0.26	2019	0.16 (MC)	0.06 (LCC/WC)	0.06 (MC)	**0.59 (GC)**	0.13 (RC)

GC = Geode Creek; LCC = Last Chance Creek; MC = Muskrat Creek; RC = Ray Creek; WC = White's Creek; SRH = Sun Ranch Hatchery; HL = High Lake; EFSC = East Fork Specimen Creek.

Table 9. Median total lengths (mm) and ranges of Westslope Cutthroat Trout (WCT), hybridized WCT, and Brown Trout captured from 1994 to 2019 in East Fork Specimen Creek, High Lake, and Grayling Creek. Letters represent Kruskal-Wallis follow-up pair-wise comparisons (familywise α = 0.5). Statistically similar groups share the same letter whereas statistically different groups do not.

		Total length (mm)			
Year	Species	*n*	Median	Range	Letters
		East Fork Specimen Creek pre-restoration			
1994	hybrids	29	167	69 – 262	abc
1997	hybrids	16	98	45 – 174	ade
1998	hybrids	24	76	32 – 219	de
2004	hybrids	68	155	54 – 254	abc
2005	hybrids	28	172	76 – 262	abc
		East Fork Specimen Creek post-restoration			
2015	WCT	81	160	93 – 230	abc
2016	WCT	87	163	106 – 273	bc
2019	WCT	260	112	53 – 317	d
	hybrids	43			
		High Lake post-restoration			
2016	WCT	14	362	174 – 440	f
2018	WCT	12	390	326 – 452	f
2019	WCT	16	107	46 – 269	abcde
		Grayling Creek pre-restoration			
2006	WCT	46	145	78 – 184	abde
2007	hybrids	15	127	60 – 352	ae
	Brown Trout	86			
2008	hybrids	96	134	28 – 306	abe
	Brown Trout	2			
2009	hybrids	185	114	27 – 247	d
	Brown Trout	17			
2010	hybrids	10	150	71 – 256	abc
	Brown Trout	28			
		Grayling Creek post-restoration			
2018	WCT	450	162	70 – 297	ab
	hybrids	7			
2019	WCT	416	168	71 – 309	c
	hybrids	4			

Table 10. Monthly mean water temperatures (°C) in East Fork Specimen and Grayling creeks June – September in 2018 and 2019 and watershed elevations (m).

	East Fork Specimen Creek (2,231 – 2,675 m elevation)		Grayling Creek (2,131 – 2,400 m elevation)	
Month	2018	2019	2018	2019
Jun	5.8	4.5	8.0	6.8
Jul	6.4	7.0	10.2	10.1
Aug	5.0	6.1	9.0	9.9
Sep	3.9	4.4	6.9	8.6

Figures

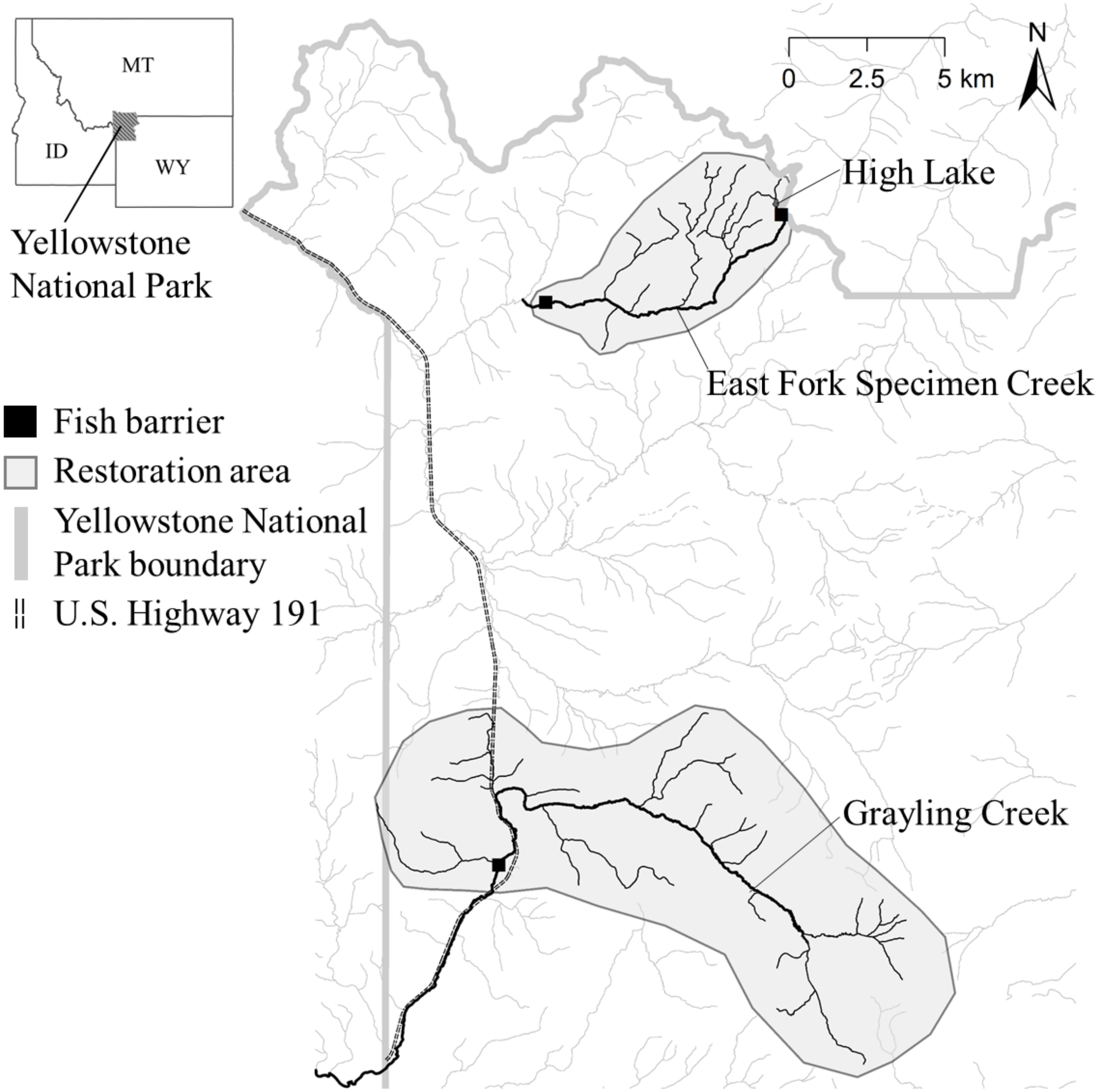


Figure 1. East Fork Specimen Creek and Grayling Creek native fish restoration areas in Yellowstone National Park, Montana and Wyoming. Westslope Cutthroat Trout were translocated into East Fork Specimen Creek and High Lake following piscicide application. Both Westslope Cutthroat Trout and Arctic Grayling were translocated into Grayling Creek. Three barriers prevent upstream movements of fish into the isolated waters: 1) a natural waterfall located in the outlet of High Lake, 2) a temporary man-made log barrier in main-stem East Fork Specimen Creek, and 3) a permanent waterfall barrier that was modified by blasting and installing a concrete splash pad in main-stem Grayling Creek. Barriers isolate 11.2 main-stem km and High Lake (surface area of 2.9 ha) in the East Fork Specimen Creek restoration area and 17.1 main-stem km in the Grayling Creek restoration area.

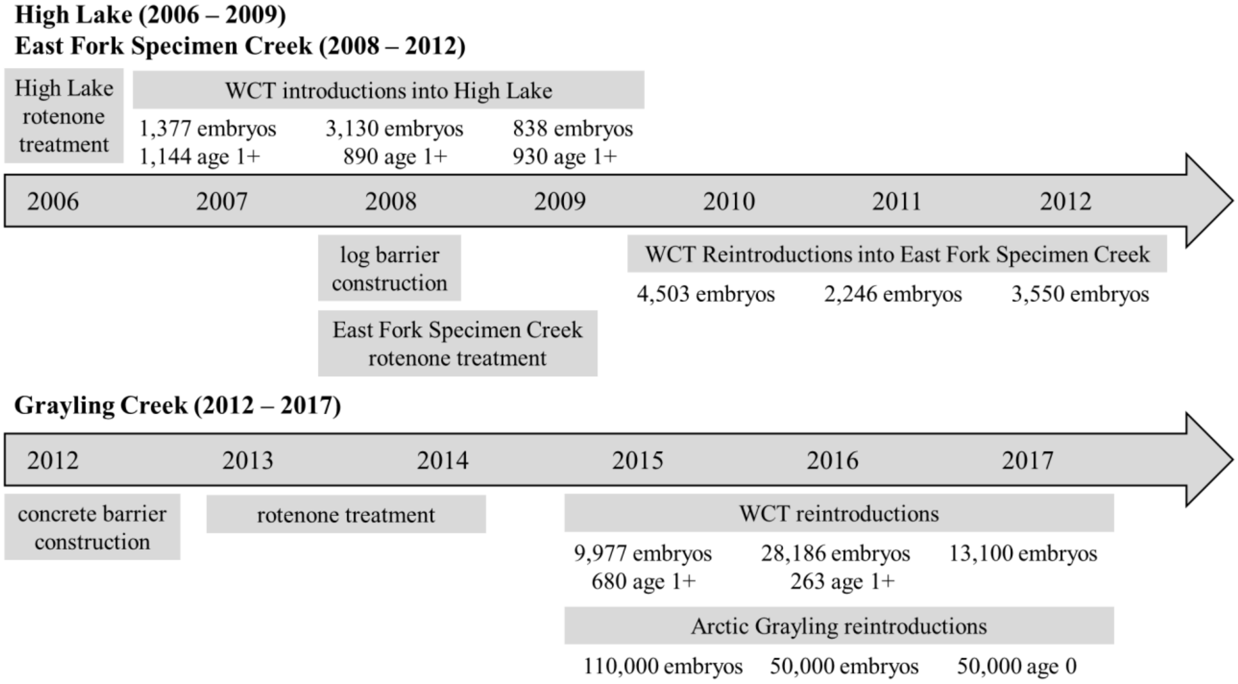


Figure 2. Native fish restoration activity timelines in the East Fork Specimen Creek and Grayling Creek watersheds. Westslope Cutthroat Trout and Arctic Grayling embryo introductions were facilitated using remote-site incubators whereas age-1+ (age 1 and older) Westslope Cutthroat Trout from Geode Creek, Yellowstone National Park, were translocated using a hatchery truck or helicopter; age-0 Arctic Grayling fry, instead of embryos, were translocated in 2017 because conditions in Grayling Creek were unsuitable when embryos were ready for transportation. Fish were translocated into all three waterbodies for three consecutive years following barrier construction and piscicide application.

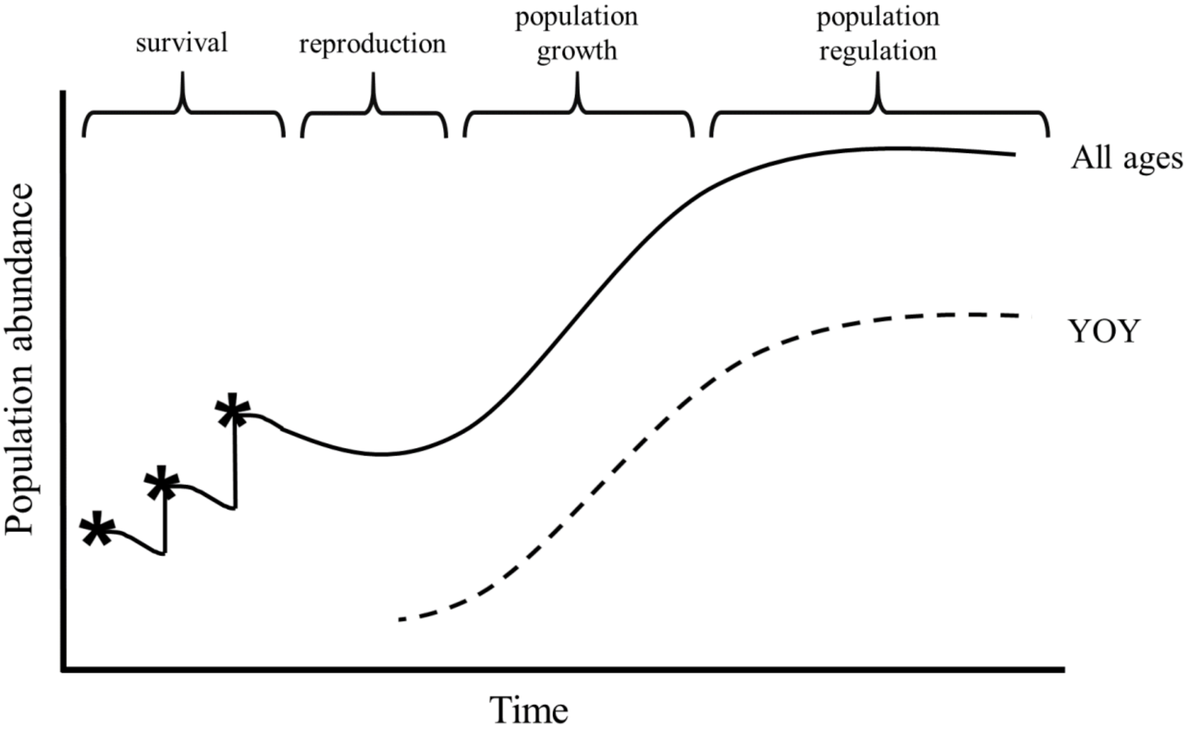


Figure 3. Theoretical relationship between population abundance, time, and stages of population recovery of a translocated native fish population whereby most individuals were translocated as embryos into a watershed. The asterisks represent translocation events, the solid line represents the population size of all ages, and the dashed line represents population size of naturally reproduced young of year (YOY). Our stages of population recovery correspond to observed growth (or not) in the translocated population. Population growth is modeled similarly to what would be observed using recruitment models used in fisheries such as the Beverton-Holt recruitment model (Beverton and Holt 1957; Maceina and Pereira 2007).

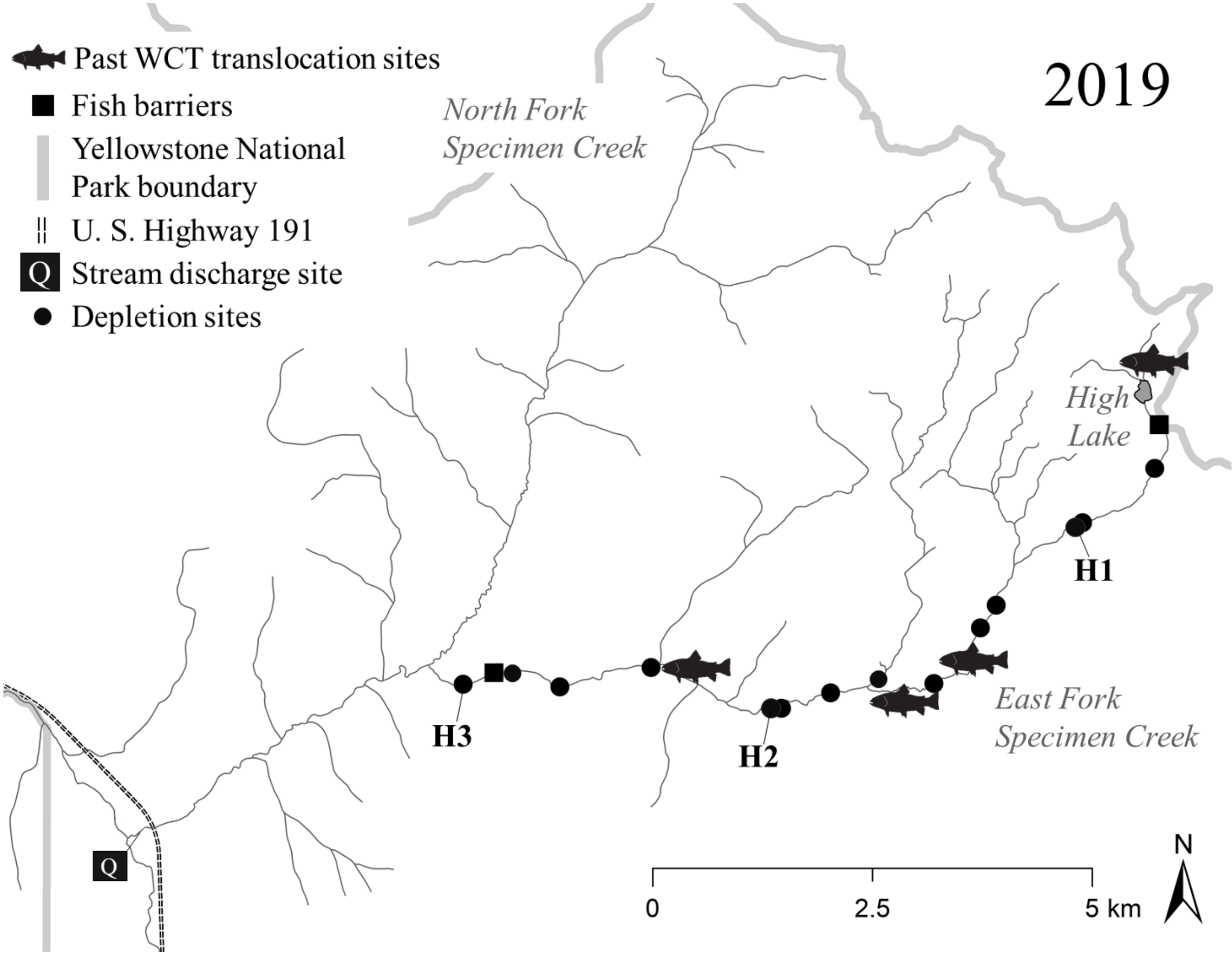


Figure 4. East Fork Specimen Creek sites sampled August 19 – 24, 2019, using backpack electrofishing gear. Crews multi-pass-depletion sampled 14 sites: 10 random sites within each km, one tributary site, and three historical sites (denoted by the letter "H") that were also sampled in 2004 and 2005. East Fork Specimen Creek was not sampled in 2018 because fire activity precluded crews from safely entering the area. Past translocation sites of Westslope Cutthroat Trout (WCT) from 2007 – 2011 are shown by the fish icons. Stream discharge (Q) was frequently measured on Specimen Creek proper, which was downstream of the East Fork Specimen Creek restoration area.

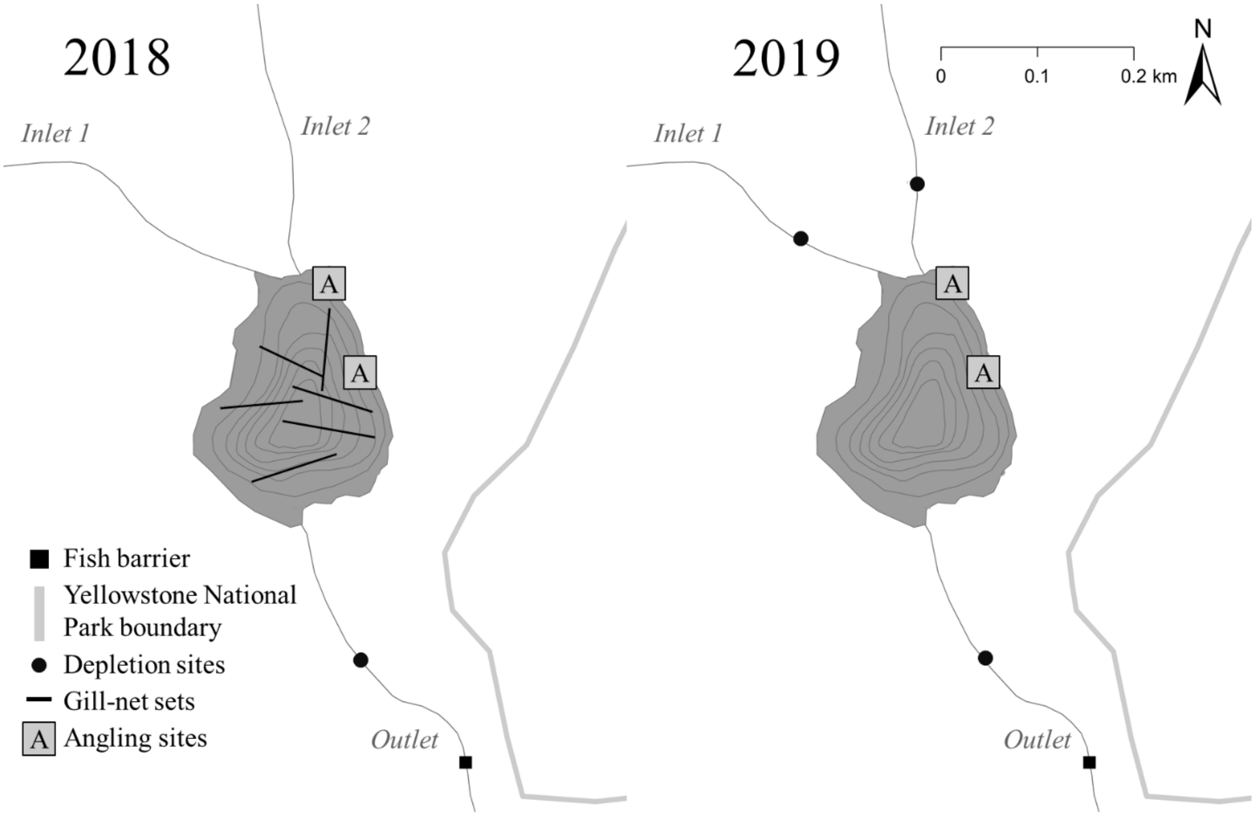


Figure 5. High Lake sites sampled July 16 – July 19, 2018 (left), and July 9, 2019 (right). Crews mark-recapture sampled High Lake using gill nets (*n* = 6) in 2018; one multi-pass-depletion site in the outlet was also sampled using backpack electrofishing gear and one expert-skilled angler used fly fishing gear along the north and northeast shores for one to two hours in both years. Whereas crews did not mark-recapture sample High Lake in 2019 because so few fish were captured with gill nets in 2018, crews sampled two additional multi-pass-depletion sites in the inlets. Bathymetry lines represent 1-m increments.

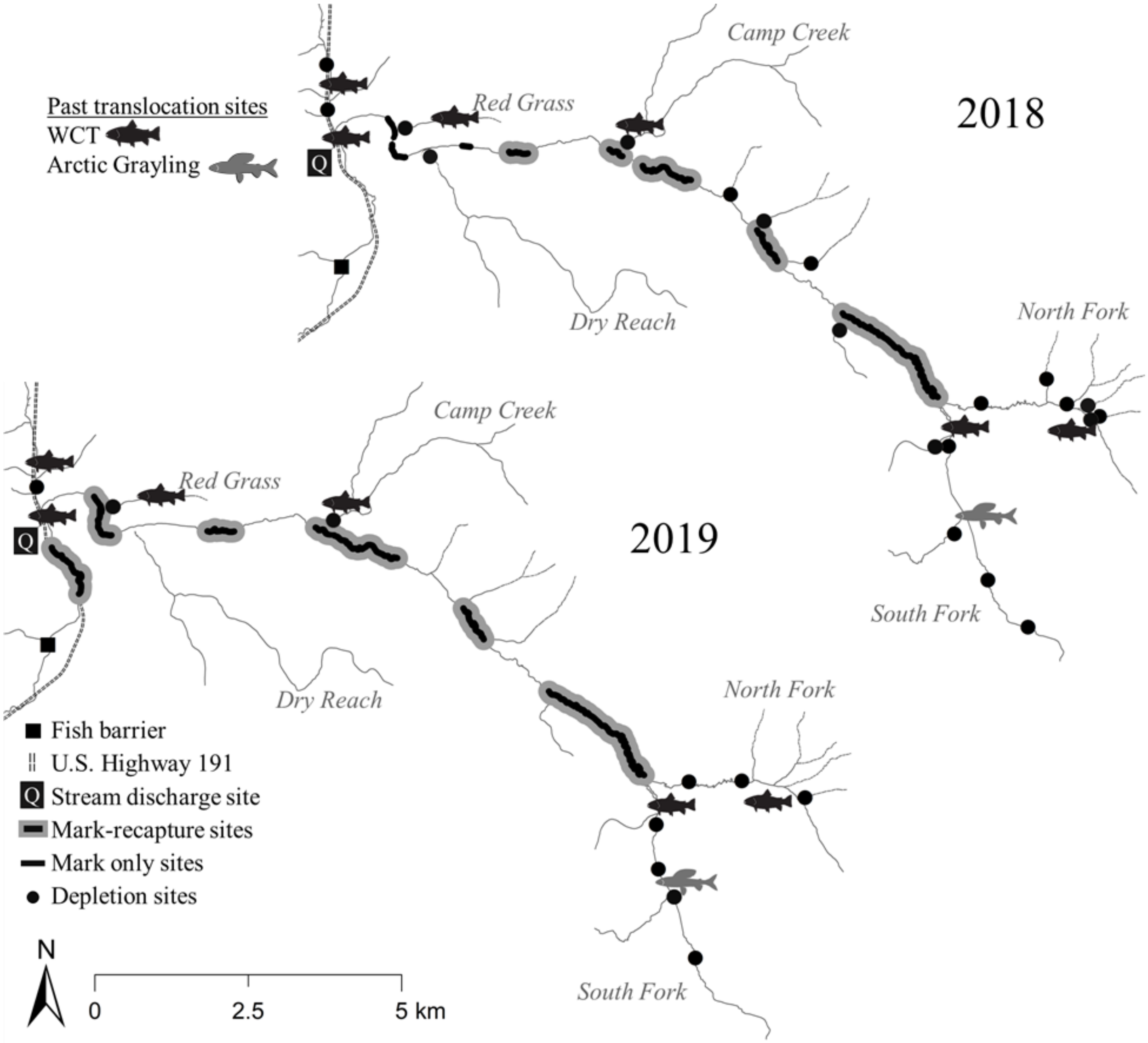


Figure 6. Grayling Creek sites sampled July 30 – August 14, 2018 (top), and July 22 – August 8, 2019 (bottom). Crews mark-recapture sampled 30% of the main stem in 2018 and 45% in 2019 using tow-barge electrofishing gear. Crews also multi-pass-depletion sampled 20 tributary sites in 2018 and 10 in 2019 using backpack electrofishing gear. Past translocation sites of Westslope Cutthroat Trout (WCT) and Arctic Grayling from 2015 – 2017 are shown by the fish icons. Stream discharge (Q) was frequently measured on Grayling Creek at a site upstream of the fish barrier.

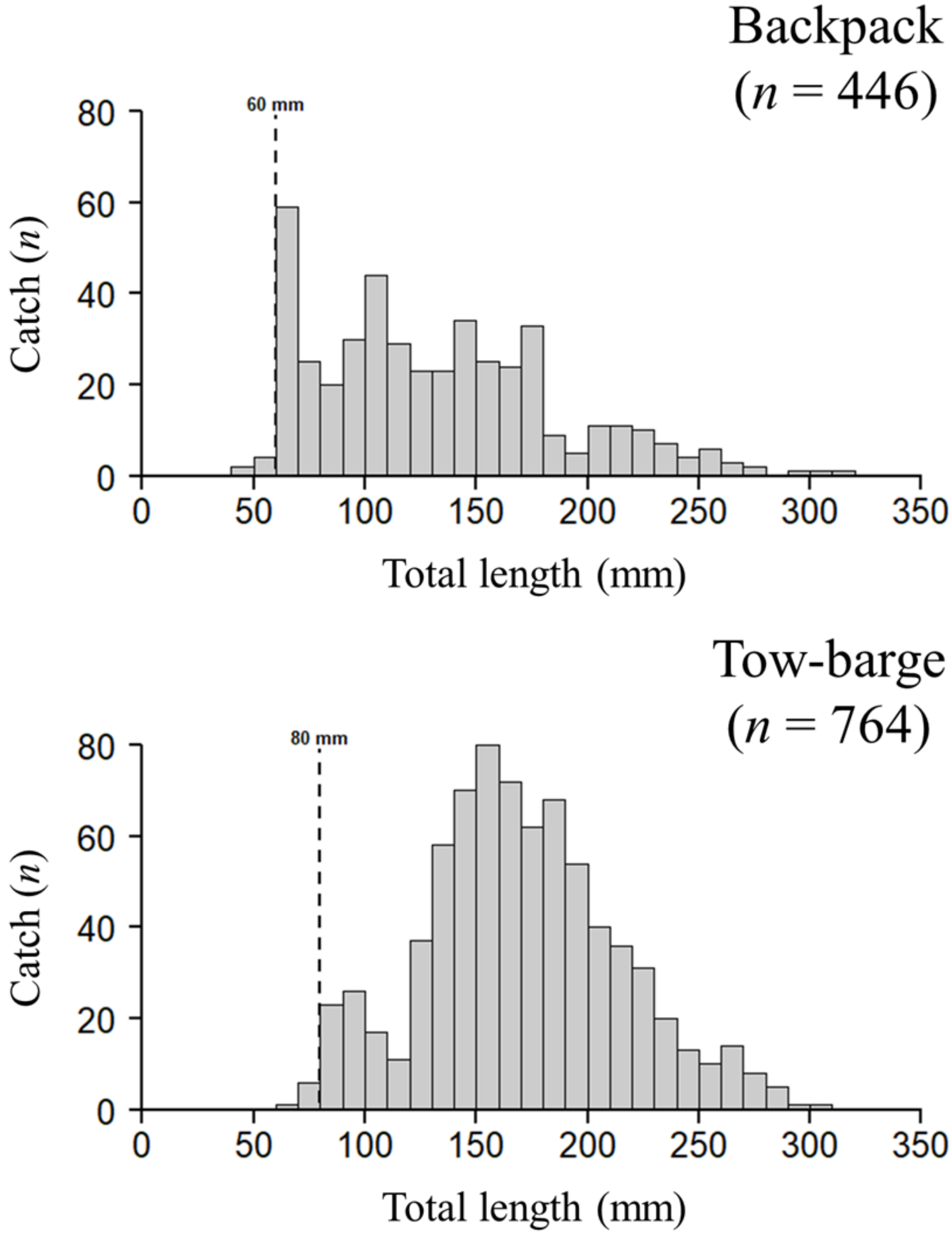


Figure 7. Catch-frequency distributions by total lengths (TLs; 10-mm bins) of fish captured using backpack electrofishing (top) and tow-barge electrofishing (bottom) in the East Fork Specimen Creek and Grayling Creek watersheds in 2018 and 2019. Captures of fish < 60 mm TL occurred rarely during backpack electrofishing and captures of fish < 80 mm TL occurred rarely during tow-barge electrofishing. Therefore, fish < 60 and < 80 mm TL were excluded from multi-pass-depletion and mark-recapture abundance estimates, respectively.

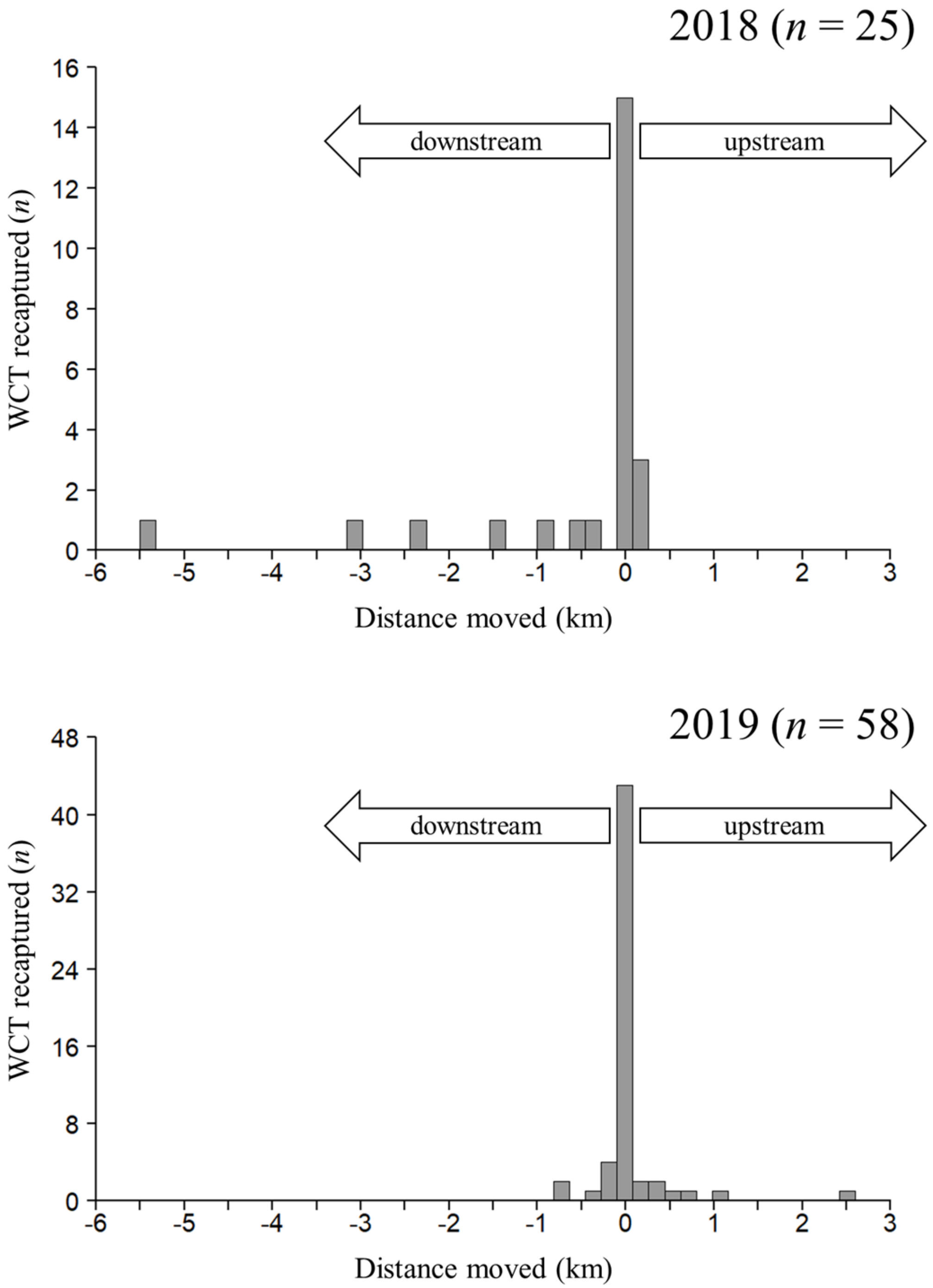


Figure 8. Frequencies of distances moved by recaptured Westslope Cutthroat Trout from original capture locations in Grayling Creek in 2018 (top) and 2019 (bottom). Downstream and upstream movements are represented by negative and positive kilometer values, respectively. Whereas most recaptured WCT did not move (0 km) from their original sample location, several moved downstream in 2018 and caused a violation of the closure assumption associated with mark-recapture models.

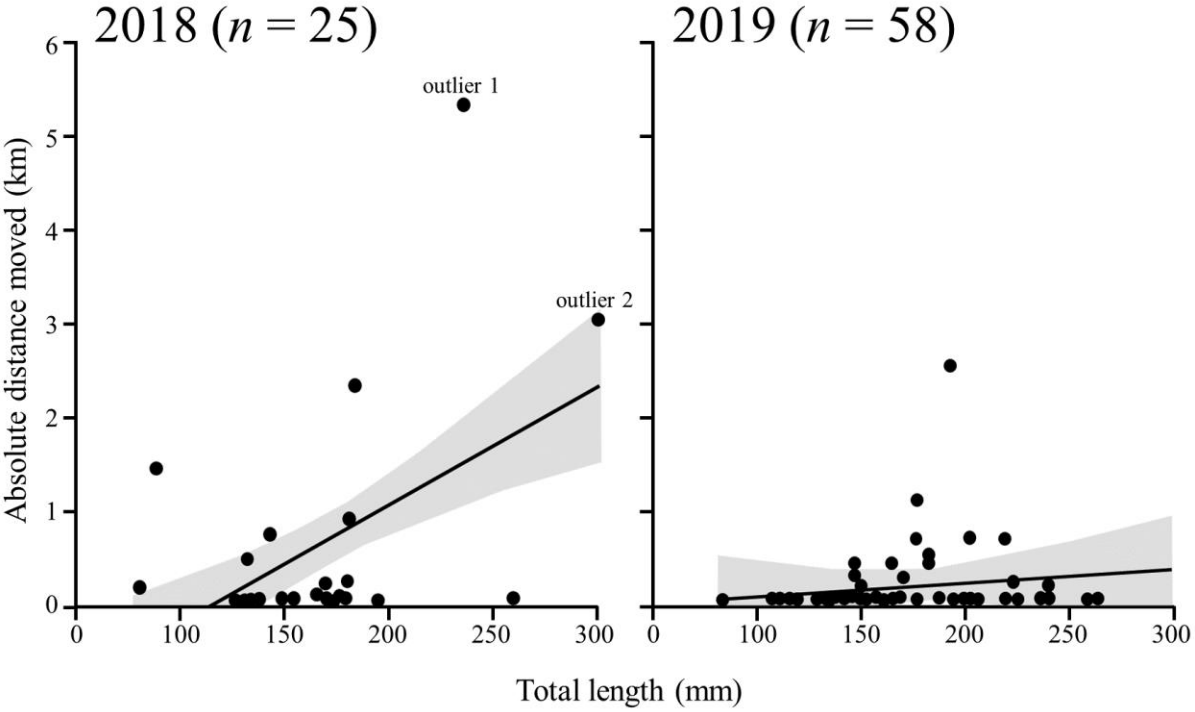


Figure 9. Linear relationships between absolute distances moved (km) from original capture locations and total lengths (TL; mm) of recaptured Westslope Cutthroat Trout in Grayling Creek in 2018 (left) and 2019 (right). Model residuals, model fit, and associated 95% confidence intervals are shown by black circles, thick black lines, and gray shading, respectively. The positive linear relationship in 2018 was driven by two outliers (Cook's Distance > 1) that moved the longest distances ($\text{WCT}_{\text{outlier1}}$ 237 mm TL, moved 5.37 km; $\text{WCT}_{\text{outlier2}}$ 297 mm TL, moved 3.01 km); no relationship between distance moved and TL was evident in 2019.

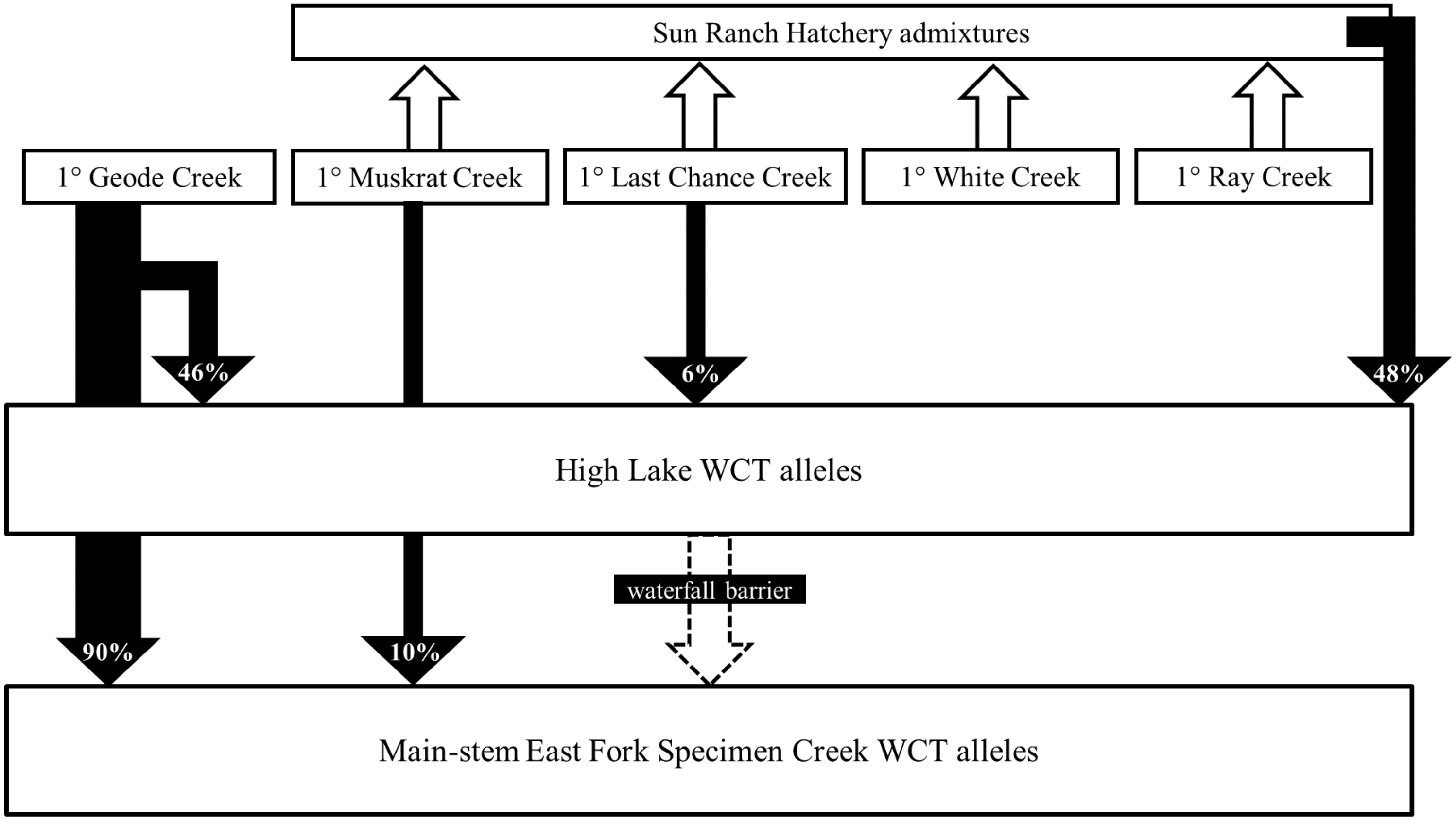


Figure 10. Proportions of Westslope Cutthroat Trout from donor sources translocated into the East Fork Specimen Creek watershed. Black arrows represent direct translocations into High Lake and East Fork Specimen Creek and associated percentages represent the proportions of fish or embryos translocated from donor sources into the waterbody. White arrows represent four primary donor sources that were used to supplement the Sun Ranch Hatchery stock. Muskrat Creek and Last Chance Creek donor sources were translocated directly and indirectly by way of the Sun Ranch Hatchery. Therefore, estimated proportions translocated from Muskrat and Last Chance creeks are confounded. Furthermore, fish translocated into High Lake could have contributed alleles to main-stem East Fork Specimen Creek by emigrating over the waterfall barrier (dashed arrow).

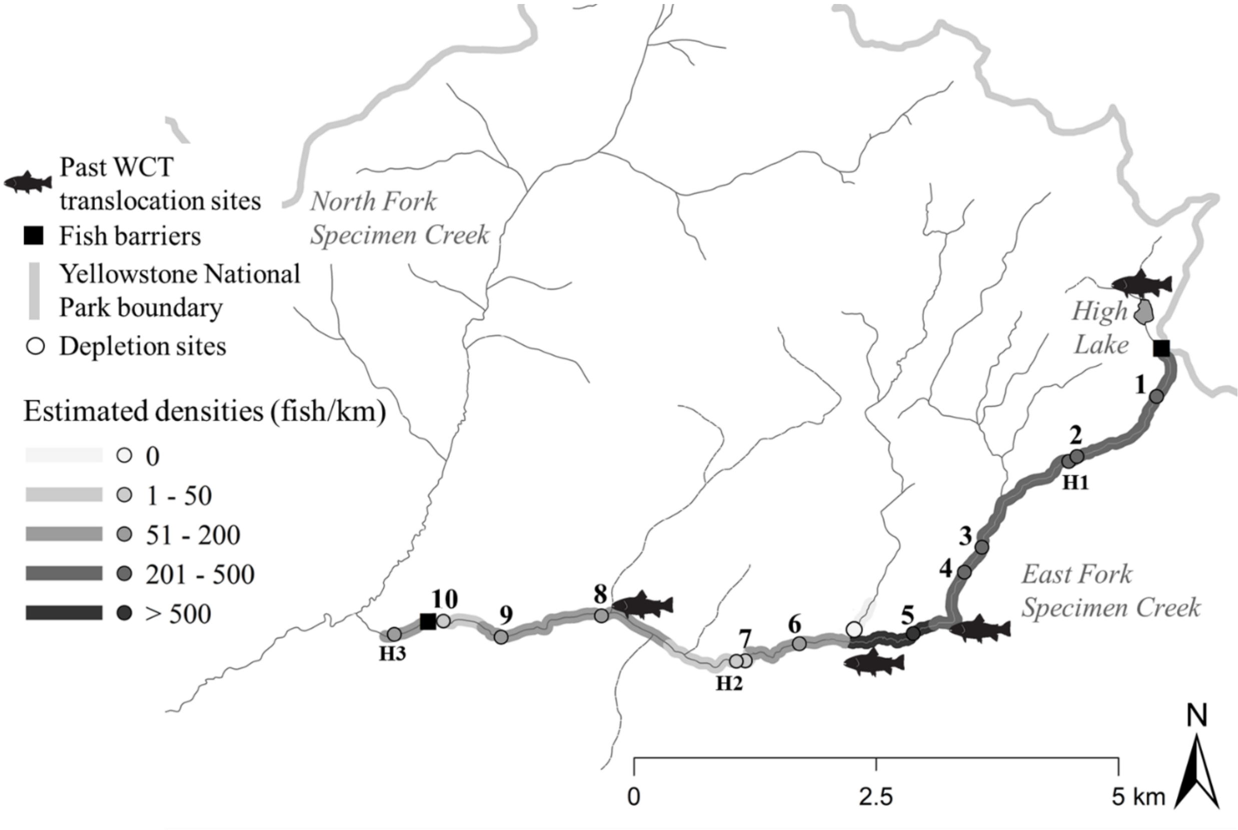


Figure 11. Extrapolated estimated densities (fish/km) of Westslope Cutthroat Trout in East Fork Specimen Creek in 2019. Removal sites are represented by circles ($n = 14$). Estimated densities are represented by shades of gray. Sites historically sampled in East Fork Specimen Creek are represented by an 'H' ($n = 3$); Site H3 is located downstream of the restoration area. No fish were captured at the tributary site (represented by the open circle between sites 5 and 6); therefore, it was assumed to have a density of zero fish/km. Densities were extrapolated to the entire stratum that each random site was chosen from. The Carle-Strub estimator was used to calculate site abundances.

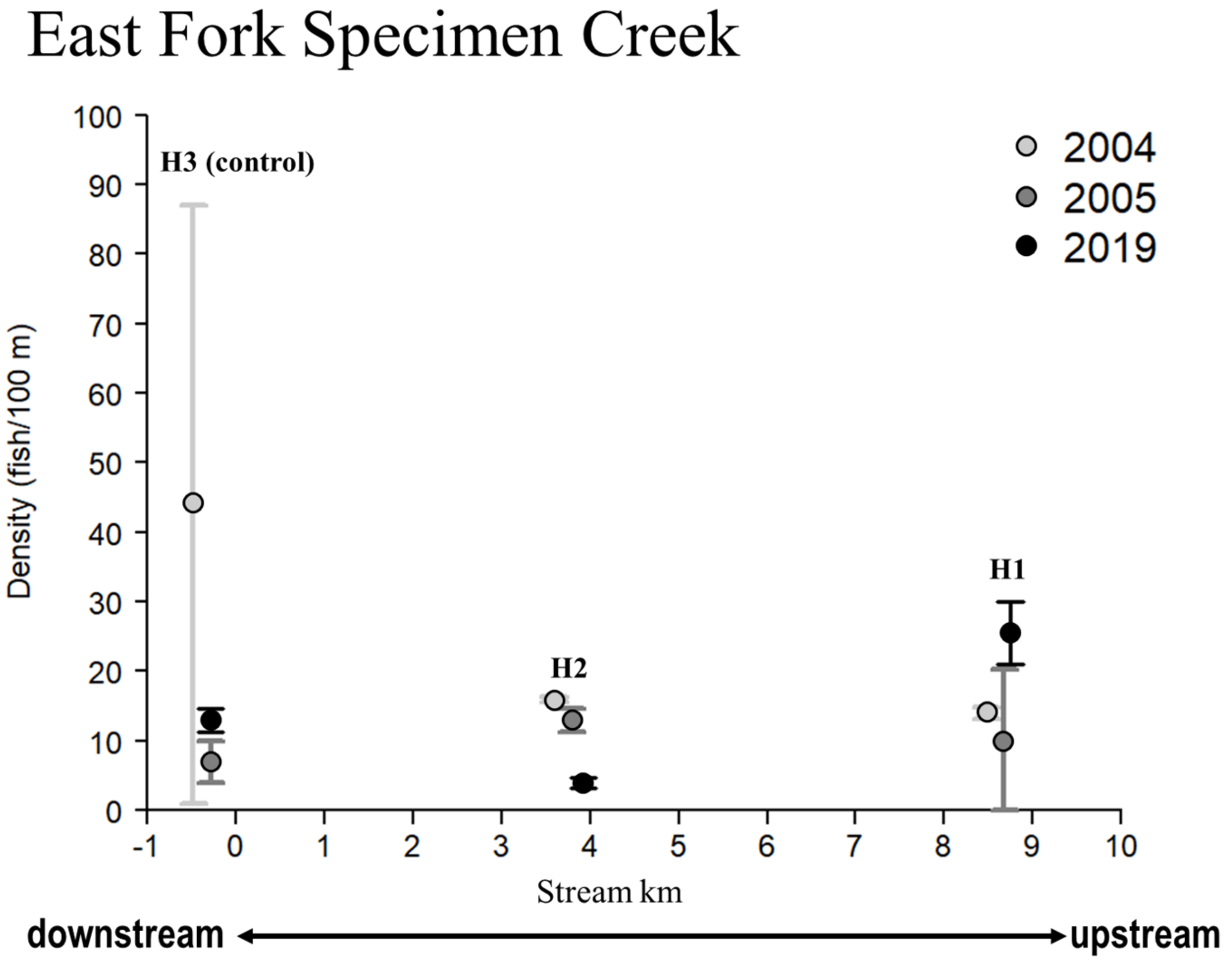


Figure 12. Estimated densities (fish/100 m) at pre- (2004 and 2005) and post-restoration (2019) sites in East Fork Specimen Creek. Sites H1 and H2 were within the restoration area whereas Site H3 was downstream of the restoration area. Bars represent 95% confidence intervals. The error associated with the 2004 pre-restoration density estimate at Site H3 was too large to make valid comparisons.

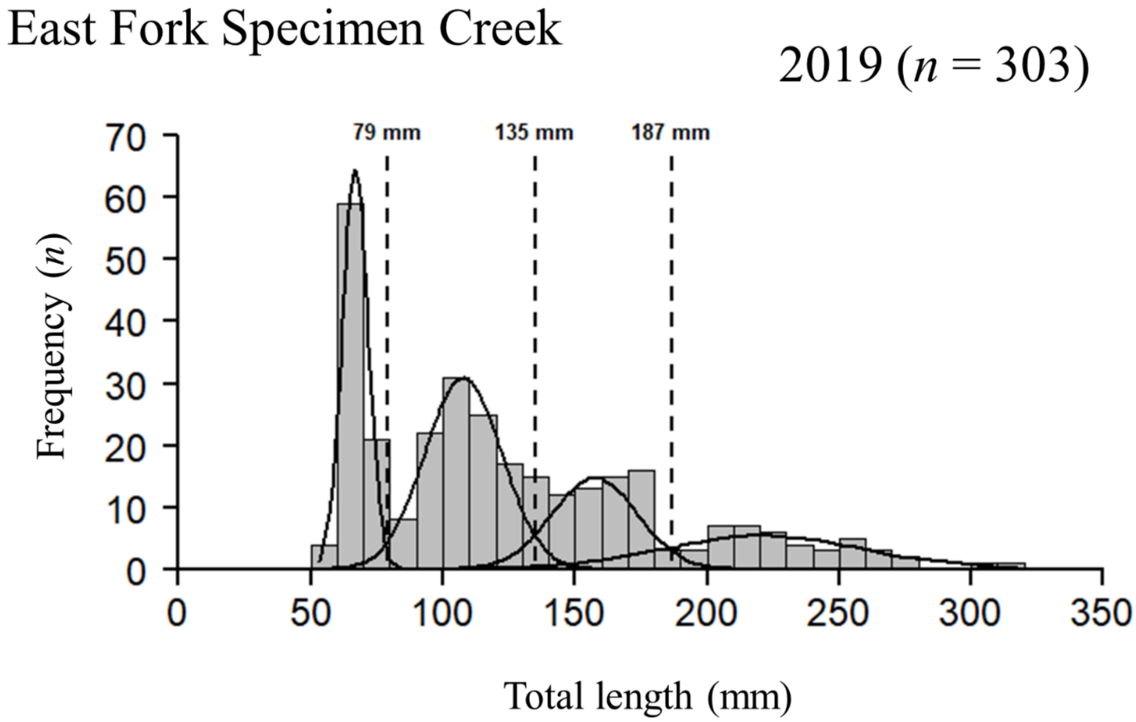


Figure 13. Age groups defined by Gaussian curves fit to a total length-frequency distribution (10-mm bins) of Westslope Cutthroat Trout captured in East Fork Specimen Creek in 2019.

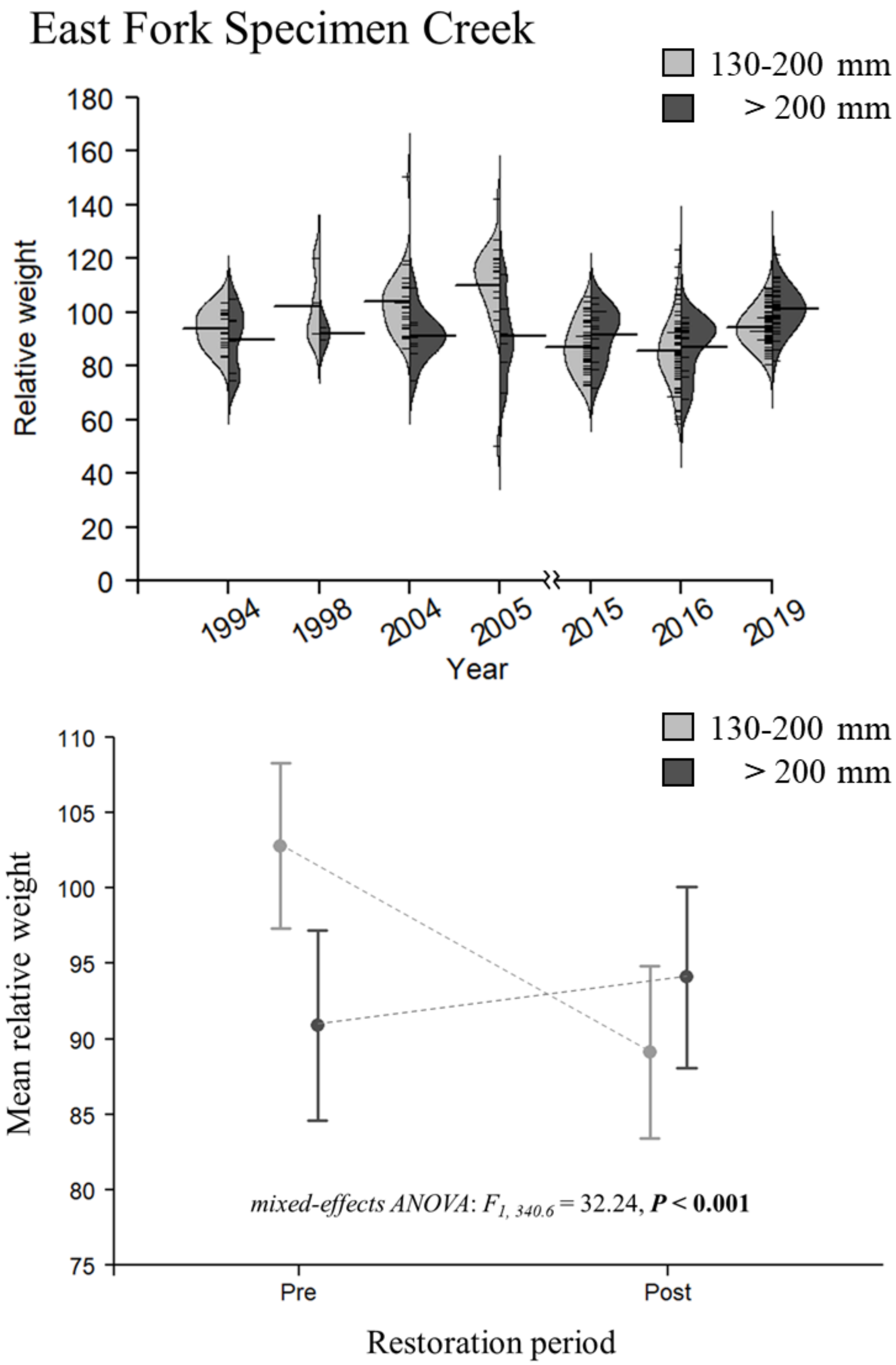


Figure 14. Relative weights of individual Westslope Cutthroat Trout in East Fork Specimen Creek in pre- (1994, 1998, 2004, and 2005) and post-restoration (2015, 2016, and 2019) years (top) and mean relative weights and 95% confidence intervals (bars) of pre- and post-restoration WCT populations, after accounting for year as a random effect and total-length category (130 – 200 mm or > 200 mm) as a fixed effect (mixed-effects ANOVA model; bottom).

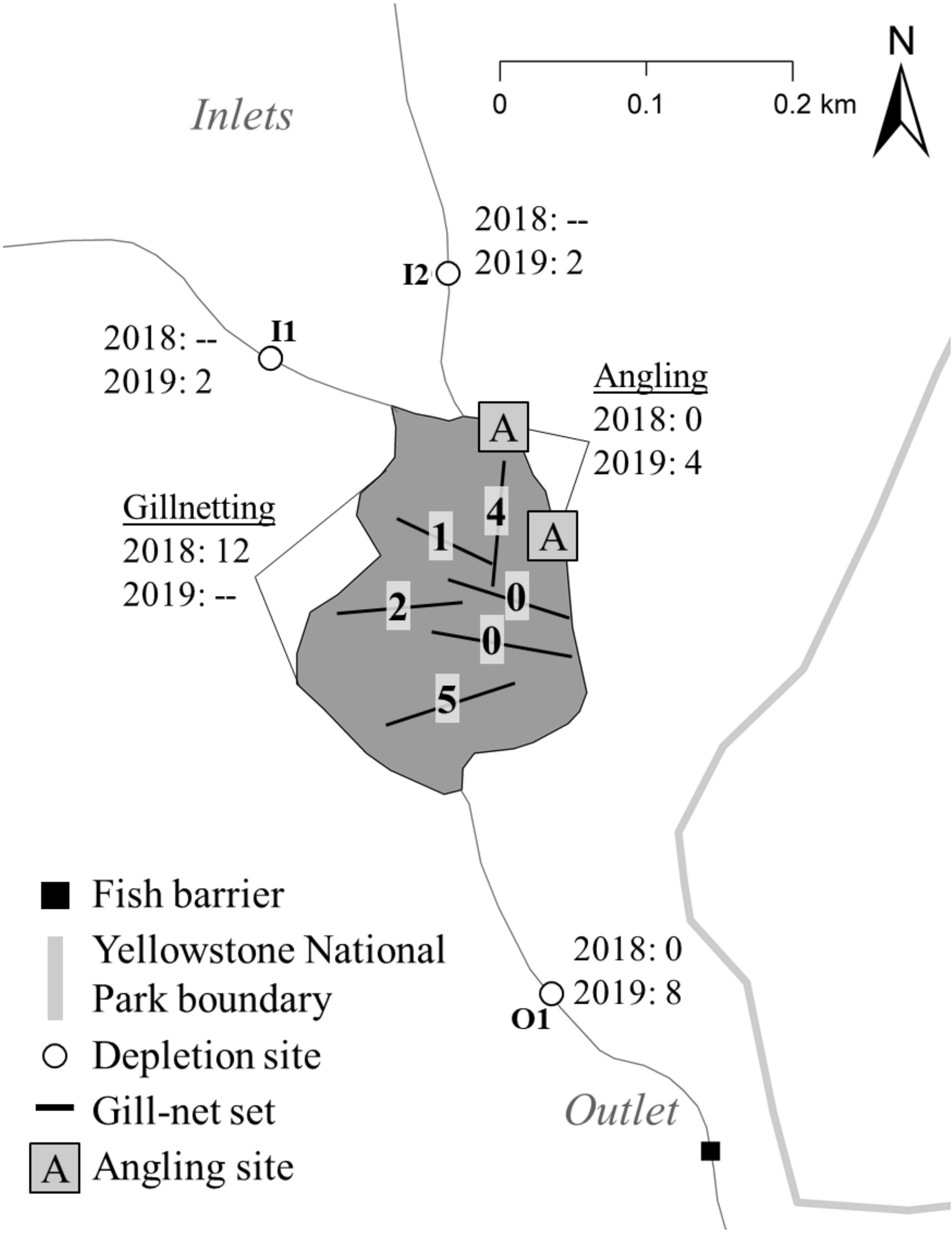


Figure 15. Capture locations of Westslope Cutthroat Trout (WCT) in High Lake in 2018 and 2019 during gillnetting, multi-pass-depletion sampling, and angling. Gill-net sets are represented by black lines (*n* = 6), multi-pass-depletion sites by circles (*n* = 3), and angling sites by the squares marked “A” (*n* = 2). Numbers of WCT captured are shown next to each sample location in each year.

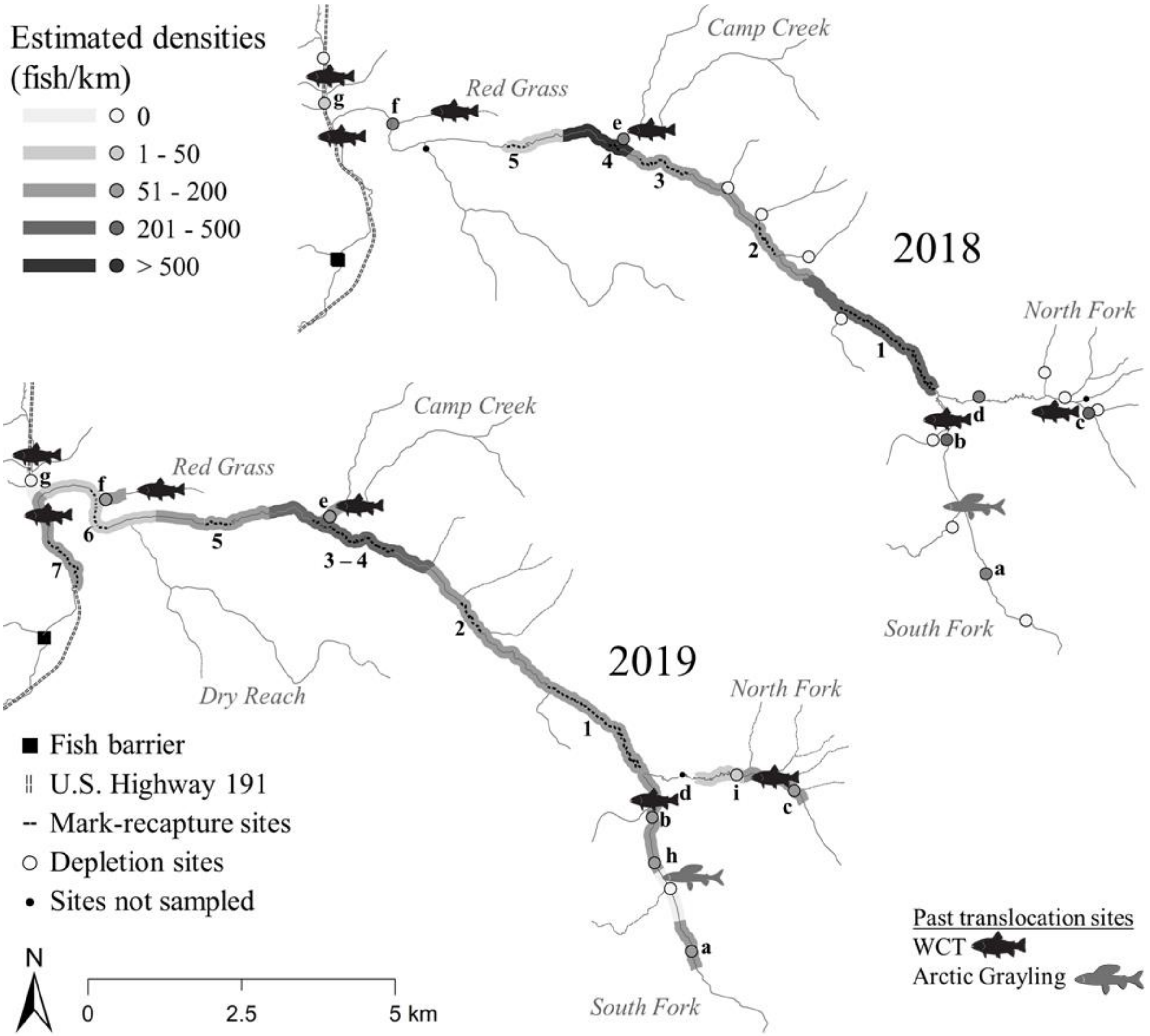


Figure 16. Extrapolated estimated densities (fish/km) of Westslope Cutthroat Trout in Grayling Creek in 2018 (top) and 2019 (bottom). Main-stem mark-recapture sections are represented by dashed lines (n = 5, 2018; n = 7, 2019) and tributary multi-pass-depletion sites are represented by circles (n = 20, 2018; n = 10, 2019). Tributary sites where no fish were captured in the first electrofishing pass (n = 11, 2018; n = 2, 2019) were assumed to have a density of zero fish/km. Sites where water was too low (n = 2, 2018) or too high (n = 1, 2019) to effectively sample are represented by small black circles. Estimated densities are represented by shades of gray. Densities in the main stem were extrapolated half the distance downstream or upstream to the next-nearest section. Densities in tributaries in 2019 were extrapolated to the entire stratum that the random site was chosen from. Tributary abundance estimates were not extrapolated in 2018 because estimates were associated with large error. Estimated abundances and their associated 95% confidence intervals for each sampled unit are shown in Tables 6 and 7. The Lincoln-Petersen bias-adjusted estimator was used to calculate abundance for main-stem sections and the Carle-Strub estimator with a quasi-Poisson generalized linear model was used for tributary sites; 99% confidence intervals instead of 95% prediction intervals were used for Carle-Strub one-pass tributary sites in 2019 (all sites except Site c).

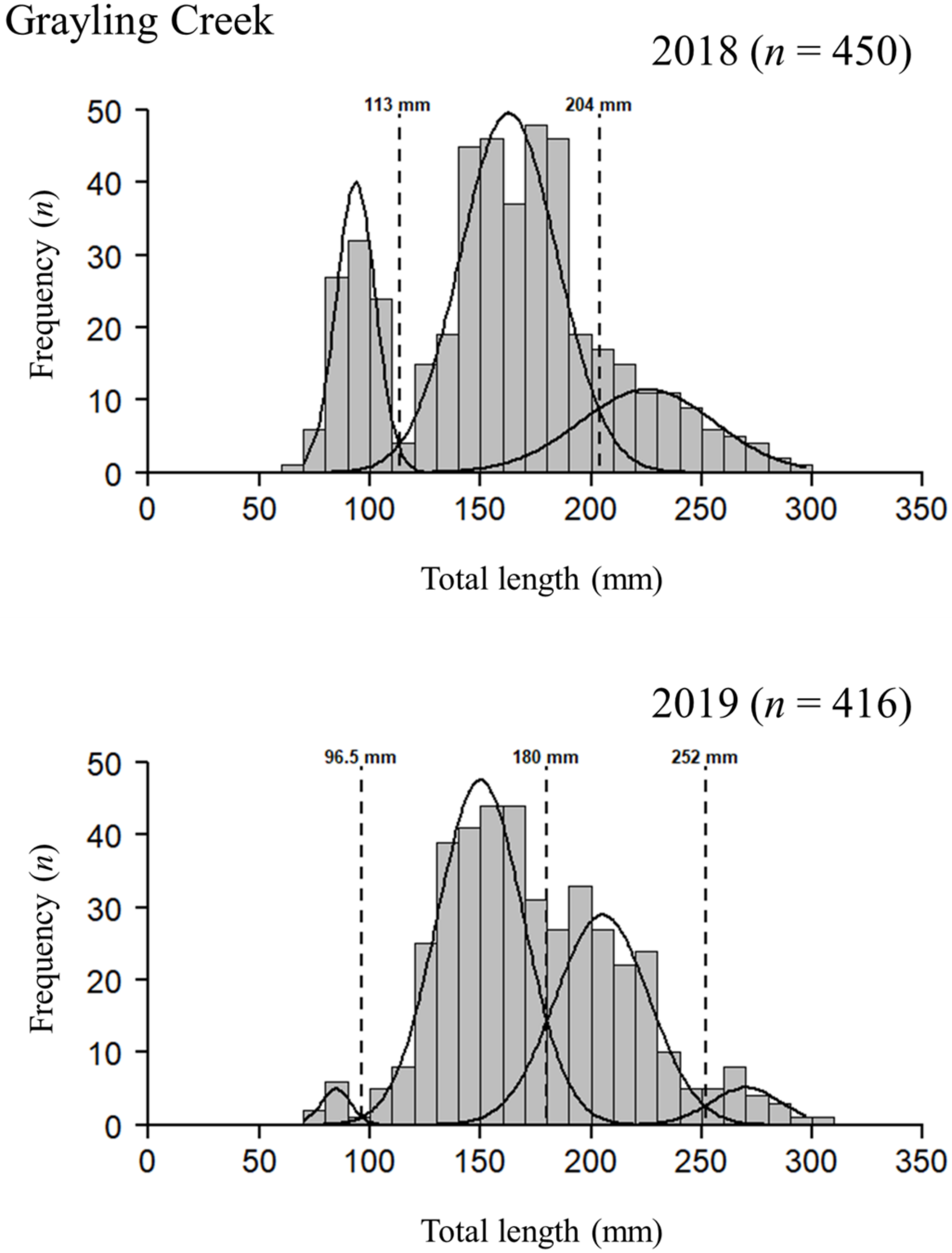


Figure 17. Age groups defined by Gaussian curves fit to total length-frequency distributions (10-mm bins) of Westslope Cutthroat Trout captured in Grayling Creek in 2018 (top) and 2019 (bottom).

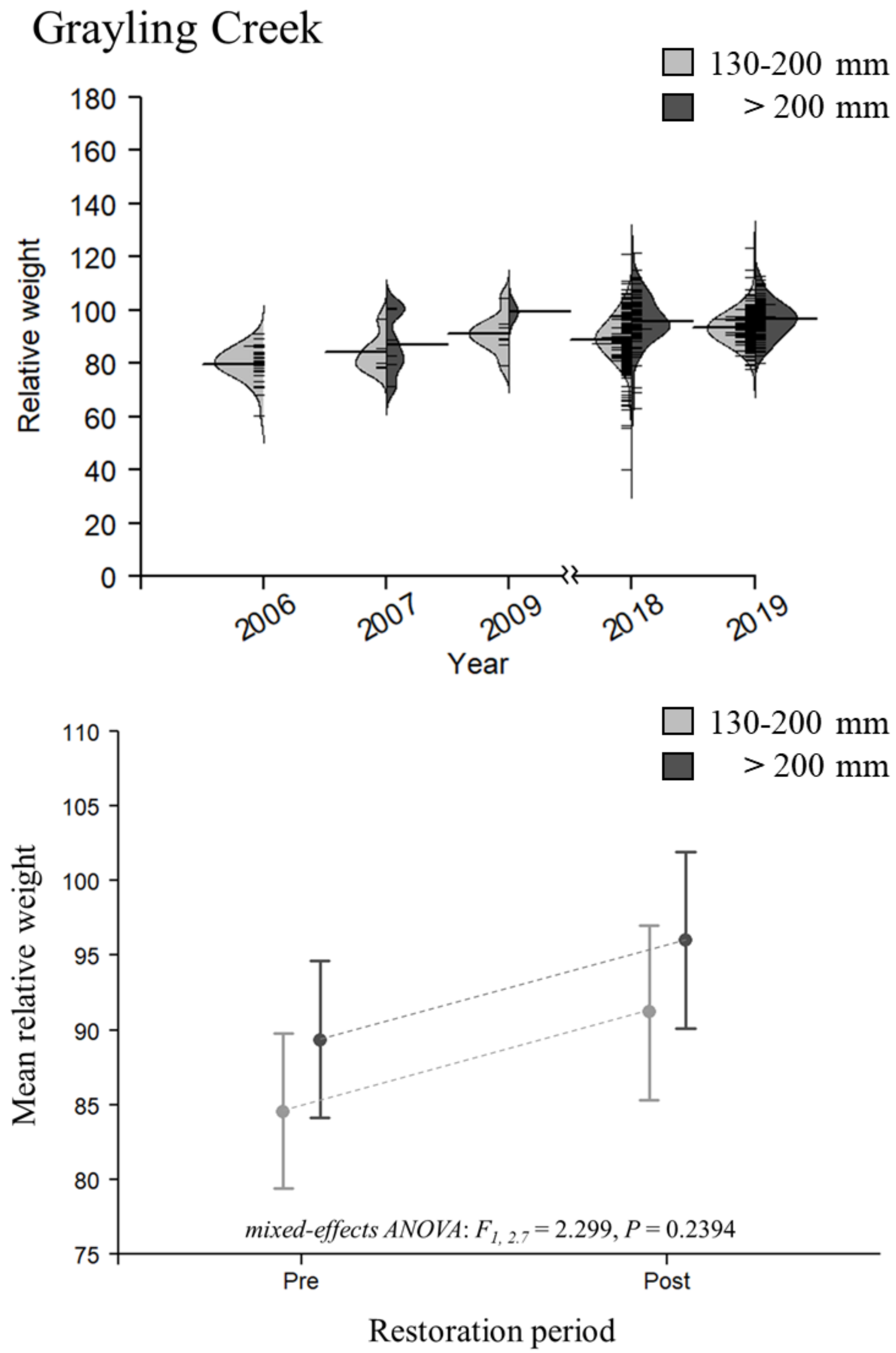


Figure 18. Relative weights of individual Westslope Cutthroat Trout (WCT) in Grayling Creek in pre- (2006, 2007, and 2009) and post-restoration (2018 and 2019) years (top) and mean relative weights and 95% confidence intervals (bars) of pre- and post-restoration WCT populations, after accounting for year as a random effect and total-length category (130 – 200 mm or > 200 mm) as a fixed effect (mixed-effects ANOVA model; bottom).

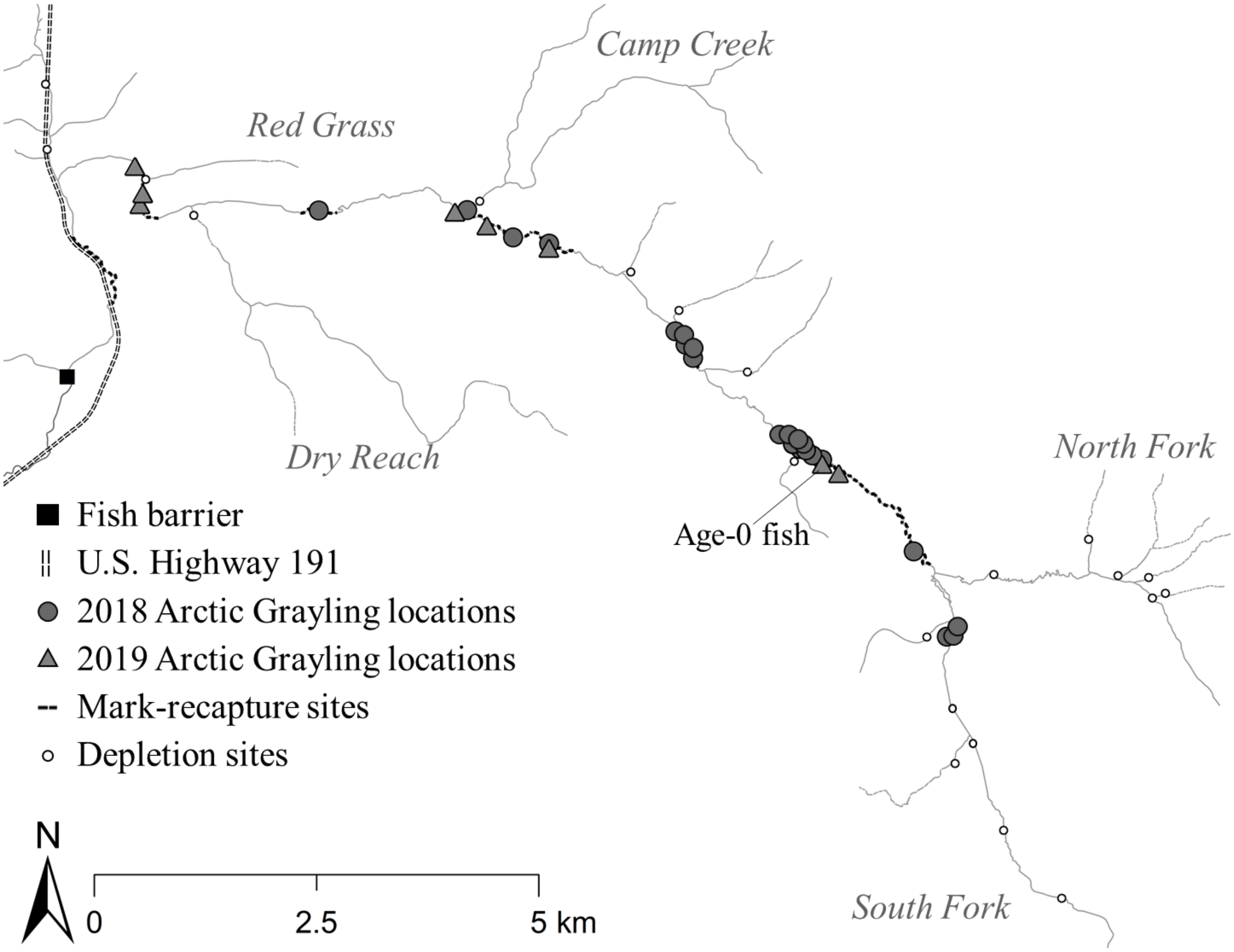


Figure 19. Capture locations of Arctic Grayling in Grayling Creek in 2018 (circles, *n* = 22) and 2019 (triangles, *n* = 8). A group of age-0 fish was observed in Grayling Creek in 2019; one specimen was preserved and later identified as an Arctic Grayling through genetic analysis providing the first documentation of natural reproduction of fluvial Arctic Grayling in Grayling Creek in decades.

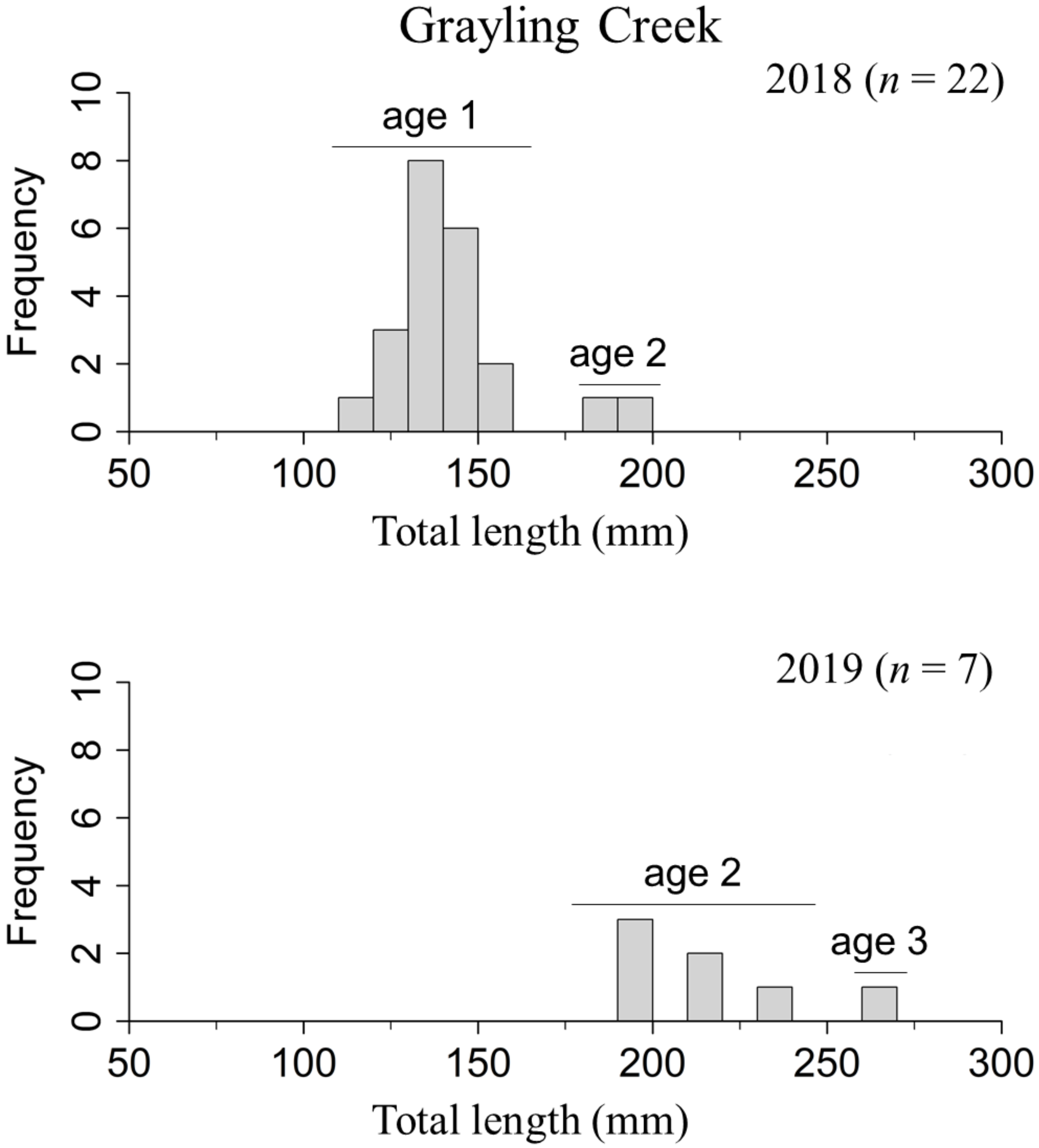
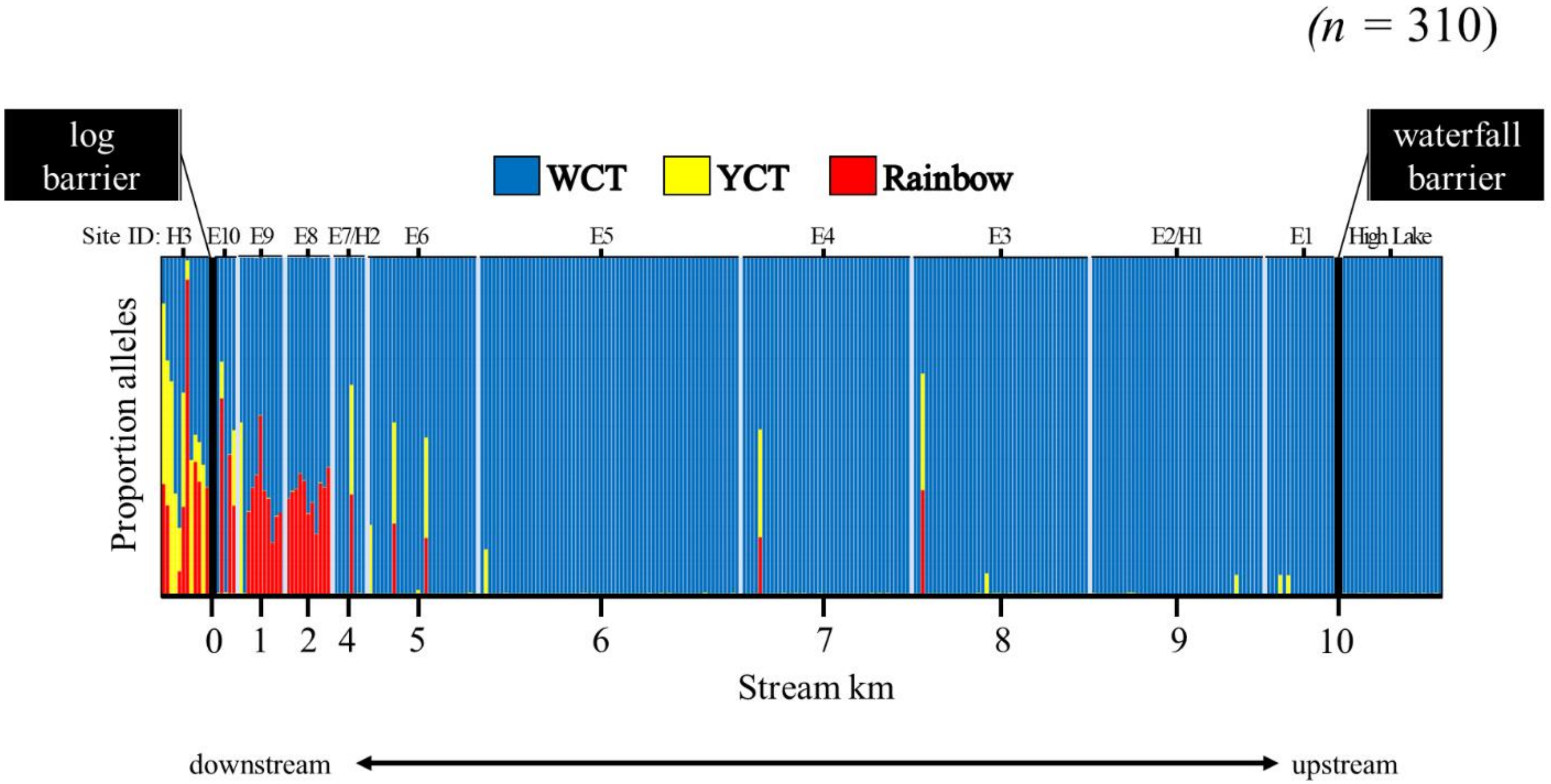


Figure 20. Total length-frequency distributions (10-mm bins) and estimated ages of Arctic Grayling captured in Grayling Creek in 2018 (top) and 2019 (bottom). Age-1 fish in 2018 and age-2 fish in 2019 were assumed to have recruited from the fry translocations made in 2017. Age-2 fish in 2018 and age-3 fish in 2019 were assumed to have recruited from the remote-site incubator translocations made in 2016. No fish thought to have recruited from the remote-site incubator translocations in 2015 were captured.



Structure analysis, *k* = 3
Campbell 2019

Figure 21. Summary of percent admixture of Westslope Cutthroat Trout (WCT), Yellowstone Cutthroat Trout (YCT), and Rainbow Trout alleles of fish captured in East Fork Specimen Creek and High Lake in 2018 and 2019. About 11.5% of fish were identified as hybrids in 2019 within the restoration area above the log barrier.

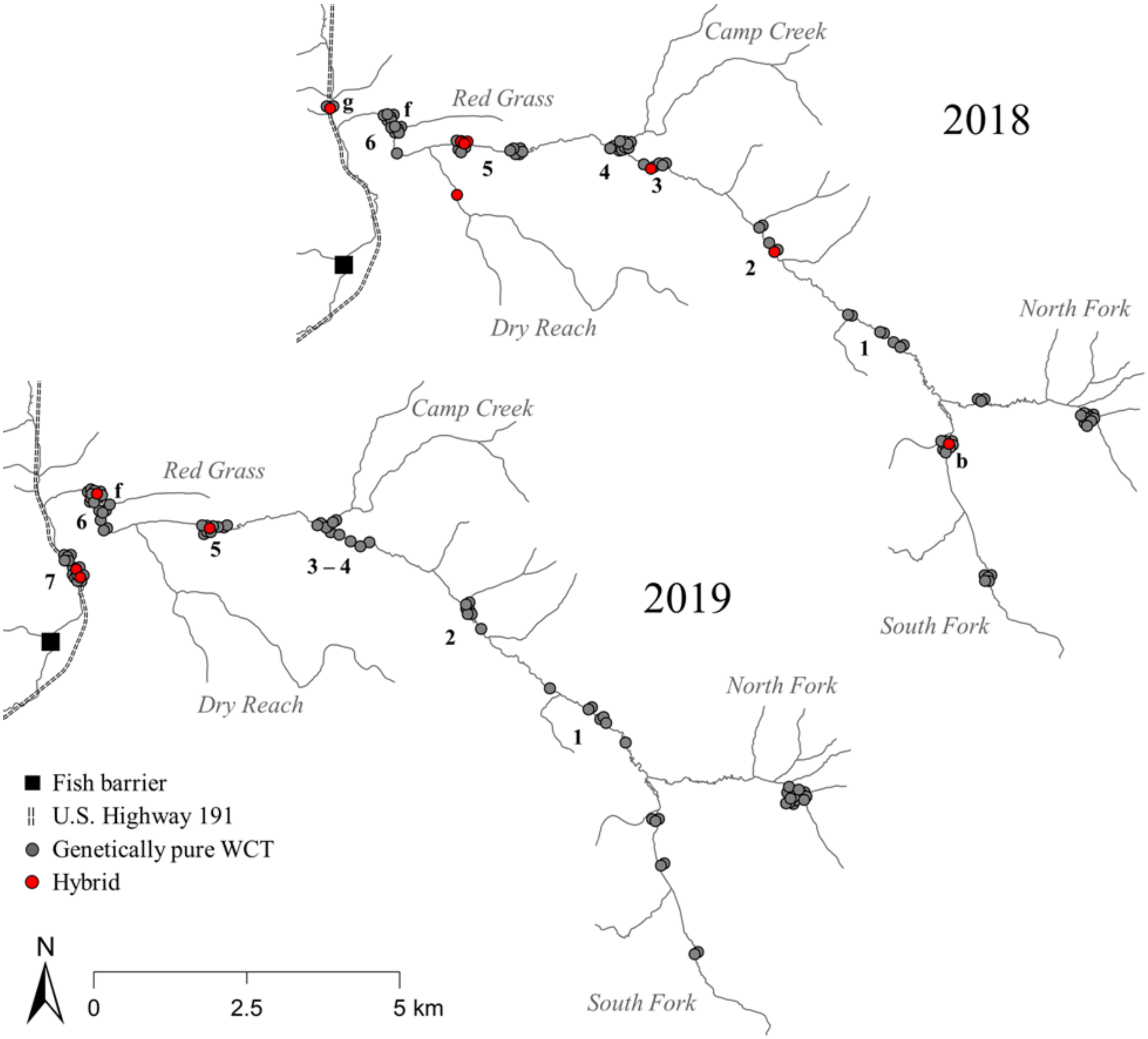


Figure 22. Capture locations of hybridized Westslope Cutthroat Trout in Grayling Creek in 2018 (top; *n* = 8) and 2019 (bottom; *n* = 4) represented by red circles. Gray circles represent locations of genetically pure WCT that were successfully genotyped. About 7.8% and 4.2% of fish were identified as hybrids in 2018 and 2019, respectively. The hybrid located in the Dry Reach tributary in 2018 was captured during preliminary sampling by the National Park Service whereas the others were identified during our study.

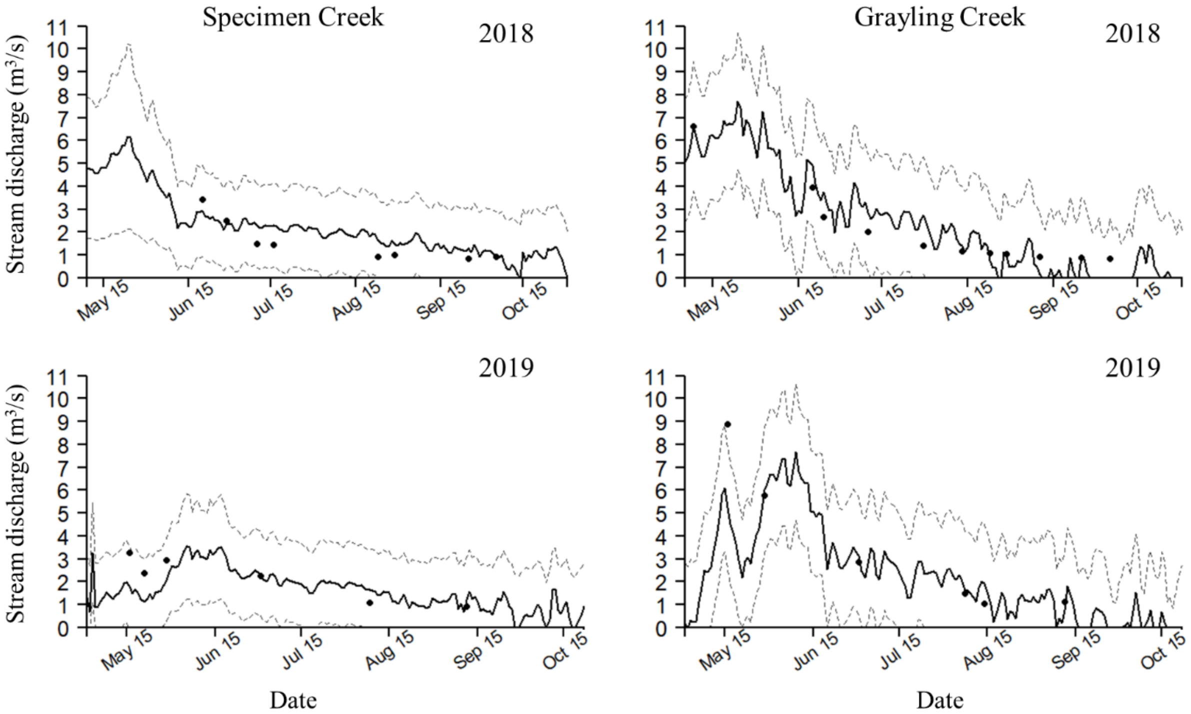


Figure 23. Estimated daily mean stream discharges (m^3/s; black lines) and 95% confidence intervals (gray lines) in Specimen (left) and Grayling (right) creeks in 2018 (top) and 2019 (bottom). Simple linear regression of stream discharge measurements (black dots) and mean daily water pressure (kPa) was used to predict stream discharge on days when measurements were not collected. Because stream discharge was measured at a site in Specimen Creek proper (downstream of the East Fork Specimen Creek restoration area), measurements are higher than what they would have been for only East Fork Specimen Creek. Stream discharge was measured at a site that was within the Grayling Creek restoration area.

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