DEVELOPMENT OF A STANDARDIZED MONITORING PROTOCOL TO ASSESS THE EFFICACY OF NONNATIVE FISH SUPPRESSION IN THE LAMAR RIVER WATERSHED, YELLOWSTONE NATIONAL PARK

by

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ABSTRACT

Hybridization between native Cutthroat Trout and introduced Rainbow Trout is pervasive throughout western North America, and this hybridization has resulted in reduced abundances and range contractions of native Cutthroat Trout subspecies. In the Lamar River watershed in Yellowstone National Park, Yellowstone Cutthroat Trout × Rainbow Trout hybrids are abundant in the lower Lamar River watershed because of past stocking efforts. To mitigate the threat of hybridization in the Lamar River watershed, the National Park Service has acted to remove Rainbow Trout and hybrids and block the upstream movement of these nonnative taxa into the upper watershed. A standardized monitoring protocol is desired to assess the response of fish populations to these management actions and to monitor existing populations of Yellowstone Rocky Mountain Cutthroat Trout. We evaluated the efficacy of electrofishing, snorkeling, and angling to estimate the absolute abundances and catch-per-unit-effort of mixed-stock aggregations of trout in main-stem locations. We also evaluated the efficacy of targeted, juvenile sampling to estimate the abundances of juvenile trout of each taxon. We also assessed the accuracy and limitations of a field-identification key developed for differentiating among Yellowstone Rocky Mountain Cutthroat Trout, Rainbow Trout, and Yellowstone Rocky Mountain Cutthroat × Rainbow Trout hybrids in the Lamar River watershed. We observed agreement between the proportion of nonnative trout sampled by electrofishing and snorkeling in Slough Creek. Catch-per-unit effort estimates were highly variable for Yellowstone Rocky Mountain Cutthroat and nonnative trout, but error was reduced when minimum section lengths of 3,600 m in Slough Creek and 2,200 m in the Lamar River were sampled. In both streams, error in mark-recapture estimates was reduced when angling was incorporated. Statistical power to detect declines in Yellowstone Cutthroat and nonnative trout abundance was low for CPUE and mark-recapture surveys unless 50% declines occurred for both taxa. Electrofishing surveys are not feasible for estimating the abundance of juvenile trout because of high error in taxa identification (30% correct-identification rate). However, we differentiated among adult fish with high rates of accuracy (98%). These results will guide future long-term monitoring of trout populations in the Lamar River watershed.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Freshwater Biological Invasions

Human-facilitated biological invasions are prevalent worldwide (Vitousek et al. 1997) and have resulted in novel species assemblages with reduced numbers of native species (Strayer 2010). Fresh water composes only 0.8% of the surface of the Earth, yet almost 6% of all described species are supported by freshwater ecosystems (Dudgeon et al. 2006). Anthropogenic activities have reduced freshwater biodiversity at a rapid rate (Albert et al. 2021), with the intentional and unintentional introduction of nonnative fishes as a significant contributing factor (Gozlan et al. 2010). Intentional introductions are often intended to enhance and diversify recreational angling opportunities (Pister 2001), create or improve aquaculture operations (Kerr et al. 2005), control undesired species by biological control (Kumar and Hwang 2006), or are the result of aquarium releases (Strecker et al. 2011). Unintentional introductions are often the result of aquaculture or baitfish escapement (Ludwig and Leitch 1996; Glover et al. 2017) and are facilitated by human transportation and the construction of commercial waterways (Jacobs and Keller 2017). Despite the motives (or lack thereof) for nonnative fish introductions, these actions can result in the establishment of self-sustaining, invasive populations. Established, invasive populations can drastically change aquatic biological communities by direct and indirect means, causing damage to ecosystems and economies (Koel et al. 2005; Pimentel et al. 2005). These introductions, along with other stressors including habitat degradation and fragmentation, climate change, and exploitation, have contributed to the decline of native aquatic species and subsequent homogenization of aquatic species assemblages across global, aquatic landscapes (Rahel 2000; Dudgeon et al. 2006).

Management of Nonnative Fish Populations

Fisheries managers have progressively acknowledged the ecological value of preserving native fishes (Rahel 1997), and many state and federal fish and wildlife programs are tasked with preserving their long-term persistence. Common tools for managing nonnative fishes include isolation, containment, eradication, and suppression of established populations (Rahel and Smith 2018). Isolation is often achieved by constructing artificial barriers to prevent the movement of nonnative fish from nearby source populations (Thompson and Rahel 1998). Similarly, containment of nonnative populations often includes exclusion by barrier construction or other means of deterring movement (Clarkson 2004). Eradication is typically achieved using chemical piscicides (antimycin and rotenone) and is often used in conjunction with isolation strategies (Buktenika et al. 2013). Eradication of nonnative fish populations is also possible by mechanical means such as repeated electrofishing or gillnetting in small lakes or streams with limited habitat complexity (Knapp and Matthews 1998; Pacas and Taylor 2015); however, established populations are notoriously difficult to eradicate in large lakes and interconnected, fluvial systems (Simberloff 2014; Shepard et al. 2014). Mechanical reduction is an alternative strategy when eradication is too costly or improbable because of the complexity or size of the system or its proximity to other nonnative source populations (Koel et al. 2020). These reduction, eradication, and isolation strategies are commonly used in the western United States, where native Cutthroat Trout *Oncorhynchus clarkii* subspecies are threatened by predation, displacement, competition, and hybridization with nonnative fishes (Al-Chokhachy et al. 2014; Meyer et al. 2017; Kovach et al. 2018).

Rainbow Trout

Rainbow Trout *O. mykiss* have been stocked globally for recreational purposes since the late 1800s and are one of the most widely introduced fishes in the world (Halverson 2010). Rainbow Trout are popular sport fish, but their introductions adversely affect native, freshwater species. For example, introduced Rainbow Trout displace and alter the distributions of amphibians in ponds and lakes (Hecnar and M'Closkey 1997; Knapp and Matthews 2000) and prey upon, compete with, and displace native fishes (McIntosh 2000; Seiler and Keeley 2009; Morita 2018). Rainbow Trout threaten the long-term persistence of native Westslope Cutthroat Trout *O. lewisi* and Yellowstone Rocky Mountain Cutthroat Trout *O. virginalis* by hybridization in the western United States (Kruse et al. 2000; Peacock and Kirchoff 2004; Gresswell 2011). Cutthroat and Rainbow Trout are closely related and often exhibit spatial and temporal reproductive overlap (Muhlfeld et al. 2009b) facilitating hybridization between the two taxa. This reproductive sympatry has resulted in the loss of locally adapted gene complexes and genetic diversity (Kovach et al. 2015), reduced fitness (Muhlfeld et al. 2009a), altered life-history expression and growth rates (Strait et al. 2020; Bourett et al. 2022), and in some cases, the genomic extinction of native Cutthroat Trout subspecies (Allendorf and Leary 1988).

Yellowstone Rocky Mountain Cutthroat Trout

The Yellowstone Rocky Mountain Cutthroat Trout (hereafter Yellowstone Cutthroat Trout), a subspecies of Rocky Mountain Cutthroat Trout native to the Intermountain West, historically occupied the upper Snake and Yellowstone River watersheds (Behnke 2002). The Yellowstone Cutthroat Trout has been widely stocked outside of its native range for recreational purposes; however, habitat fragmentation and degradation, climate change, exploitation, and invasive species introductions (including hybridization; Gresswell 2011) have contributed to the reduced abundance and distribution of the subspecies. The subspecies currently occupies 43% of its native range (Endicott et al. 2016), with only 23% of the range occupied by non-hybridized populations (Endicott et al. 2016). Yellowstone National Park is at the center of the native range of the Yellowstone Cutthroat Trout and supports genetically unaltered and economically important populations of the subspecies (Al-Chokhachy et al. 2018). Despite the protected and relatively undisturbed status of aquatic habitats in this National Park, Yellowstone Cutthroat Trout populations are threatened by competition and displacement by nonnative Brook Trout *Salvelinus fontinalis* (Ertel et al. 2017), predation by nonnative Lake Trout *Salvelinus namaycush* (Ruzycki et al. 2003), and hybridization with nonnative Rainbow Trout (Heim et al. 2020b).

Lamar River Watershed

The Lamar River watershed was formerly a fluvial stronghold for genetically unaltered Yellowstone Cutthroat Trout. However, the National Park Service and other agencies intentionally stocked Rainbow Trout in the Lamar River watershed in the early 1900s to diversify sportfishing opportunities for visiting anglers (Varley 1981). Some of these Rainbow

Trout were stocked into the historically fishless headwaters of Buffalo Creek, a large tributary of Slough Creek in the Absaroka-Beartooth Wilderness of Montana. By the 1930s, the National Park Service shifted its focus from stocking nonnative fishes towards native fish conservation, adopting a policy "prohibiting the introduction of exotic species in national park or monument waters now containing only native species" (Madsen 1937). Although stocking of Rainbow Trout ceased nearly a century ago, legacy populations persist, especially in the headwater streams and lakes of the Buffalo Creek watershed. These fish continue to invade downstream reaches of Slough Creek and the Lamar River where they are hybridizing with native Yellowstone Cutthroat Trout. Rainbow Trout × Yellowstone Cutthroat Trout hybrids (hereafter "hybrids") are now abundant in the lower Lamar River watershed, and because of the seasonal, fluvial connectivity of the system, appear to be invading the upper watershed where Yellowstone Cutthroat Trout populations of high conservation priority are present (Ertel 2017; Al-Chokhachy et al. 2018; Heim 2019). In addition to its conservation value, the watershed is one of the most popular angling destinations in the park, with over 10,000 anglers from around the world visiting the watershed annually to catch Yellowstone Cutthroat Trout (Heim et al. 2020a).

The National Park Service is attempting to curtail the spread of invasive Rainbow Trout in the lower Lamar River watershed to slow the rate of hybridization in the upper watershed (Ertel et al. 2017). It has conducted single- and multiple-pass electrofishing to selectively remove Rainbow Trout and hybrids from the middle Lamar River and upper Slough Creek since 2013. Mandatory-kill angling regulations for Rainbow Trout and hybrids were instituted in 2014. A barrier was also constructed in lower Slough Creek in 2017 to slow upstream movement by Rainbow Trout and protect Yellowstone Cutthroat Trout populations in the three upper meadows

(Ertel et al. 2017). However, an overflow channel near the barrier reopened in a 2020 flood event; [K. Wellstone, personal observation]; the effects of this failure in facilitating further invasion by Rainbow Trout is unknown. Buffalo Creek was recently identified as the primary source of Rainbow Trout invading the Lamar River watershed (Heim et al. 2020b). The National Park Service and partner agencies plan to eradicate the Rainbow Trout population from Buffalo Creek in 2024 and 2025 using rotenone—a chemical piscicide. Following the rotenone treatment, genetically unaltered Yellowstone Cutthroat Trout from nearby fluvial populations are to be stocked in Buffalo Creek.

Fish suppression and eradication efforts require quantitative measures of native and invasive fish population abundance over time to assess their efficacy (Syslo et al. 2016; Budy et al. 2020; Koel et al. 2020) but pilot studies may be required to assess the precision and bias of such measures in the absence of prior monitoring. Additionally, evaluating tradeoffs in resource expenditures versus precision and bias is necessary to inform feasible, long-term monitoring (Quist et al. 2006; Al-Chokhachy et al. 2009; Dauwalter et al. 2010).

Project Goal

The National Park Service desires a strategy for monitoring the success of nonnative Rainbow Trout and hybrid control actions in the Lamar River watershed. Specifically, the National Park Service desires a monitoring program that can detect whether their management actions will have reduced the abundance of Rainbow and hybrid trout, or increased the abundance of native Yellowstone Cutthroat Trout, in the lower Lamar River and Slough Creek. Heretofore, the study area had not been effectively sampled in a standardized way (NPS, unpublished data); therefore, our goal was to determine the sampling gears, methods, and amount of effort needed to estimate the abundances of Yellowstone Cutthroat, Rainbow, and hybrid trout in the lower Lamar River watershed to be able to adequately detect changes in abundance resulting from the suppression actions. In chapter 2, we describe our evaluation of the efficacy of electrofishing, snorkeling, and angling to estimate the absolute abundances and catch-per-unit-effort of mixed-stock aggregations of trout in main-stem locations. We also evaluate the efficacy of targeted, juvenile sampling to estimate the abundances of juvenile trout of each taxon. In Chapter 3, we describe our evaluation of a field-identification key specifically designed for the Lamar River by determining the rate of correctly identifying trout taxa. In chapter 4, we make recommendations to aid National Park Service monitoring and management efforts in the watershed.

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CHAPTER TWO

EVALUATING TROUT POPULATION MONITORING STRATEGIES IN A HIGH-ELEVATION, MEDIUM-SIZED RIVER SYSTEM: IMPLICATIONS FOR DETECTING TRENDS IN TROUT ABUNDANCE

Introduction

Monitoring

Monitoring is an essential component of fisheries management. Fisheries biologists often implement monitoring programs to estimate trends in the abundances and distributions of fish species of interest (Pope et al. 2010). Repeated measures of these estimates across time, space, or both can be compared to determine whether management actions such as habitat restoration, changes to harvest regulations, and invasive species suppression or eradication are having the desired effect on the population(s) of interest (Noble et al. 2007). Assessing the effectiveness of invasive species suppression and eradication efforts usually requires quantification of native and invasive fish abundances, or indices of abundances, over time (Syslo et al. 2016; Budy et al. 2020; Koel et al. 2020) and associated precision, bias, and statistical power to detect trends (Quist et al. 2006; Al-Chokhachy et al. 2009; Dauwalter et al. 2010; Dzul et al. 2013).

Components of Monitoring

Statistical power ($1-\beta$) is defined as the probability of correctly rejecting a null hypothesis; that is, the probability of rejecting the null hypothesis when it is truly false (probability of not committing a type-II error; β = type-II error). In the context of population monitoring, statistical power determines the probability of the study design to detect changes in the population when they occur (Wagner et al. 2013). Components that affect statistical power include sampling design, effect size, sample size, and precision (Brown and Guy 2007). Sampling design is the structure of how the samples are collected, effect size is the desirable level of change between response or among response variables, sample size is the number of sampling units (e.g., sites or individuals) included in the study, and precision is the level of variability observed (i.e., the level of agreement among repeated samples) in the estimates (Gotelli and Ellison 2013).

Natural demographic fluctuations of fish populations, sampling error, and sampling variation influence precision in a data set (Allen and Hightower 2010). Sampling error is often influenced by gear efficiency, crew experience, and the abundance estimator used in the study (Hangsleben et al. 2012; Gibson-Reinemer et al. 2016). Sampling variation is influenced by spatial and temporal heterogeneity in sampling units (multiple sites) or temporal heterogeneity in the sampling unit (single site). Among multiple sites, variation in parameter estimates (e.g., catch rates) and the abundance estimator used may lead to high variability across sites, leading to low precision (Hangsleben et al. 2012). At a single site, temporal heterogeneity in catch rates and the variance of the abundance estimator used may affect precision similarly (Dauwalter et al. 2009). Sampling error often masks fluctuations in fish populations, further confounding the difficulty and cost of detecting trends in population parameters (Ham and Pearsons 2000).

Abundance Estimation Methods

Estimating abundance is often a primary objective for management agencies and researchers because abundance reveals the outcome of dynamic rate functions such as mortality and recruitment (Pope et al. 2010). Mark recapture is a commonly applied method for estimating the absolute abundances of fish populations (Otis et al. 1978). However, this estimator requires repeat sampling events to achieve precise estimates and is therefore often costly (Russell et al. 2012). Catch-per-unit-effort (CPUE) is an index of abundance commonly used to monitor fish populations and assemblages. Indices typically require less effort than absolute abundance estimates, allowing for increased replication and expansion of the geographic scope of the study. For example, reducing effort by using indices can be a cost-effective way of sampling more sites to reduce the variance of estimates for more effective trend detection (Mitro and Zale 2000; Hedger et al. 2013; Hanks et al. 2018). However, indices of abundance are inherently biased, because they do not allow for the estimation of capture probability. Consequently, the use of abundance indices assumes that changes in CPUE are proportional to changes in the absolute abundance of the population of interest (Hubert and Fabrizio 2007). Capture probabilities often change depending on the field conditions, sampling gears used, and personnel experience (Thompson and Rahel 1996; Rosenberger and Dunham 2005; Simonson et al. 2022); therefore, efforts to standardize sampling such as sampling during the same field conditions, using the same gears, and providing proper personnel training are essential to drawing the correct inference when using indices of abundance (Curry et al. 2009). Inland stream salmonid monitoring programs typically standardize sampling efforts by conducting surveys during base-discharge levels (spring, summer, and autumn, depending on the objective, focal species, or flow regime) at the same time each year to avoid changes in environmental conditions and fish behavior. These fisheries are often sampled at either a single site or a network of sites to estimate the absolute abundance or CPUE of the salmonid populations present. Estimation at a single site may be used when the site is representative of the area of interest and is often favored when the location of the site is cost-effective and feasible given the access or conditions of the reach. Multiple sites are often sampled using a probabilistic approach such as random or systematic sampling when managers want to infer results to a large area of stream or watershed (Larson et al. 2001).

Life-Stage Monitoring

Monitoring programs often focus on adult fish abundance or CPUE in mixed-stock aggregations of main-stem rivers to make inferences about the status or trend of a fishery. However, assessing multiple life stages of fish populations can elucidate more detailed information on population dynamics. The abundance of age-0 (hereafter "juvenile") salmonids is a measure of the number of individuals successfully hatched each year and can be used to determine spawning success, predict future recruitment to a population, and evaluate the population status of species of interest (Allen and Hightower 2010). This can be particularly important when applied concurrently with nonnative species suppression programs because it allows agencies and researchers to make early predictions about the success of their management efforts (Caudron and Champigneulle 2010; Kanno et al. 2016). Furthermore, in salmonid metapopulations, juveniles often represent distinct populations rather than mixed-stock aggregations, allowing for more targeted population assessments (Cegelski et al. 2006; Uthe et al. 2016). However, estimating juvenile abundance is often difficult because of low capture probabilities and patchy distribution of juvenile fish in river systems (Korman et al. 2010). Estimating juvenile salmonid abundance in medium and large rivers (> 6 m wide) confounds these difficulties because of the ineffectiveness of traditional sampling methods for sampling juvenile fish (Hayes and Baird 1994; Hitt et al. 2020). Targeted juvenile sampling may be an alternative or supplemental option to monitoring adult abundance to expand the scope of inference of a monitoring program, if feasible.

Sampling Gear

Conducting a pilot study to identify locations where certain gears can be used for abundance and CPUE estimation is an essential step in the monitoring process (Noble et al. 2007). Stream-sampling gears are often limited by water depth, velocity, and accessibility (Bonar et al. 2009). In systems that are difficult or costly to access with certain sampling gears, the evaluation of methods for sampling fish is particularly important for developing effective monitoring programs (Korman et al. 2010; Neebling and Quist 2011; Pregler et al. 2015).

Electrofishing, angling, and snorkeling are commonly used for describing the distribution and abundances of salmonids in clear, coldwater streams in the Intermountain West (Thurow et al. 2006; O'Neal 2007; Meyer et. Al. 2022); however, the applicability and effectiveness of these gears may only be feasible in certain locations. Multiple gears are often used concurrently to sample fish populations in large river systems where conditions are not suitable to a single gear (Zajicek and Wolter 2018; Dunn and Paukert 2020). Angling has been used by agencies to estimate the abundance of fishes, particularly in locations where traditional data collection (e.g., electrofishing) is costly or not feasible (Zubik and Fraley 1988; Vidal et al. 2018), and citizen participation is often leveraged to reduce the cost of these efforts (Williams et al. 2015; Mycko et al. 2018; Oremland et al. 2022). A unique opportunity exists in Yellowstone National Park to leverage the support of volunteer anglers for structured data collection (Detjens et al. 2017). Therefore, incorporating the use of angling data for estimating abundance of Yellowstone Cutthroat, Rainbow, and hybrid trout in main-stem locations was an important aspect of our study.

Objective

The objective of our study was to determine a methodology and amount of effort needed to detect changes in the abundances of Yellowstone Rocky Mountain Cutthroat Trout *O. virginalis bouvieri* (hereafter Yellowstone Cutthroat Trout), Rainbow Trout *O. mykiss*, and Yellowstone Cutthroat × Rainbow Trout hybrids (hereby “hybrids”) in the lower Lamar River watershed. We started by evaluating the use of snorkeling and targeted juvenile electrofishing as potential monitoring methods. Next, we compared snorkeling and electrofishing estimates from paired sampling efforts in locations where snorkeling was feasible. Finally, we evaluated the precision of sampling designs implemented with electrofishing and angling gear for estimating the CPUE and absolute abundance of trout taxa in Slough Creek and the Lamar River and estimated the amount of effort needed to detect declines in the abundance of the taxa with sufficient statistical power.

Methods

Study Area

The Lamar River watershed in Yellowstone National Park originates in the Absaroka-Beartooth Mountains and has a drainage basin of 1,731 km^2. The Lamar River flows for 78 km from its headwaters before reaching its confluence with the Yellowstone River. Most of the watershed is federally protected by the National Park Service and U.S. Forest Service. For this study, we refer to the sections of the Lamar River watershed (Figure 2.1) as (1) the upper watershed, encompassing all streams above the Lamar River canyon, (2) the lower Lamar River, encompassing the section of the Lamar River below the downstream end of the Lamar River Canyon to the confluence of the Yellowstone and Lamar rivers, (3) upper Slough Creek (a tributary to the Lamar River), encompassing everything above the Slough Creek barrier, (4) lower Slough Creek, encompassing everything below the Slough Creek barrier to the Lamar River and Slough Creek confluence, and (5) Buffalo Creek (a tributary to Slough Creek). The upper and lower Lamar River watersheds are separated by the Lamar River canyon, a hypothesized seasonal fish barrier (Figure 2.1). Yellowstone Cutthroat Trout are native to the Lamar River watershed and have persisted there for over 10,000 years (Benkhe 2002). The other native fishes present are Mottled Sculpin *Cottus bairdii*, Plains Sucker *Pantosteus jordani*, and Longnose Dace *Rhinichthys cataractae*. The National Park Service and other agencies intentionally stocked nonnative Rainbow Trout in the Lamar River watershed in the early 1900s to diversify sportfishing opportunities for visiting anglers (Varley 1981). However, by the 1930s the National Park Service shifted its focus from stocking nonnative fishes towards native fish conservation, adopting a policy “prohibiting the introduction of exotic species in national park or monument waters now containing only native species" (Madsen 1937). Although stocking of Rainbow Trout ceased nearly a century ago, legacy populations still exist, and they continue to invade, hybridizing with native Yellowstone Cutthroat Trout. Hybrids are now abundant in the lower Lamar River watershed, and because of the seasonal, fluvial connectivity of the system, appear to be invading the upper watershed where Yellowstone Cutthroat Trout populations of high conservation priority are present (Ertel 2017; Al-Chokhachy et al. 2018; Heim 2019). Hybrid trout are primarily concentrated in the lower watershed, including Buffalo and Slough creeks and the lower Lamar River (Ertel et al. 2017). However, hybridization has been identified with increasing frequency in the upper watershed in recent years (Heim et al. 2020). This increased prevalence of hybridization is of concern to the National Park Service, which is tasked with preserving the persistence of genetically unaltered Yellowstone Cutthroat Trout populations in the upper Lamar River watershed. Hybrid trout spawn primarily in lower Buffalo and lower Slough creeks, with individuals moving more than 10 km from the upper watershed, through Lamar River canyon, to the lower watershed in the spring (Heim 2019). Buffalo Creek was identified as the primary source of Rainbow Trout in the watershed (Heim et al. 2020), and the National Park Service and agency partners plan to eradicate the Rainbow Trout population from Buffalo Creek in 2024 and 2025 using rotenone—a chemical piscicide. Following the rotenone treatment, genetically unaltered Yellowstone Cutthroat Trout from nearby fluvial populations will be stocked in Buffalo Creek.

Selection of Sampling Locations. We scouted Slough Creek and the Lamar River prior to sampling, focusing our efforts on the lower Lamar River watershed downstream of Buffalo Creek according to National Park Service guidance. Our criteria for sampling included finding locations that were suitable for multiple sampling methods and safely accessible. Our selected sampling area comprised a 4.3-km section of lower Slough Creek and a 2.8-km section of the lower Lamar River (Figure 2.1).

Our Slough Creek section encompassed an unconstrained valley with a gradient of 0.004%. The vegetation in the low-gradient valley is primarily composed of Idaho fescue *Festuca idahoensis* and big sagebrush *Artemesia tridentata*. Riparian vegetation includes tall willow *Salix gyeriana* and sandbar willow *S. exigua* and *S. melanopsis*. Large herds of bison *Bison bison* graze the valley year-round, and browse pressure is evident along much of the stream banks. The streambed is primarily composed of large cobbles, gravel, and sand, with large meanders creating deep scours in the outside bends, point bars, and run-riffle-pool complexes. The average stream width of the valley part of the sampling section was 31.7 m. The lower Lamar River section had a gradient of 0.016% where large tertiary formations compose a canyon. The stream bed is composed of large boulders, cobbles, gravel, and sand. Dispersed large, woody debris and bedrock created deep plunge pools and long runs with scattered boulders. The average width of the canyon part of the sampling section was 31.2 m.

Pilot Snorkeling Study

We conducted a pilot snorkeling study in July 2021 to determine the efficacy of snorkeling as a sampling method for adult Yellowstone Cutthroat, Rainbow, and hybrid trout in the Lamar River and Slough Creek. We repeated the study in Slough Creek, but not the Lamar River, in July 2022 based on the results from 2021. We defined the minimum snorkeling thresholds to evaluate the utility of snorkeling as a potential sampling method in each stream.

These thresholds were defined as (1) underwater visibilities ≥ 3 m (Schill and Griffith 1984 [1.5 m is often used as the minimum threshold, but we determined this would be too low for snorkelers to identify key morphological characteristics such as white fin tips, coloration, and spotting patterns (Heim et al. 2020; Meyer et al. 2017)]); and (2) minimum water depths of 15 cm and the ability of snorkelers to see the bottom of the stream (Thurow 1994). We measured the visibility of each site by showing snorkelers the silhouette of a 25-cm, model trout marked with parr marks and spots underwater as they drifted downstream past it while holding a measuring tape. The minimum distance that parr marks and spots became visible to the snorkelers was recorded as the minimum visibility for each reach (Thurow 1994). Visibilities were measured to the nearest 10 cm, within fifteen minutes of the start or end of the survey, in depths that were representative of each site.

We conducted snorkel surveys at six 400-m sites in 2021 in Slough Creek, but the site furthest upstream was omitted from the study in 2022 because of the difficulty of transporting gear to the location (Figure 2.2) and safety concerns during snorkel surveys; therefore, analyses included only five sites. We used these sites (hereafter "reference sites") to compare the observed counts and proportions of trout taxa between snorkel and electrofishing surveys. We conducted snorkel surveys during the day (between 0900 and 1700 hours), 24 hours prior to electrofishing in Slough Creek. Snorkelers visually surveyed each reach by drift-diving downstream. Six snorkelers equipped with dry-suits, masks, and snorkels counted fish as they passed over them. Most sites were too swift to effectively sample in an upstream direction; water velocities made it difficult for snorkelers to maintain an upstream direction without being forced downstream by the current. Upstream sampling was only possible in riffle areas and backwaters, but many riffles did not meet the minimum sampling depth. Snorkelers were randomly assigned to lanes to avoid bias in counting lanes. Snorkelers maintained their respective lanes by spacing themselves equidistantly along a 9-m long polyvinyl chloride (PVC) pipe (O'Neal 2007; Figure 2.3) in Slough Creek. Occasionally, snorkelers separated the pipe to split into groups in areas of different depths. In sections of stream too shallow to swim, snorkelers stood up, walked downstream, and waited until disturbed substrate cleared from the water before entering the next floatable section. Snorkelers noted whether they could see the bottom of the stream in each site. Trout were identified as Yellowstone Cutthroat, Rainbow, or hybrid trout. Lengths of trout were categorized as < 100 mm, 100–249 mm, 250–350 mm, and > 350 mm. All fish observations were recorded on dive slates attached to an arm of each snorkeler and transferred to a data sheet at the end of each reach. To avoid double counting, snorkelers used hand gestures to signal whether they had marked a fish that moved across multiple snorkeling lanes.

We compared the number of trout identified to a taxonomic group (e.g., identified using morphological characteristics such as white fin tips, coloration, and spotting patterns [Heim et al. 2020; Meyers et al. 2017]) to the number of trout marked as unknown. These comparisons were used to assess how measured visibility, ability of snorkelers to see the bottom of the stream, and width of the stream affected the visual extent to which these characteristics were detectable. Snorkel surveys were conducted similarly in the Lamar River, but a PVC pipe was not used there (Figure 2.4) because of the presence of large boulders in the snorkeling lanes at some sites. We snorkeled seven 400-m sites and one 250-m site in late September 2021 in the Lamar River (Figure 2.2). Logistical constraints in 2021 precluded us from electrofishing after snorkeling in the Lamar River; therefore, we did not conduct any comparisons between electrofishing and snorkeling surveys.

Electrofishing CPUE

We conducted electrofishing surveys in a 4.3-km section of Slough Creek, divided into 18 sites, in 2021 and 2022 to calculate CPUE (trout/100 m) of Yellowstone Cutthroat, Rainbow, and hybrid trout (Rainbow and hybrid trout hereby referred to collectively as “nonnative trout”); electrofishing surveys were conducted in late July in both years. Snorkel reference sites also served as starting and ending points during these surveys to compare electrofishing and snorkeling estimates; these sites encompassed multiple electrofishing sites, hence the discrepancy between the number of snorkel-reference and electrofishing sites. We conducted a single-pass electrofishing survey in a 2.6-km section of the Lamar River, divided into 12 sites, in late September 2022 to calculate CPUE of the trout taxa. We used VVP-15B electrofishers (Smith-Root, Vancouver, Washington) powered by 3,500- or 5,000-watt generators mounted to the frames of two 3-m, inflatable, self-bailing rafts in both streams. The electrofishers transferred power to the water through two electrode arrays attached to the bow of each raft (300 V, 30% duty cycle, and 30 Hz). The rafts floated downstream as one person in each operated the oars to steer and maintain a speed consistent with, or slightly faster than, the water velocity (Bonar et al. 2009). Two people netted fish from the bow of each raft using long-handled dip nets. Trout were processed at the downstream end of each site in a trailing raft. We identified each trout to taxon, recorded its total length (mm), and scanned it for an existing half-duplex passive integrated transponder (PIT) tag (Biomark FS-2001 ISO proximity reader; Biomark Inc., Boise, Idaho). A syringe (Biomark MK7) was used to implant 23-mm tags into the dorsal sinuses of large, unmarked fish (> 120 mm). Small fish (70–120 mm) were implanted with 12-mm tags in their abdominal cavities using a scalpel. We used PIT-tag recoveries to evaluate the independence of sites (to assess if trout were moving among sites after being released, and therefore being included in calculations of the CPUE estimates of other sites). Trout were released at least 50 m upstream of the processing location to avoid recapture downstream.

Abiotic Measurements

We measured water temperature and conductivity was measured during electrofishing surveys. Water temperature was measured using Onset HOBOware data loggers (UA-001-08) placed at the upstream and downstream ends of the sections. Conductivity was measured using an Apera Instruments handheld conductivity meter (EC850).

Mark Recapture

Slough Creek. We conducted additional electrofishing and angling sampling in late July and early August 2022 to estimate the abundances of Yellowstone Cutthroat and nonnative trout. Four capture “events” (one event = a complete sampling of the 4.3-km section) were conducted over a 4-week period. We conducted two electrofishing events followed by two angling events (Table 2.1), with one week between each survey. Electrofishing methods were the same as described above for our CPUE analysis; the first electrofishing event was used for both the CPUE and mark-recapture analyses. Angling events involved 4–7 anglers divided into two groups. We randomly assigned each group to one of five stream segments within the 4.3-km section over a 4-day period. Each 4-day period of angling constituted a capture event, and anglers sampled the segments in the same order during the second week to maintain consistency. Anglers proceeded upstream within their assigned segment, sampling strategically to ensure all available habitat was fished. They alternated among habitats and the use of fly and spinning gear to reduce bias in gear selectivity, lure presentation, and angler experience. Angled fish were released at their capture location. We identified each trout to taxon, recorded its total length (mm), and scanned it for an existing PIT tag. During each capture event, Yellowstone Cutthroat Trout without existing PIT tags were tagged using the methods described previously. We removed nonnative trout after the first capture event in each stream to assist with NPS nonnative trout suppression efforts. Yellowstone Cutthroat Trout captured during the electrofishing survey were released at the downstream end of each site. Yellowstone Cutthroat Trout captured during the angling surveys were released at the capture location.

Lamar River. We conducted a 2-event, mark-recapture study over a 2-week period (Table 2.1) in a 2.6-km section of the Lamar River in 2022 (Figure 2.1) using techniques similar to those used in Slough Creek. Electrofishing surveys were conducted in late September. Angling was conducted by three anglers starting 10 days after electrofishing. Anglers started at the downstream end of the section and proceeded upstream, sampling the section over a 4-day period. We identified each trout to taxon, recorded its total length (mm), and scanned it for an existing PIT tag. We removed nonnative trout after the first capture event in each stream to assist with NPS nonnative trout suppression efforts. Yellowstone Cutthroat Trout captured during the angling surveys were released at the capture location.

Juvenile Sampling Pilot Study

Slough Creek. We conducted three-pass-depletion, juvenile-trout electrofishing surveys at six sites of Slough Creek in October 2021 to determine the efficacy of estimating the absolute abundance of juvenile Yellowstone Cutthroat, Rainbow, and hybrid trout along channel margins. We sampled in October to allow individuals to reach their maximum growth potential for the year, ostensibly improving our ability to visually distinguish among trout taxa (Koenig 2006; Meyer et al. 2017). Sites were 20-m long bank-habitat units extending 2 m perpendicular from the bank towards the main current (Figure 2.5). Block nets were not used during these surveys; instead, we assumed sites were biologically closed because of the short sampling period (1 hour) and evidence that short-term juvenile emigration is limited (Mitro and Zale 2000; Korman et al. 2009). Two people operated backpack electrofishers while two netters captured trout with long-handled dip nets. Crews moved upstream, carefully sampling the substrate, and ensuring to shock interstitial spaces slowly among large substrates, when present. We conducted three electrofishing passes at each site unless we caught zero fish on the second pass (1 site). If more fish were caught on the third pass than on the second, we conducted a fourth pass (2 sites). During surveys, fish were placed in live boxes placed at least 5 m outside of the survey reach to avoid exposure to the electrical field during subsequent passes. At the end of each electrofishing pass, trout were identified to taxa, their total lengths were measured (mm), and small tissue samples were collected from their anal fins for genetic analyses (results of genetic analyses are detailed in Chapter 3).

Lamar River. We conducted juvenile-trout electrofishing surveys at 16 sites in the Lamar River in October 2021 using the same methods as in Slough Creek. However, some sites were extended to 50 m in length to increase the opportunity to catch fish. We also conducted opportunistic sampling in habitats deemed ideal for juvenile trout (boulder substrate with interstitial spaces along the bank).

Data Analysis

All data analyses were conducted using R statistical software (R Development Core Team, 2009) or Program MARK (White and Burnham 1999).

Snorkel-Electrofishing Comparison Analysis

We evaluated the accuracy of the taxonomic identifications assigned during snorkeling by comparing the raw counts and proportions of each taxon assigned by snorkeling versus electrofishing at sites where both techniques were used; the identifications of electrofished individuals were assumed to be correct because the fish were examined by hand. We first compared counts of each taxonomic group from each method at each site to a 1:1 line to determine if both methods recorded similar counts. We also compared the proportions of nonnative trout identified in reference reaches by snorkeling and electrofishing using a generalized linear mixed model. The model used a binomial random variable for the response, in which each trout observed was modeled as a Bernoulli trial with 1 = nonnative trout observed and 0 = Yellowstone Cutthroat Trout. We included method (snorkeling or electrofishing) as the explanatory variable, site as a fixed variable, and year as a random variable to account for non-independence of samples among years. Prediction intervals were calculated using the *ggpredict* function in the *ggeffects* package (Lüdecke et al. 2021). We assumed the observed proportions of nonnative trout observed by each sampling methods to be statistically indistinguishable if the null hypothesis (H_0) was accepted ($P > 0.05$) and 95% prediction intervals overlapped.

CPUE Analysis

Catch-per-unit-effort at each site was calculated as number of trout per 100 m in our simulated section (we multiplied the quotient by 100 to standardize our measurements to the same units) using the equation

$$\mathrm{CPUE} = \frac{c}{f_{distance}} \cdot 100\,,$$

where c = the number of trout caught and $f_{distance}$ = site length (m).

Mark-Recapture Analysis

Slough Creek. We fit closed mark-recapture models for Yellowstone Cutthroat Trout in Slough Creek with Program MARK using the *RMark* package (Laake 2013). For capture probability (*p*), three full-likelihood models were considered where *p* was constant (M_0) and *p* varied by capture event (M_t; Otis et al. 1987). We used Akaike's Information Criterion adjusted for sample size (AICc) to evaluate model fit among the models (Burnham and Anderson 1998). The lowest ΔAICc score was used to determine the best-ranked model compared to the lesser-ranked models. Goodness-of-fit testing was conducted using the CAPTURE interface in Program MARK. We calculated the weighted average for the derived estimates of $\widehat{N}$ and associated 95% confidence intervals because of the similar performances of the M_0 and M_t models. Nonnative trout abundance estimates were also calculated using only the first two (electrofishing) events and a Chapman-modified, Lincoln-Peterson (hereafter "LP") estimator (Chapman 1952) using the *mrClosed* function in the *FSA* package (Ogle 2019). The LP estimator was calculated as

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

where $\widehat{N}$ = the estimated trout abundance, n_1 = the number of fish caught during the first (marking) sampling event, n_2 = the number of fish caught during the second (recapture) event, and m_2= the number of marked fish recaptured during the second sampling event. We also estimated abundance of Yellowstone Cutthroat Trout from the first two sampling events using the LP estimator to compare to the models analyzed in Program MARK. All trout abundance estimates were standardized to density (trout/kilometer).

Lamar River. We estimated Yellowstone Cutthroat Trout and nonnative trout abundances in the Lamar River using the LP estimator because we only had two sampling events. All trout abundance estimates were standardized to density (trout/kilometer).

Simulations

CPUE. We bootstrapped (with replacement) our CPUE estimates of each taxon from each stream to determine the sampling intensity (i.e., continuous site length) needed to precisely characterize trout CPUE in each stream. For our Slough Creek simulations, we averaged the CPUE estimates from each site across both years (2021 and 2022) to account for differing sampling conditions. For the Lamar River, we used our single year of data (2022). For each number of sites (e.g., 5, 6, 7, etc.), we conducted 10,000 simulations in which we (1) summed the site lengths (e.g., 2 sites; 330 + 250 m = 580 m) and total number of individuals captured, (2) calculated CPUE as

$$CPUE_{sim\ i} = \frac{\sum individuals}{\sum site\ lengths} \cdot 100,$$

and (3) calculated the mean and standard deviation of all CPUE estimates for each sample size (number of sites) across the 10,000 simulations. The coefficient of variation (CV) was used to compare the effects of section length on the precision of CPUE. We calculated the CV of our CPUE simulations as

$$CV = \frac{SD}{\widehat{CPUE}}$$

where SD = the population standard deviation of the simulations and $\widehat{CPUE}$ = mean CPUE across simulations for each sample size.

Mark Recapture. We used abundance estimates of Yellowstone Cutthroat and nonnative trout ($\widehat{N}$) and angling and electrofishing *p* to simulate alternative sampling designs and evaluate the precision of the resulting abundance estimates (Kéry and Royle 2016). Electrofishing *p* was estimated directly from the mark-recapture analysis ($\frac{m_2}{n_2}$) , and angling *p* was estimated by dividing the number of each taxon caught during angling surveys by the abundance estimate ($\frac{n_2}{\widehat{N}}$). For Yellowstone Cutthroat Trout, we constructed a hypothetical population using our abundance estimate ($\widehat{N}$) of 399 individuals and our estimates of *p* (0.14 for electrofishing and 0.15 for angling) to inform our simulations. For nonnative trout, we used the $\widehat{N}$ of 224 individuals and the respective *p* for each sampling method (0.10 for electrofishing and 0.22 for angling). By creating these hypothetical populations, we had known abundances and were able to compare the point estimates and confidence intervals generated by different sampling designs to the “true” population abundances. We generated *p* for each population across three simulated capture events ($t = 3$) assuming a study where captured trout receive a unique identifier (i.e., a PIT tag) during each event. For example, if a trout was simulated to be captured on the first occasion, not captured on the second occasion, and captured on the third occasion, it would have a capture history of 101. For each simulated capture event, we incorporated uncertainty into *p* by randomly selecting from a beta distribution centered around our specified *p* (SD = 0.02) for each method using the *rbeta2* function in the *IPMbook* package (Schaub et al. 2023). We constructed this beta distribution based on a range of capture probabilities we determined to be realistic for each sampling gear. We used Program MARK with the *RMark* package to estimate abundance of the constructed populations using a full-likelihood M_t model (Otis et al 1978). We also estimated abundance using two events to evaluate agreement among our empirical estimates from the LP estimator and the simulations (EA) because maximum likelihood for the M_t model and LP estimator are equivalent when $t = 2$ (Otis et al. 1978). We evaluated sampling designs that incorporated combinations of angling and electrofishing including three angling events (3A), two angling events and one electrofishing event (2AE), and two electrofishing events and one angling event (2EA). Simulations were repeated 10,000 times for each sampling design. We used the simulation CVs and median 95% confidence interval lengths as measures of precision for comparing sampling designs. The CV of each sampling design was calculated as

$$CV = \frac{SD}{\hat{N}},$$

where SD = the population standard deviation of the simulations and $\widehat{N}$ = mean abundance estimates across simulations for each sampling design. Median confidence interval lengths were calculated as the median upper – median lower 95% confidence intervals.

Power Analyses

We used different methodologies to determine the magnitude of change each sampling design could detect. We evaluated linear declines in CPUE by using time as a substitute for site replication because of the limited geographic scope of our study area. Therefore, we had no intra-annual variation in our estimates. For mark-recapture estimates, uncertainty is "built in" to the estimator, and therefore, we were able to use the confidence intervals to determine whether a change could be detected.

CPUE. We used Monte Carlo simulations (recommended by Link and Hatfield 1990) to determine our statistical power to detect declining trends in the CPUE of Yellowstone Cutthroat and nonnative trout in Slough Creek and the Lamar River. We used our bootstrapped CPUE estimates to simulate declines in trout CPUE at a single site of varying lengths. We used an exponential decay function to simulate declines in CPUE using the equation

$$N_i = N_1 \cdot (1 + r)^t,$$

where N_i = CPUE at time step i (e.g., year 5), N_1 = the initial CPUE estimate (year 1), r = the rate of change for the prespecified declines, and t = the number of years. We simulated 25%, 50%, and 75% declines over 5-, 10-, 15-, and 20-year time periods. To account for demographic fluctuations in trout population abundances, we incorporated stochasticity into the estimate based on the "proportional standard error" of CPUE at the initial time step calculated as ($\frac{1}{\sqrt{CPUE}}$; Gerrodette 1987) and selected random points from a normal distribution centered around the projected CPUE at time t+1 (Figure 2.6); this process was repeated 10,000 times for each scenario (site length, percentage decline, and number of years). The slope parameters of log-linear regression models were used to determine whether the decline was negative and detectible (H_0: $\hat{b} = 0$; $P < 0.05$) for each simulation (Thompson et al. 1998; Dauwalter et al. 2009). Statistical power was calculated as the proportion of simulations that were negative and detectible out of our total simulations (Gibbs 1998). We used 0.8 (i.e., 80% of the simulations) as our threshold for acceptable power.

Mark Recapture. We used our empirical and simulated abundance estimates and associated precision (95% confidence intervals) to determine whether we could detect 50 and 75% declines in Yellowstone Cutthroat and nonnative trout abundances across our simulated sampling designs. Rather than using a null hypothesis testing framework, we determined our ability to detect declines based on non-overlapping confidence intervals of our reduced and initial estimates. For the Lamar River, we repeated our simulations for each sampling design based on populations that were 50 and 25% (hereby "reduced estimates") of the initial estimates. We then compared the median $\widehat{N}$, lower 95%, and upper 95% confidence intervals of all 10,000 simulations of our reduced estimates to those of our initial estimates. We were unable to conduct the same population simulations for the LP estimates from Slough Creek because of low recapture rates (Table 2.1). We only conducted the simulations for Yellowstone Cutthroat Trout based on our estimated $\widehat{N}$ and p from RMark.

Juvenile Trout Abundance Analysis

Juvenile abundance estimates were calculated with depletion models (Carle and Strub 1978) using the *removal* function in the *FSA* package (Ogle et al. 2019).

Results

Snorkeling Pilot Study

Slough Creek. We determined that conditions in Slough Creek were conducive to snorkeling in 2021 but not in 2022. Mean snorkeler visibility was 3.3 m in 2021 and snorkelers observed 178 trout in 6 sites, 23 of which (12%) were identified as unknown (morphological characteristics could not be confirmed). Mean snorkeler visibility was 2.6 m in 2022 and snorkelers observed 135 trout in 5 sites, 55 of which (41%) were identified as unknown. A 0.7-m reduction in snorkeler visibility resulted in a nearly 4-fold increase in the proportion of trout that could not be identified to taxon. Snorkelers were unable to see the bottom of deep pools in three of five sites in both years. These results indicate that snorkeling may be useful for monitoring, but only when snorkeler visibility is ≥ 3 m.

Lamar River. We determined that the lower Lamar River is not conducive to monitoring by snorkeling. The mean snorkeler visibility was 2.2 m and snorkelers observed 27 trout in seven sites, 22 of which (81%) were identified as unknown. Water velocities in this section made snorkeling difficult, and snorkelers were unable to slow themselves to observe trout long enough to identify morphological characteristics. Water visibility did not meet the minimum requirement of 3 m, snorkelers were unable to see the bottom of the stream in 3 sites, and most trout could not be identified to taxon.

Snorkel-Electrofishing Comparison Analysis

We did not see agreement between the numbers of Yellowstone Cutthroat, Rainbow, and hybrid trout sampled (counts) by electrofishing and snorkeling in Slough Creek for 2021. Electrofishing and snorkeling counts in reference sites were not related when compared to a 1:1 line, and snorkeling counts were generally higher than electrofishing counts (Figure 2.7). Seventy-four trout were sampled by electrofishing, and 155 (excluding unknown) trout were observed during snorkel surveys in six reference sites in 2021. We sampled fewer trout during snorkeling and electrofishing surveys in 2022, with 63 trout sampled by electrofishing and 48 (excluding unknown) trout observed during snorkel surveys in five reference sites in 2022. We observed agreement between the observed proportions of trout taxa in the sample of each method, but the agreement was stronger in 2021. The total proportion of nonnative trout in reference sites was 0.65 (95% CI = 0.52 – 0.77) in 2021 for electrofishing surveys and 0.66 (95% CI = 0.58 – 0.74) for snorkeling. In 2022, we estimated the total proportion of nonnative trout as 0.79 (95% CI = 0.59 – 0.92) for electrofishing and 0.49 (95% CI = 0.37 – 0.60) for snorkeling. We have moderate evidence to suggest that proportions of nonnative trout did not differ between sampling methods based on results from the GLM ($\beta = 0.77$; $P = 0.10$; DF = 19; Figure 2.8).

Abiotic Measurements

Average water temperature was 17° C in 2021 and 16.8° C in 2022 and average conductivity was 162 µS in 2021 and 124 µS in 2022 during the electrofishing survey in the Slough Creek section. Average water temperature was 13.4° C and average conductivity was 217 µS during the 2022 electrofishing survey in the Lamar River section.

CPUE Estimates

Slough Creek. Estimates of Yellowstone Cutthroat and nonnative trout CPUE were relatively low in Slough Creek. The frequency of zero catches of Yellowstone Cutthroat Trout increased from 4 to 13 from 2021 to 2022. Mean CPUE of Yellowstone Cutthroat Trout was 0.67 (SE = 0.24) in 2021 and 0.20 (SE = 0.09) in 2022. The frequency of zero catches of nonnative trout was 7 for both years. However, nonnative trout CPUE decreased from 1.14 (SE = 0.32) in 2021 to 0.86 (0.31) in 2022 (Table 2.2).

Lamar River. Catch-per-unit-effort estimates were higher for both taxa in the Lamar River than in Slough Creek. Yellowstone Cutthroat Trout CPUE was 2.22 (SE = 0.42) with only one site having zero catches. Nonnative trout CPUE was 1.33 (0.42) and two sites had zero catches (Table 2.2).

Mark Recapture Estimates

Slough Creek. Yellowstone Cutthroat Trout abundance estimates were low (17 – 24% that of nonnative trout), depending on the model used. The weighted-average Yellowstone Cutthroat Trout abundance was 17 trout/km (95% CI of 12 – 22 trout/km). Estimates of abundance from the LP estimator were 24 trout/km (95% CI = 4 – 956 trout/km) for Yellowstone Cutthroat Trout and 100 trout/km (95% CI of 30 – 196 trout/km) for nonnative trout. Overall, recapture rates were very low for the electrofishing events (Table 2.1), resulting in wide confidence intervals for the LP estimator. However, Yellowstone Cutthroat Trout abundance estimates were precise when we conducted two extra capture events by angling, indicating that electrofishing alone may not be sufficient for estimating trout abundance in Slough Creek.

Lamar River. Yellowstone Cutthroat Trout abundance was 153 trout/km (95% CI = 93 – 230 trout/km) and nonnative trout abundance was 86 trout/km (95% CI = 43 – 185 trout/km). Angling captured more individuals of each taxon than electrofishing (Table 2.1).

Simulations

CPUE. The coefficient of variation of Yellowstone Cutthroat and nonnative trout CPUE estimates decreased with longer section lengths. In Slough Creek, the greatest reduction in error occurred when section length increased from 250 m to 500 m, and we observed a consistent decline in error as site length increased (Figure 2.9). In the Lamar River, the greatest reduction in error occurred when section length was increased from 200 to 400 m, and we observed a consistent decline in error as site length increased (Figure 2.10).

Mark Recapture. For Lamar River estimates, an all-angling approach yielded the largest reduction in error, with a CV estimate of 0.18 and confidence interval width of 253 for Yellowstone Cutthroat Trout and a CV estimate of 0.14 and confidence interval width of 117 for nonnative trout (Table 2.3).

Power Analyses

Slough Creek. The probability of detecting a decline in Yellowstone Cutthroat Trout and nonnative trout CPUE was low for anything less than a 50% decline. A 50% decline in Yellowstone Cutthroat Trout CPUE was detectible over a 10-year period when section length is ≥ 3,600 m (Figure 2.11) whereas a 75% decline would be detectible after 5 years when section length is ≥ 1,500 m. A 75% decline in nonnative trout CPUE would be detectible after 5 years if section length is ≥ 1,200 m (Figure 2.12). A 50% reduction in nonnative trout CPUE would be detectible after 5 years if section length is ≥ 3,600 m. A 25% decline in CPUE of both taxa would not be detectible, even after 25 years. Confidence intervals for a 75%-reduced population using a multiple-mark recapture approach incorporating electrofishing and angling, or an all-angling approach, did not overlap with initial estimates of Yellowstone Cutthroat Trout abundance (Figure 2.13).

Lamar River. The probability of detecting a decline in Yellowstone Cutthroat Trout and nonnative trout CPUE was higher in the Lamar River when compared to Slough Creek (Figures 2.14 and 2.15). When section length is ≥ 1,200 m, a 75% reduction in both taxa would be detectible. A 50% reduction in both Yellowstone Cutthroat and nonnative trout CPUE would be detectible after 5 years if section length is ≥ 2,200 m (Figures 2.14 and 2.15).

The all-angling sampling design was the only sampling design with the ability to detect a 50% decline in nonnative trout abundance but not Yellowstone Cutthroat Trout abundance, whereas all other simulated designs were not able to detect a 50% decline in abundance of either taxon (Figures 2.16 and 2.17). Seventy-five percent of the declines in abundance would be detectible by all other sampling designs except for a 2-event design (Figure 2.17).

Juvenile Electrofishing Pilot Study

We successfully estimated juvenile trout abundances by depletion electrofishing in Slough Creek. Capture probabilities from depletion analyses varied from 0.461 to 1 (mean = 0.69) and estimated abundances ranged from 2 to18 (mean = 6; Table 2.4). However, we were unable to reliably identify juvenile trout to taxon in the field, and the proportion of trout ≤ 100 mm correctly identified was 0.30; details of this analysis are discussed in Chapter 3. We did not catch any juvenile trout in the Lamar River. A useful monitoring method often requires accurate identification of individuals in the field, and therefore, monitoring specific juvenile trout taxa abundance does not appear feasible in the Lamar River watershed using electrofishing and taxon identification in the field.

Discussion

Monitoring the abundances of fish populations of interest is necessary, but often lacks sufficient precision for trend detection. National Park Service management actions to reduce nonnative trout abundance in the lower Lamar River watershed necessitate monitoring to determine whether these actions are causing declines in the abundances of these nonnative taxa. Additionally, detecting declines in Yellowstone Cutthroat Trout is important to determine whether additional management actions are needed to ensure their long-term persistence in the watershed. Fish monitoring programs are diverse, and each sampling location poses its own difficulties to feasible implementation of certain gear types. Our objective was to determine the methodologies and amount of effort needed to detect declines in the CPUE and absolute abundance of Yellowstone Cutthroat, Rainbow, and hybrid trout in Slough Creek and the Lamar River.

Snorkeling

The efficacy of snorkeling in Slough Creek was dependent on water visibility. Low flow conditions in 2021 probably aided snorkeler visibility, because flows had reached their base-discharge conditions. Conversely, higher flows following the 500-year flood in 2022 probably impeded snorkeler visibility. Shallower pool depths in 2021 allowed snorkelers to see the bottom of the stream in more sites than in 2022. Snorkelers were unable to identify the taxon of many individuals, which indicates any estimates from snorkeling may be biased. Comparisons of snorkeling and electrofishing often reveal agreement between the two methods (Pinter et al. 2018; Chamberland et al. 2014); however, this is often the case when snorkeling is compared to depletion electrofishing where the site is blocked-netted during the study (Wildman and Neumann 2003; Mollenhauer and Brewer 2018). Despite the short duration between snorkeling and electrofishing (24 hours), our lack of block nets could have biased our estimates because of movement in and out of the sites. Increasing site length may have minimized this bias; however, we attempted to achieve replication by sampling multiple sites. The total length of the study area was relatively short (4.3 km), limiting the number of sites that we could sample, but shortening site lengths to increase our sample size of sites would not be feasible. Shorter site lengths would have limited the effectiveness of our electrofishing methods and therefore limited our ability to make comparisons. Addressing this deficiency could entail using alternative electrofishing gear (e.g., mobile anode electrofishing from rafts) to increase capture efficiency because of high water clarity. The National Park Service could take a similar approach to Pintler et al. (2018) and Wildman and Neumann (2003) by treating each pool as a sampling unit in which to increase replication. Site length could also be lengthened, and temporal replication could be used as a surrogate for site replication (Plichard et al. 2017).

Our observations comprised mostly trout suspended in pools < 2 m deep, probably because of the relative lack of additional structure (i.e., large woody debris) in the stream. However, snorkelers were unable to identify trout to taxon in deep pools > 2 m where they could not see the bottom. Despite the measured snorkeler visibilities, the lack of light penetration, even in depths within their range of measured visibility, probably limited the effectiveness of the method.

CPUE

Slough Creek. Catch-per-unit-effort estimates in Slough Creek were often zero, particularly in 2022. The frequency of zero catches among sites indicates trout are either patchily distributed and in low abundances throughout the section, or electrofishing efficiency was variable among sites, or both. We had highly variable sampling conditions between the years, which could explain the differences in CPUE observed. We observed a historical low snowpack in 2021 that resulted in reduced base-discharge levels. Conversely, in June 2022, historical flooding (500-year event) occurred throughout the upper Yellowstone River drainage. Reduced abundances may have been caused by high rates of mortality caused by either the record drought conditions observed in 2021 or the record discharge in the Lamar River watershed in 2022. Conversely, reduced discharge in 2021 may have increased electrofishing efficiency by reducing pool depths. Higher water levels may have also decreased electrofishing efficiency in 2022. Electrofishing efficiency could have also been influenced by water temperature, turbidity, conductivity, or crew experience. Lack of complex in-stream structure in Slough Creek (i.e., general lack of large woody debris, limited channel braiding, and sparse in-stream cover) may have also affected electrofishing efficiency. Often, habitat complexity reduces electrofishing efficiency (Habera et al. 1992; Shepard et al. 2014), but in some cases, water depth and lack of in-stream cover may also reduce electrofishing efficiency. For example, we often observed trout darting away from the electrofisher as we approached, indicating potential low electrofishing power transfer. We attempted to "corral" these trout towards the downstream ends of pools where they were either captured or evaded the electrical current. Water clarity is relatively good in Slough Creek, and conductivity is within acceptable range of electrofishing standards (Bonar et al. 2009); however, our power transfer may have been poor because of the conservative voltages applied (300 V) to reduce Yellowstone Cutthroat Trout mortality. We suspect that water depth and lack of habitat complexity may have also been major factors that limited our electrofishing efficiency, with these factors being compounded by stream discharge. We can only speculate on the potential bias introduced to the study because of this environmental stochasticity and crew experience because we did not estimate capture efficiency by mark-recapture methods in 2021, did not measure in-situ power transfer of our electrofishing configurations, nor were our objectives to estimate factors associated with capture efficiency. Alternative electrofishing methods or less conservative electrofisher settings (e.g., higher voltages, duty cycles, and frequencies) may be warranted in Slough Creek to increase efficiency (Kolz 1989). For example, throwable-anode configurations, rather than the boom-mounted anodes we used, have been shown to reduce fright bias (Monahan 1991; Ensign et al. 2002). This method allows operators to target specific habitats (i.e., pools) before trout observe the approaching boat (Matt McCormack, Montana Fish, Wildlife, and Parks, personal communication).

Lamar River. Potential differences in electrofishing efficiency among years could not be evaluated because we sampled the Lamar River only in 2022; however, we suspect the same factors affecting efficiency in Slough Creek were influential there.

Mark Recapture

Low capture probabilities contributed to high variability in our estimates in both Slough Creek and the Lamar River, but a combined-gear approach yielded higher precision. Therefore, more intensive sampling designs should be explored for nonnative trout abundance estimates. A dual-gear approach is a promising alternative to electrofishing alone. Simulations suggested that we can increase precision by increasing effort (using three mark-recapture events) and using angling for at least two of the events.

Power Analysis

Catch-per-unit-effort estimates were highly variable when section length was short. We used the number of sites and their respective site lengths as a proxy for sampling a single, continuous section length. This allowed us to use finer-scale data from each site to determine the minimum site length needed to accurately characterize trout abundance using CPUE. High variability from simulations of short section lengths were probably a result of the high spatial variability in trout distributions; simulation results suggested that sampling a longer section reduces sampling variability (Gotelli and Ellison 2004; Ramsey and Schafer 2012). As section length increased, we probably captured a greater amount of the observed, site-to-site variation in our CPUE estimates.

Power to detect trends was low for both CPUE and abundance unless declines were substantial ($\geq 50\%$). Extensive sampling effort and increased capture probabilities or mark-recapture events would be required to detect more moderate ($< 50\%$) declining trends in nonnative abundance. More effective electrofishing techniques such as mobile anode configurations or implementing sampling designs that combine electrofishing and angling to increase capture probabilities may be an alternative approach to monitoring. With less than three years of data at a single site, we were unable to capture year-to-year variation of the mixed-stock aggregations in the Lamar River and Slough Creek (Dauwalter et al 2009); estimates of this variation can be estimated in the future.

Juvenile Sampling Feasibility

Slough Creek. Electrofishing along bank habitats in Slough Creek may be useful to determine the abundance of juvenile trout present; this methodology has been effective in the Henrys Fork of the Snake River (Mitro and Zale 2000). However, our inability to distinguish among the trout taxa present suggests it is not a feasible way to determine the success of nonnative trout suppression in the Lamar River watershed. Instead, genetic monitoring should be conducted to estimate the proportion of individuals in the population with Rainbow Trout ancestry or frequency of Rainbow Trout alleles present among the population. Abundance estimates were generally low (mean = 6), indicating low densities of juvenile trout in the sections we sampled. However, our abundance estimate was relatively high (18) at one site ~70 m upstream of the confluence of Slough and Buffalo creeks. Trout sampled at this site might have outmigrated from Buffalo Creek or hatched in the main stem of Slough Creek (Heim et al. 2020), indicating this location is nursery habitat for juvenile trout. Most juvenile trout were caught in large boulder substrate (>256 mm diameter) with interstitial spaces and in woody debris adjacent to the main flow. Juvenile trout were also caught in shallow pools with boulder cover adjacent to the main channel at one site. Shallow habitats with low water velocities and cover often provide rearing habitat for juvenile salmonids (Heggenes and Traaen 1988; Hubert et al. 1994; Johnson et al. 2018). Thus, sampling shallow stream margins with ample cover was probably the most important factor for capturing juvenile trout (Mitro and Zale 2002). Gear limitations precluded us from sampling deep pools, which can also serve as rearing habitat for juvenile salmonids (Mundie and Traber 1983; LaVoie and Hubert 1996), but if future methodologies rely solely on genetic methods, the intent should be to sample 25–30 unrelated trout from each stream (Mandeville et al. 2019; Kreiner et al. 2024) rather than attempting to sample the entirety of the habitat. If juvenile trout surveys are conducted for genetic monitoring, efforts should be focused in low-velocity habitats with adequate cover (boulders and large woody debris) adjacent to the main flow. These methodologies may also be useful elsewhere to determine the strength of early year classes or assess distinct populations of Yellowstone Cutthroat Trout of high conservation value (Cegelski et al. 2006; Uthe et al. 2016; Al-Chokhachy et al. 2019; Heim et al. 2020).

Lamar River. Depletion electrofishing for juvenile trout is probably not feasible in the lower Lamar River, because we did not capture juvenile trout there. Electrofishing capture efficiency may have been low (Korman et al. 2010), juvenile trout may be patchily distributed and concentrated in areas we did not sample (Mitro and Zale 2000), or the lower Lamar River may not serve as a prominent spawning location for trout and therefore have little relevance as a nursery area.

Management Recommendations

We demonstrated the importance of conducting pilot studies and using empirical data to inform long-term monitoring efforts in data-sparse locations. Detecting declines in Yellowstone Cutthroat Trout, Rainbow Trout, and hybrids may be possible if declines are ≥ 50%. We recommend a minimum section length of 3,600 in Slough Creek and 2,200 m in the Lamar River. However, because of the low conductivity and poor electrofishing efficiency in Slough Creek, we recommend exploring the use of a throwable anode electrofishing unit or using less conservative electrofishing settings to maximize power transfer. Transporting electrofishing gear to and from the sampling locations is time consuming, but after gear is in place, the entire sections can be sampled with little additional effort. Sampling three, longer reaches may be an alternative strategy to reduce error by increasing replication (Dauwalter et al. 2009). This would also allow for estimation of inter-site variability (variation in CPUE among sites).

A dual-gear approach using electrofishing and angling is recommended for monitoring Yellowstone Cutthroat and nonnative trout abundances by mark recapture in both streams. In the Lamar River, an all-angling approach may yield the most precise results, and thus the greatest probability of detecting declines of all trout taxa examined, but analysis of these data should explore potential heterogeneity in capture probabilities among individuals or size classes to avoid inaccurate results.

Tables and Figures

Table 2.1 Sampling design and capture totals for mark-recapture surveys in Slough Creek, Yellowstone National Park, in 2022. Electrofishing events represent a single pass throughout the sampling area, and angling events represent a multi-day sampling effort throughout the sampling area.

		Yellowstone Cutthroat Trout		Nonnative trout	
Event	Sampling method	Total caught	Recaptures	Total caught	Recaptures
Slough Creek					
1	Electrofishing	9		31	
2	Electrofishing	13	1	26	1
3	Angling*	17	6	33	0
4	Angling*	14	6	13	0
Lamar River					
1	Electrofishing	61		26	
2	Angling	70	10	49	5

* Nonnative trout were removed from the system after the second sampling event in Slough Creek.

Table 2.2. Catch-per-unit-effort (trout/100 m) summary statistics for Yellowstone Cutthroat Trout and nonnative trout in Slough Creek and the Lamar River from 2021 to 2022. Mean CPUE represents the mean across 18 sites in Slough Creek and 12 sites in the Lamar River. The SE represents the standard error of the CPUE estimates.

	Yellowstone Cutthroat Trout		Nonnative trout	
Stream	Mean CPUE (SE)	Sites with 0 catches	Mean CPUE (SE)	Sites with 0 catches
2021				
Slough Creek	0.67 (0.24)	4	1.14 (0.32)	7
2022				
Slough Creek	0.20 (0.09)	13	0.86 (0.31)	7
Lamar River	2.22 (0.42)	1	1.33 (0.34)	2

Table 2.3. Coefficient of variation estimates for trout abundance estimates across simulated sampling designs. Sampling design 3A represents an all-angling approach, 2AE represents two angling events and one electrofishing event, 2EA represents two electrofishing events and one angling event, and EA represents a standard Lincoln-Peterson design with one electrofishing and one angling event. The CV denotes the coefficient of variation, and CI denotes 95% confidence intervals.

Sampling design	CV	Median CI width
Yellowstone Cutthroat Trout		
3A	0.18	253
2AE	0.18	261
2EA	0.19	269
EA	0.24	491
Nonnative trout		
3A	0.14	117
2AE	0.20	154
2EA	0.32	221
EA	0.31	362

Table 2.4. Estimated abundances, capture probabilities, and associated uncertainty (standard errors; SE) of juvenile trout in Slough Creek in 20-m long bank-habitat units extending 2 m perpendicular from the bank towards the main current.

	Abundance ($\widehat{N}$)		Capture probability (p)	
Site	$\widehat{N}$ (95% CI)	SE	p	SE
1	18 (14 – 22)	21.5	0.46	0.14
2	3 (2 – 4)	0.27	0.75	0.27
3	2 (2 – 2)	0	1	0
4	5 (4 – 6)	0.44	0.71	0.22
5	5 (4 – 6)	0.39	0.62	0.21
6	4 (2 – 6)	0.97	0.57	0.32

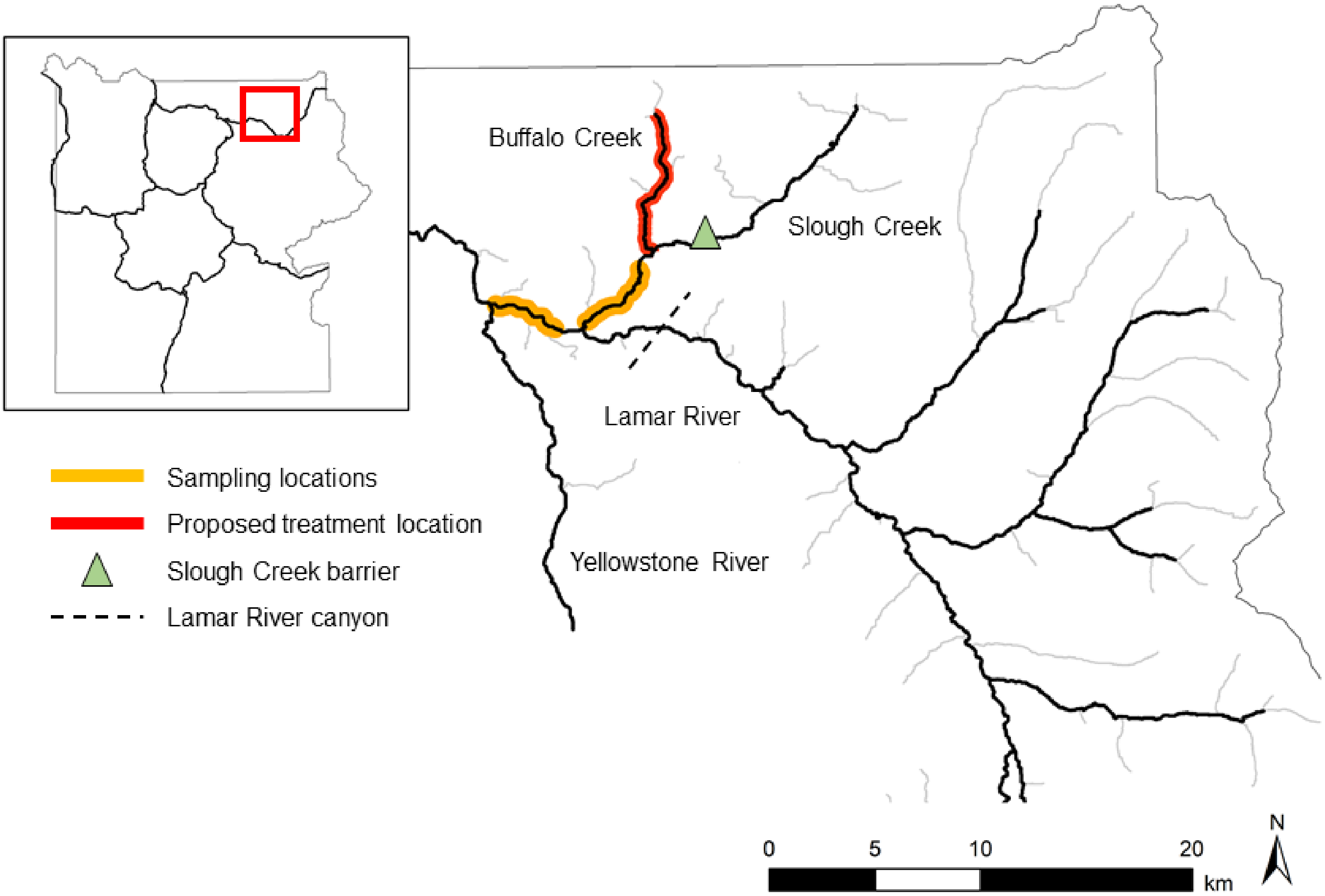

Figure 2.1. Map of the study area and section delineations of the Lamar River watershed, Yellowstone National Park.

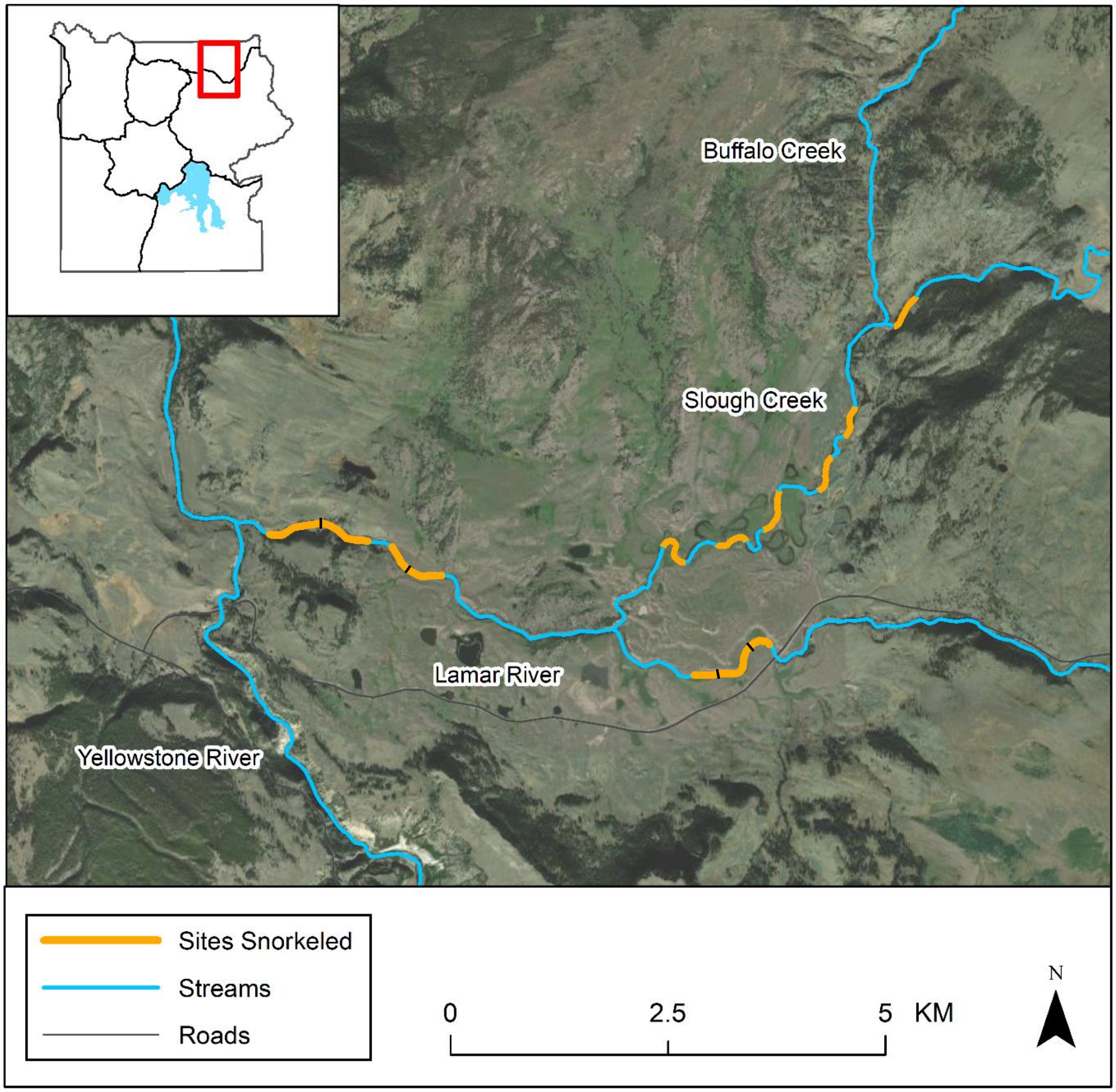

Figure 2.2. Locations sampled by snorkeling in the Lamar River and Slough Creek, Yellowstone National Park. Sites in the Lamar River were sampled in 2021, and sites in Slough Creek were sampled in 2021 and 2022. We did not sample the Slough Creek site upstream of the Buffalo Creek confluence in 2022.

Figure 2.3. Snorkelers using a PVC pipe to space themselves equidistantly and maintain their snorkeling lanes during a survey in Slough Creek, Yellowstone National Park in 2021.

Figure 2.4. Snorkelers drifting downstream to sample the Lamar River, Yellowstone National Park in 2021.

Figure 2.5. Example of a juvenile sampling site in Slough Creek, Yellowstone National Park in 2021.

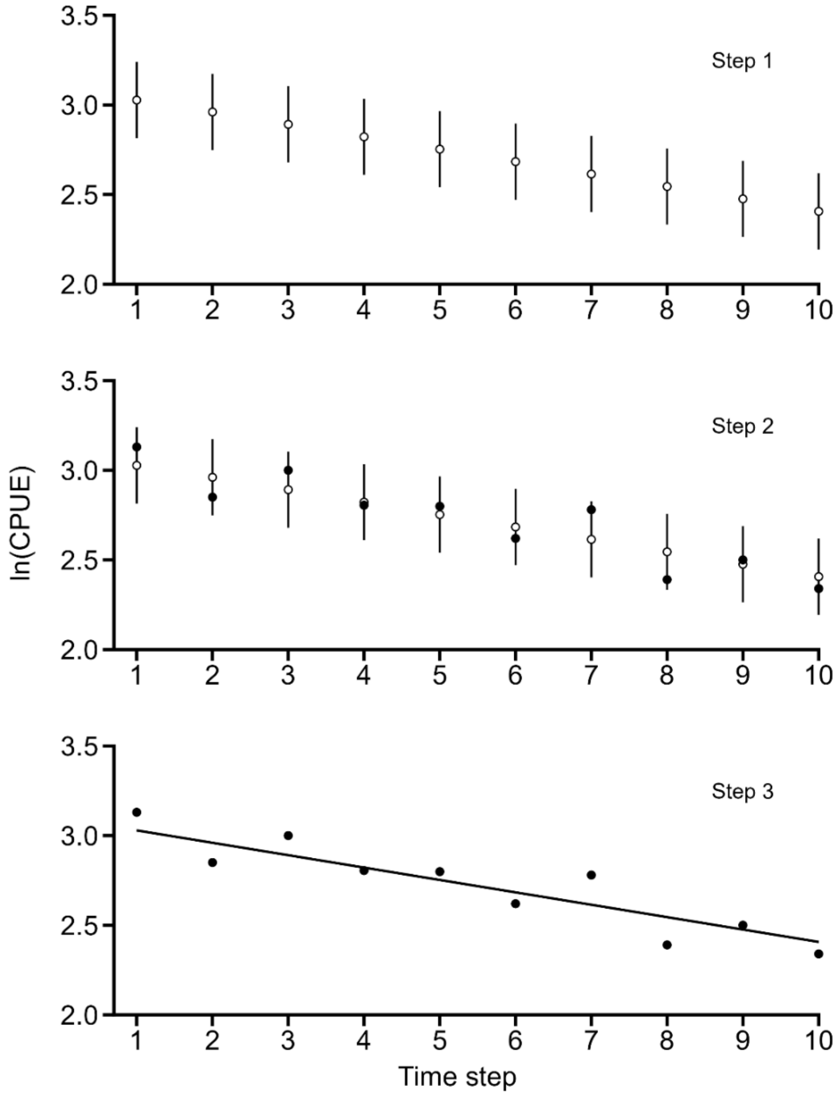

Figure 2.6. Simulation procedures for determining the statistical power of sampling designs to detect declines in CPUE of Yellowstone Cutthroat and nonnative trout in the Lamar River and Slough Creek. In step one, a decline (e.g., 50% decline) was projected onto the bootstrapped, log-transformed CPUE estimates for each sampling design over a pre-specified time period (e.g., 10 years). The white circles represent the projected CPUE in year *t* (N_t). Step two projects stochasticity in the estimate based on the standard deviation of the initial CPUE and selects random points (black circles) from a normal distribution centered around the mean of the projected CPUE. Step three fits a linear regression line to the estimates. This process was repeated 10,000 times for each sampling design (number of sites) and combination of declines (e.g., 50% decline over 10 years as shown). The proportion of slopes that were negative and significant ($P < 0.05$) from the regressions represented our statistical power to detect a trend. This figure was adapted from Gibbs (1998) and Dauwalter (2009).

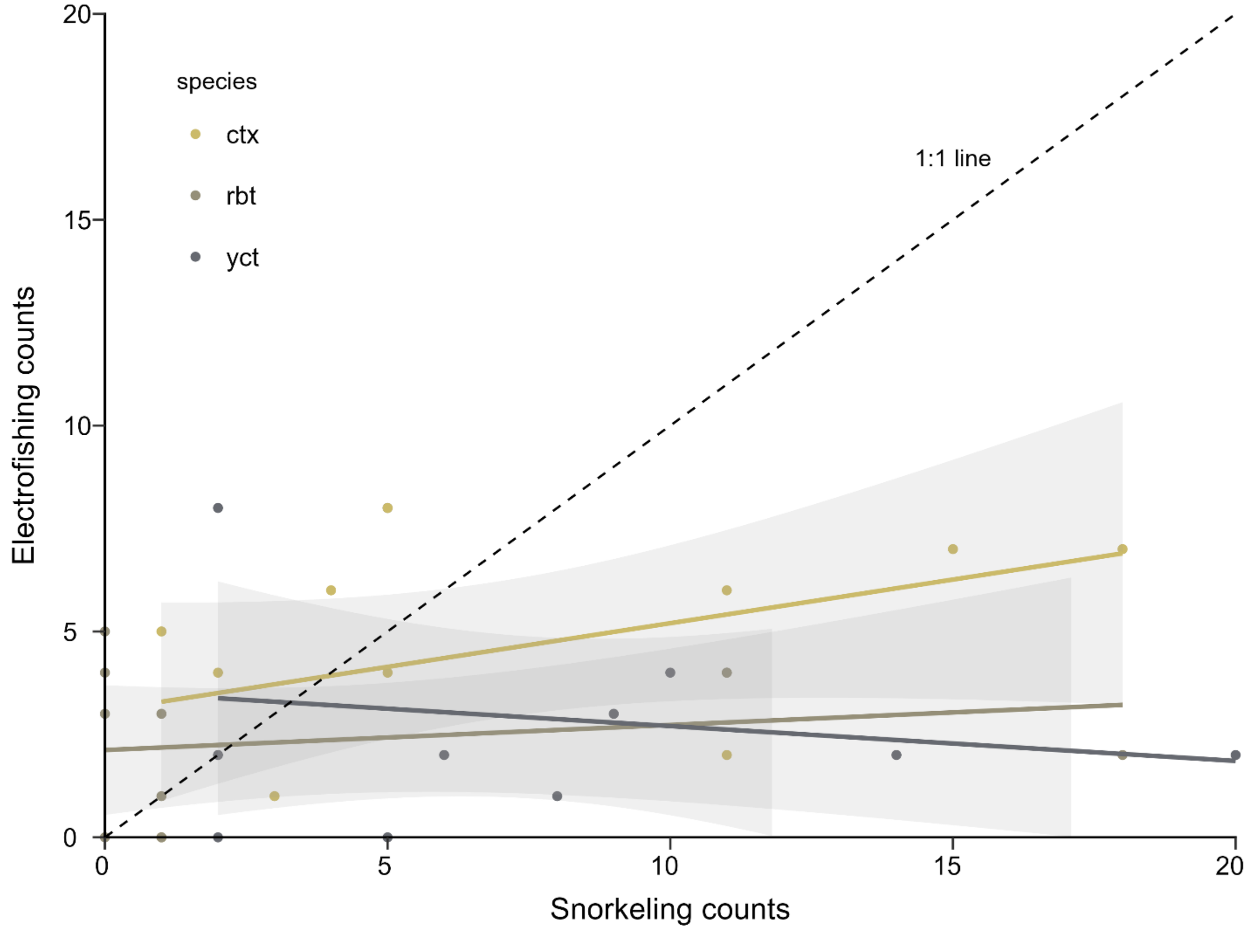

Figure 2.7. Comparison of counts of trout taxa by electrofishing and snorkeling in Slough Creek in 2021 and 2022 (n=11; one site was omitted in 2022). Ctx (yellow) represents hybrid trout, yct (blue) represents Yellowstone Cutthroat Trout, and rbt (brown) represents Rainbow Trout.

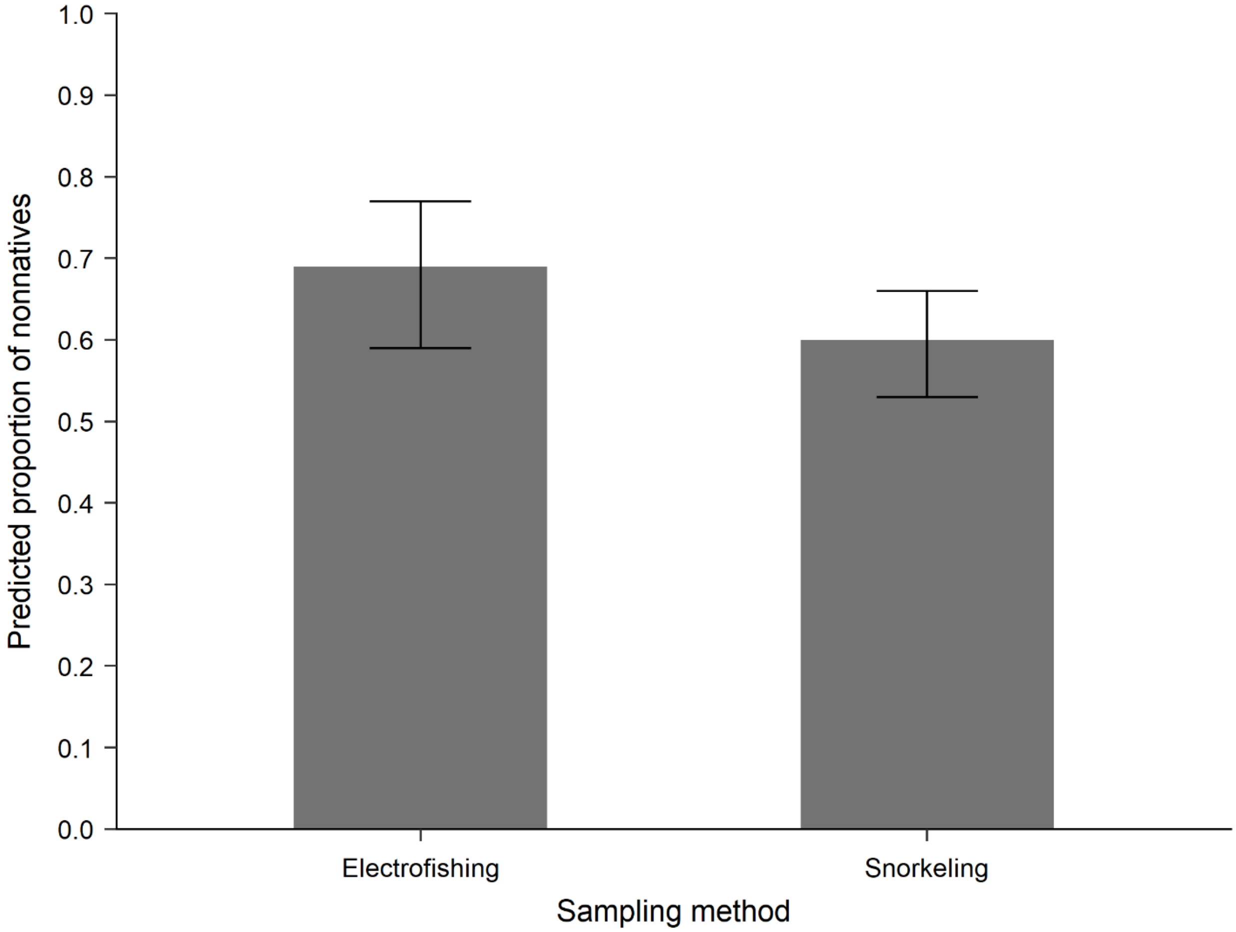

Figure 2.8. Predicted proportions of nonnative trout (Rainbow Trout and hybrids) sampled by electrofishing and angling in reference sites in Slough Creek, Yellowstone National Park in 2021 and 2022. Error bars represent 95% prediction intervals from the GLM.

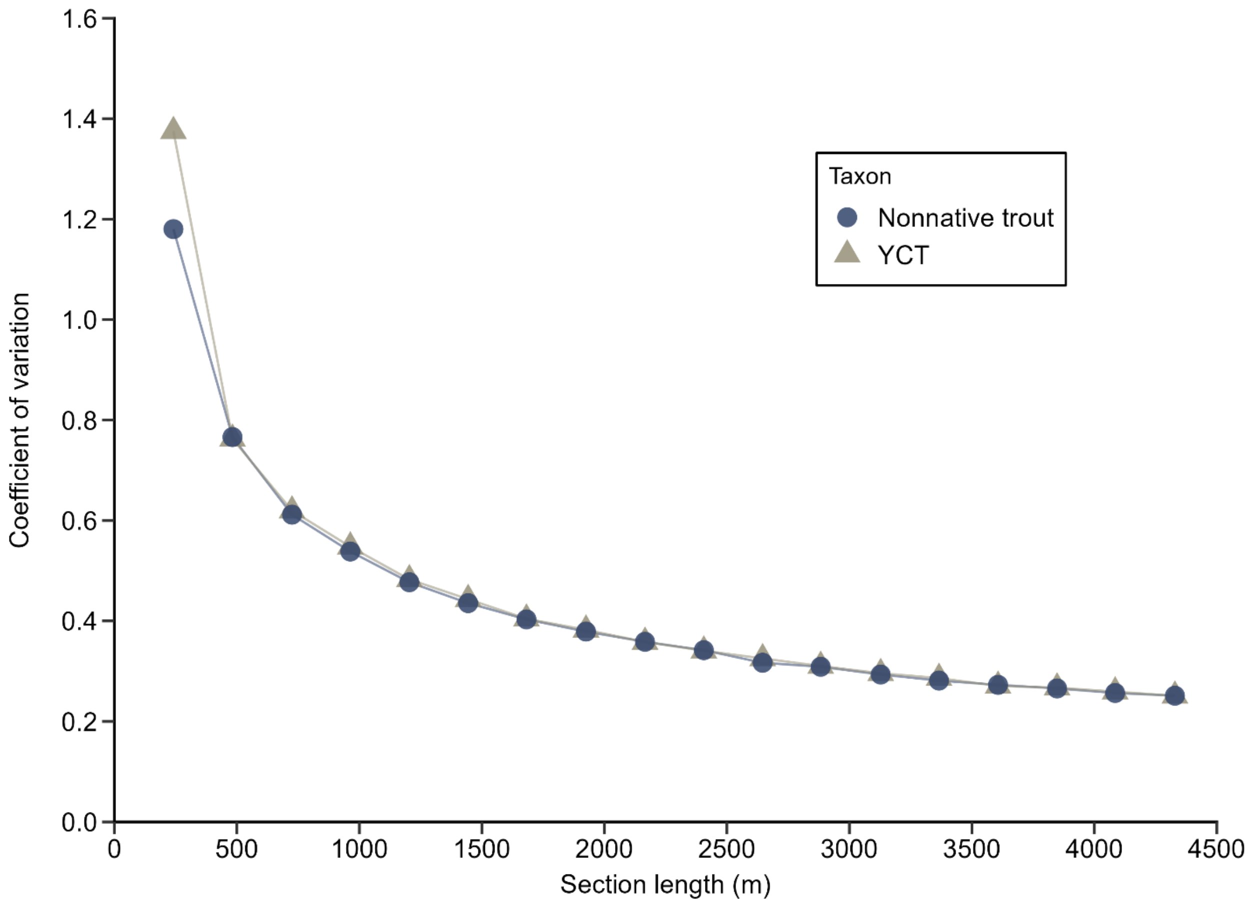

Figure 2.9. Changes in the CV of CPUE (trout/100 m) estimates for nonnative trout (circles) and Yellowstone Cutthroat Trout (triangles) in Slough Creek, Yellowstone National Park, when increasing numbers of sites are sampled.

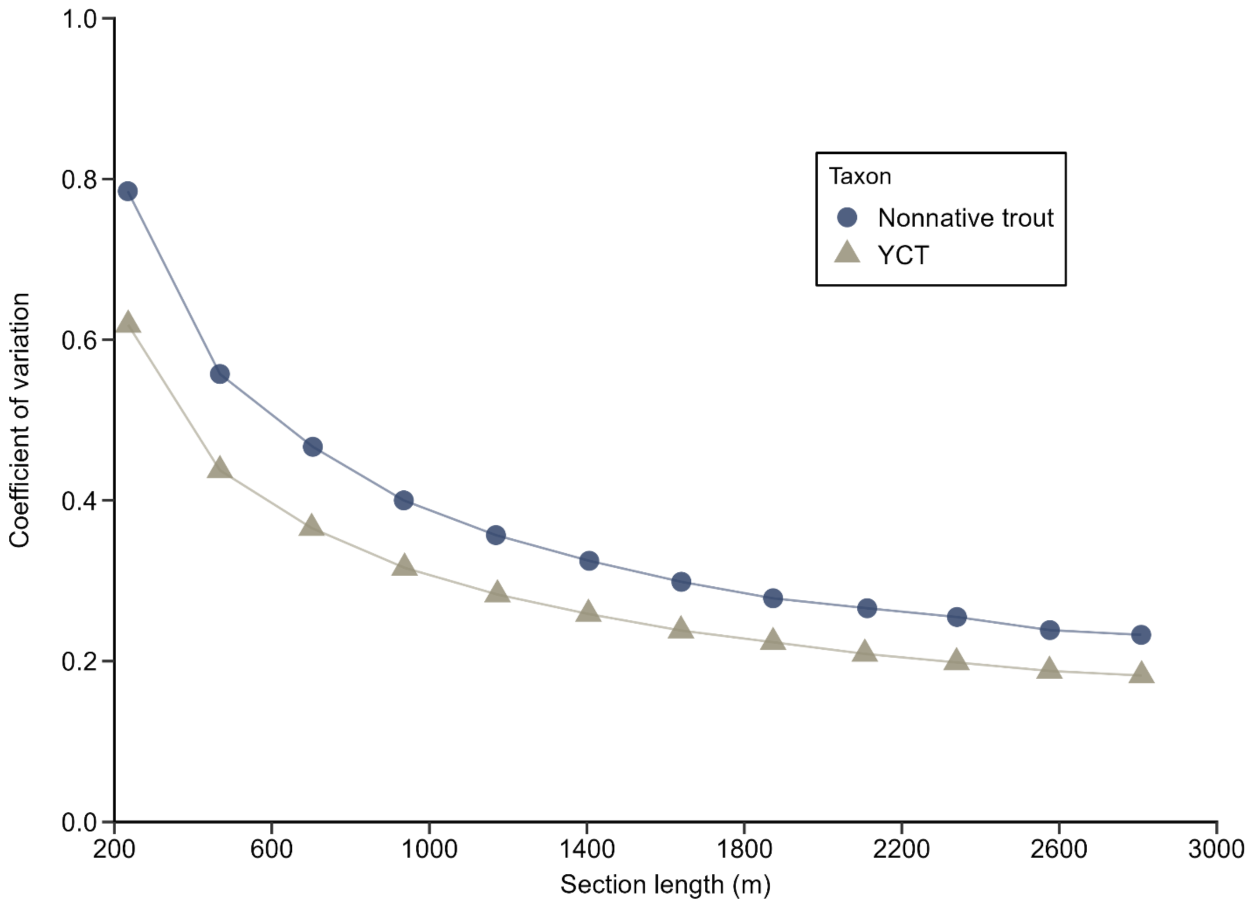

Figure 2.10. Changes in the CV of CPUE (trout/100 m) estimates for nonnative trout (circles) and Yellowstone Cutthroat Trout (triangles) in the Lamar River, Yellowstone National Park, when increasing numbers of sites are sampled.

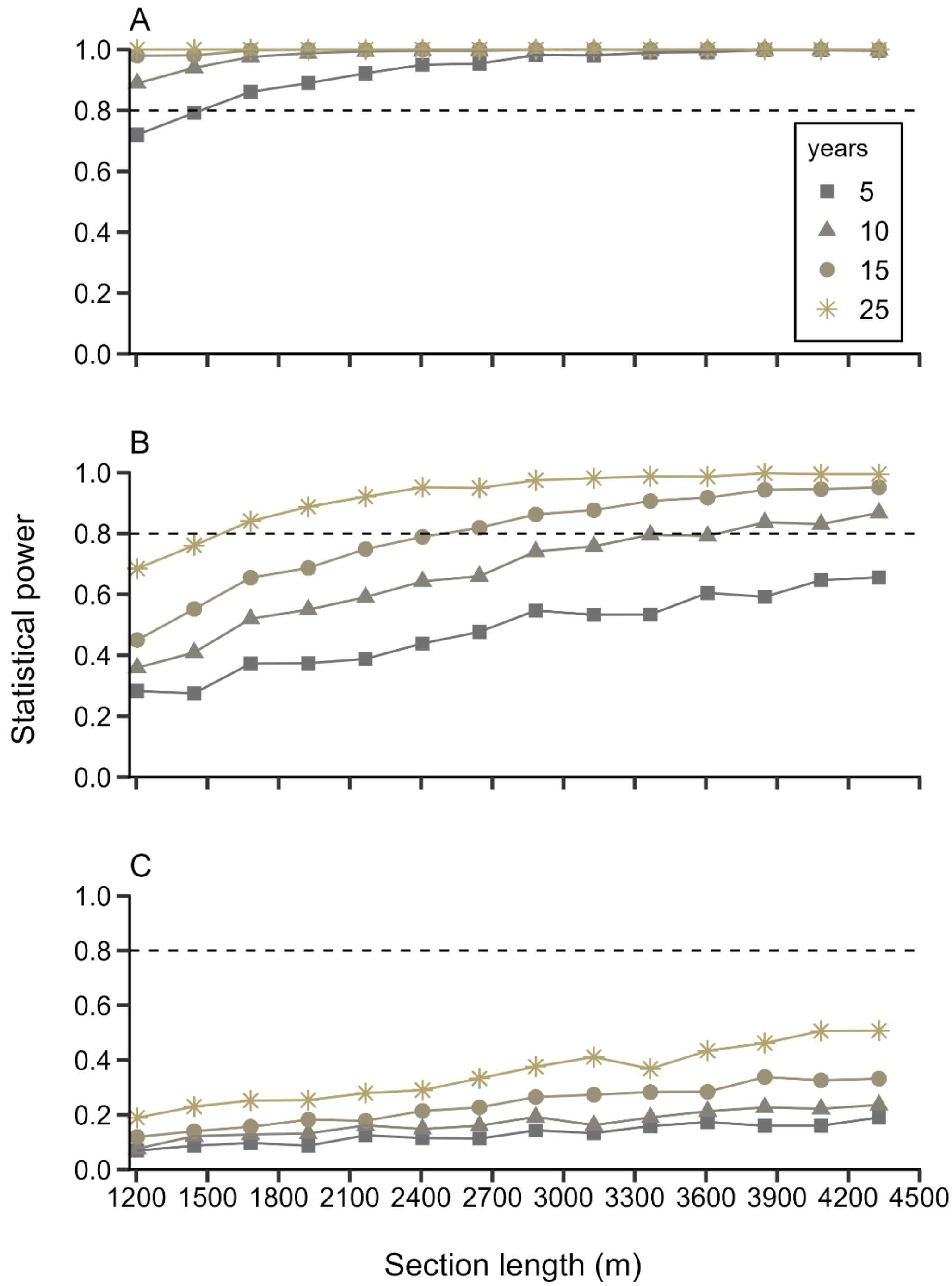

Figure 2.11. Monte Carlo simulation results for the probability of detecting (statistical power) 75% (A), 50% (B) and 25% (C) declines in Yellowstone Cutthroat Trout CPUE over 5- (squares), 10- (triangles), 15- (circles), and 25-year (asterisks) periods in Slough Creek. Results represent the proportion of simulated declines that were negative and significant ($P \leq 0.05$).

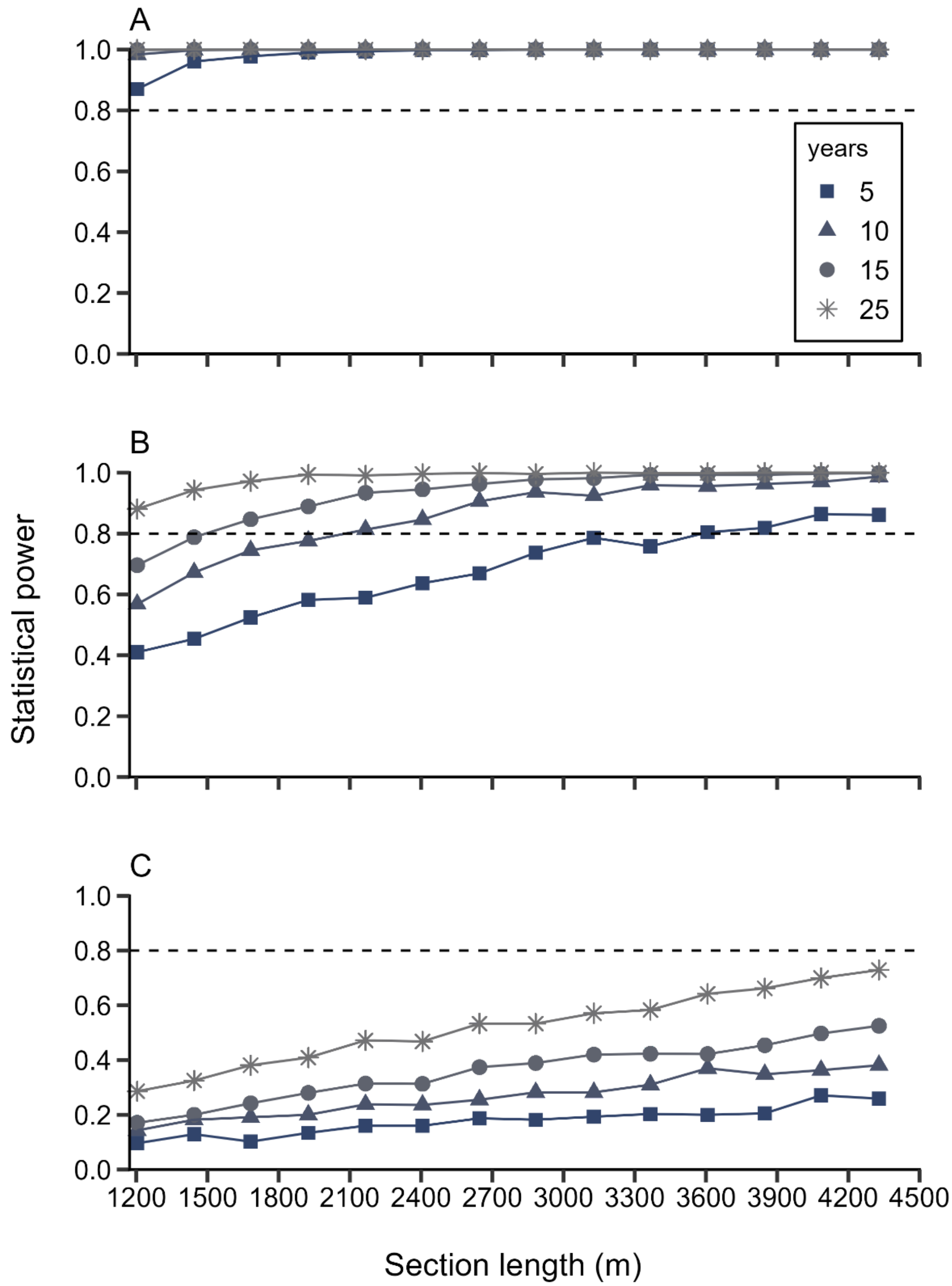

Figure 2.12. Monte Carlo simulation results for the probability of detecting (statistical power) 75% (A), 50% (B) and 25% (C) declines in nonnative trout CPUE over 5- (squares), 10- (triangles), 15- (circles), and 25-year (asterisks) periods in Slough Creek. Results represent the proportion of simulated declines that were negative and significant (P ≤ 0.05).

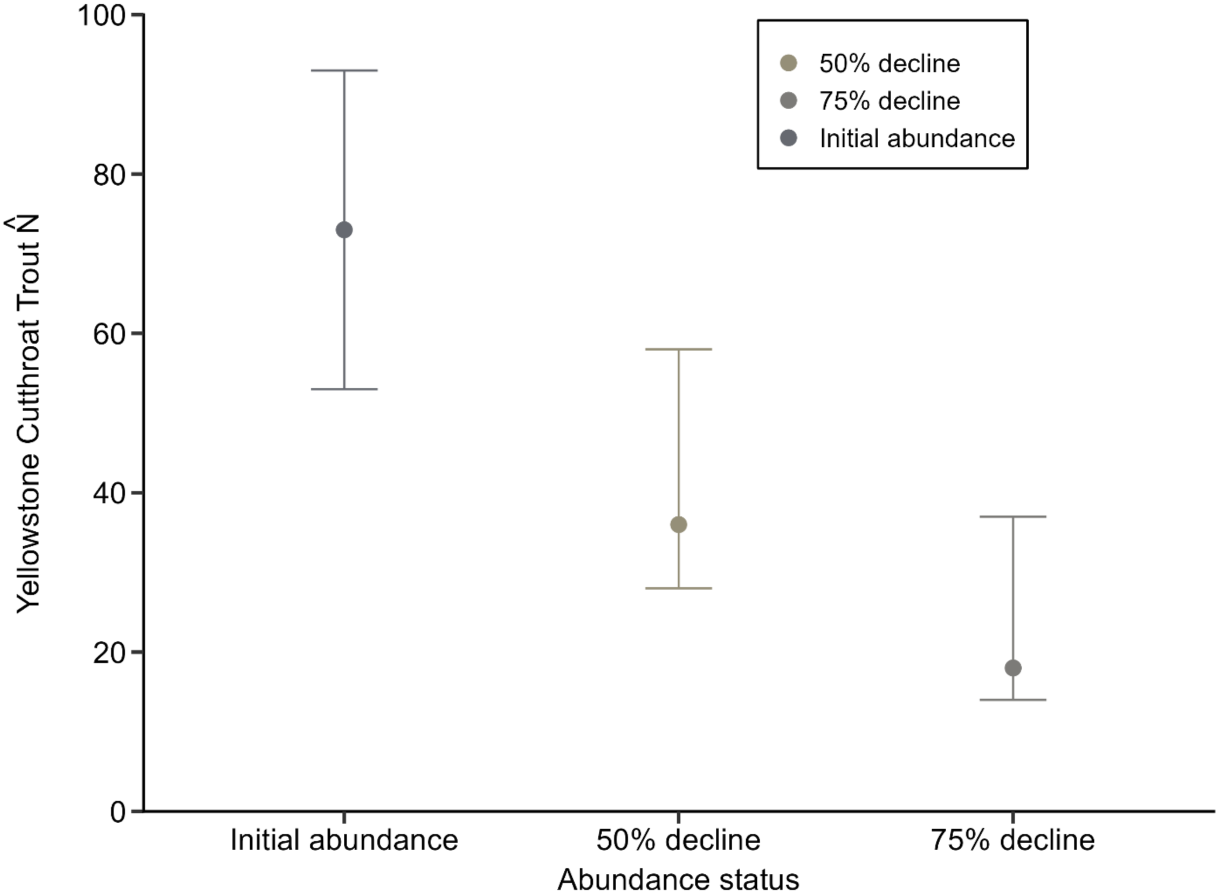

Figure 2.13. Empirical (initial abundance) and simulated 50 and 75% declines in Yellowstone Cutthroat Trout abundance in Slough Creek. Fifty and 75% declines were simulated using the empirical estimates of abundance and capture probabilities from our electrofishing and angling mark-recapture study. Error bars represent 95% confidence intervals.

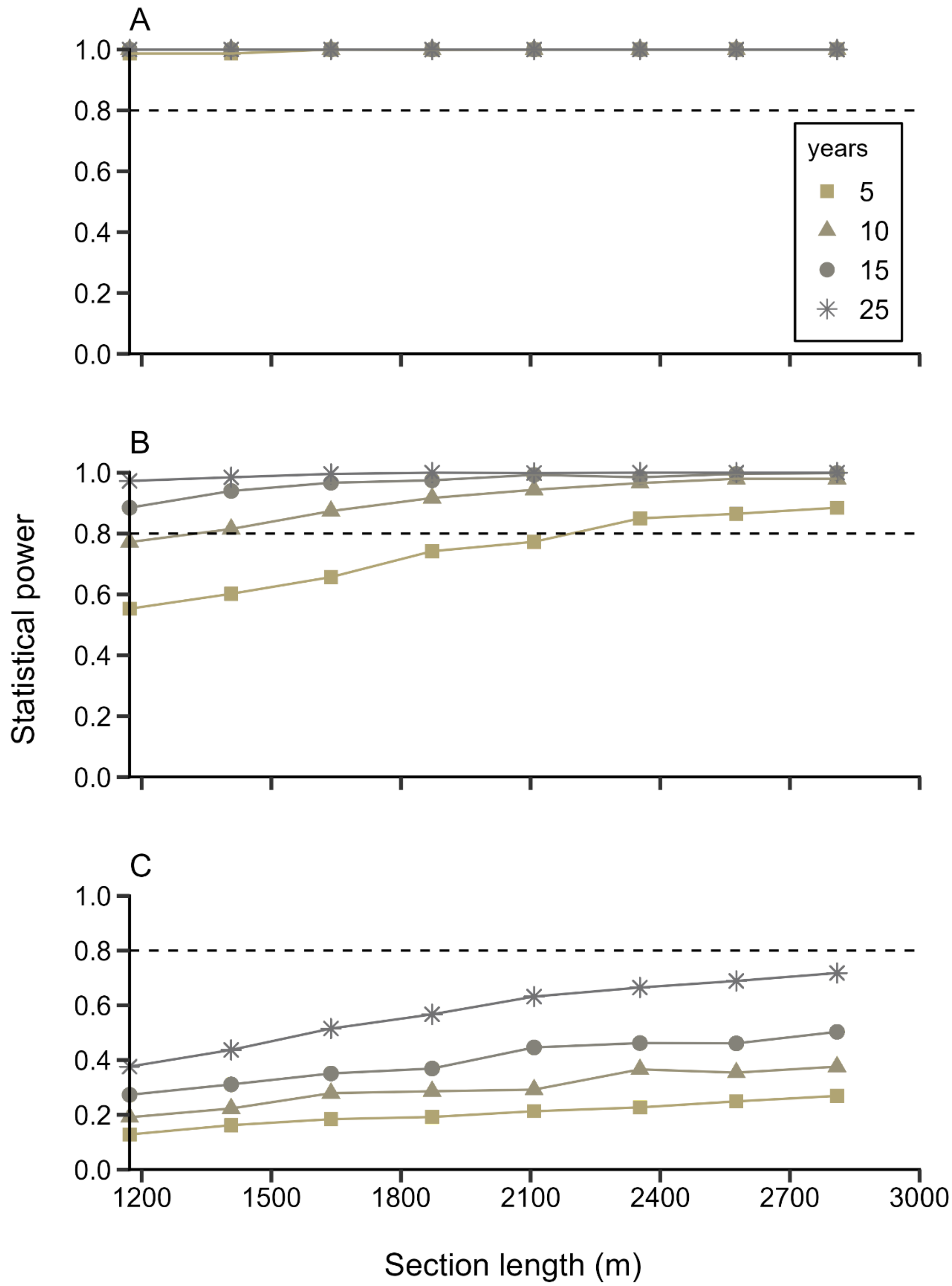

Figure 2.14. Monte Carlo simulation results for the probability of detecting (statistical power) 75% (A), 50% (B) and 25% (C) declines in Yellowstone Cutthroat Trout CPUE over 5- (gold squares), 10- (grey triangles), 15- (brown circles), and 25-year (blue asterisks) periods in the Lamar River. Results represent the proportion of simulated declines that were negative and significant (P ≤ 0.05).

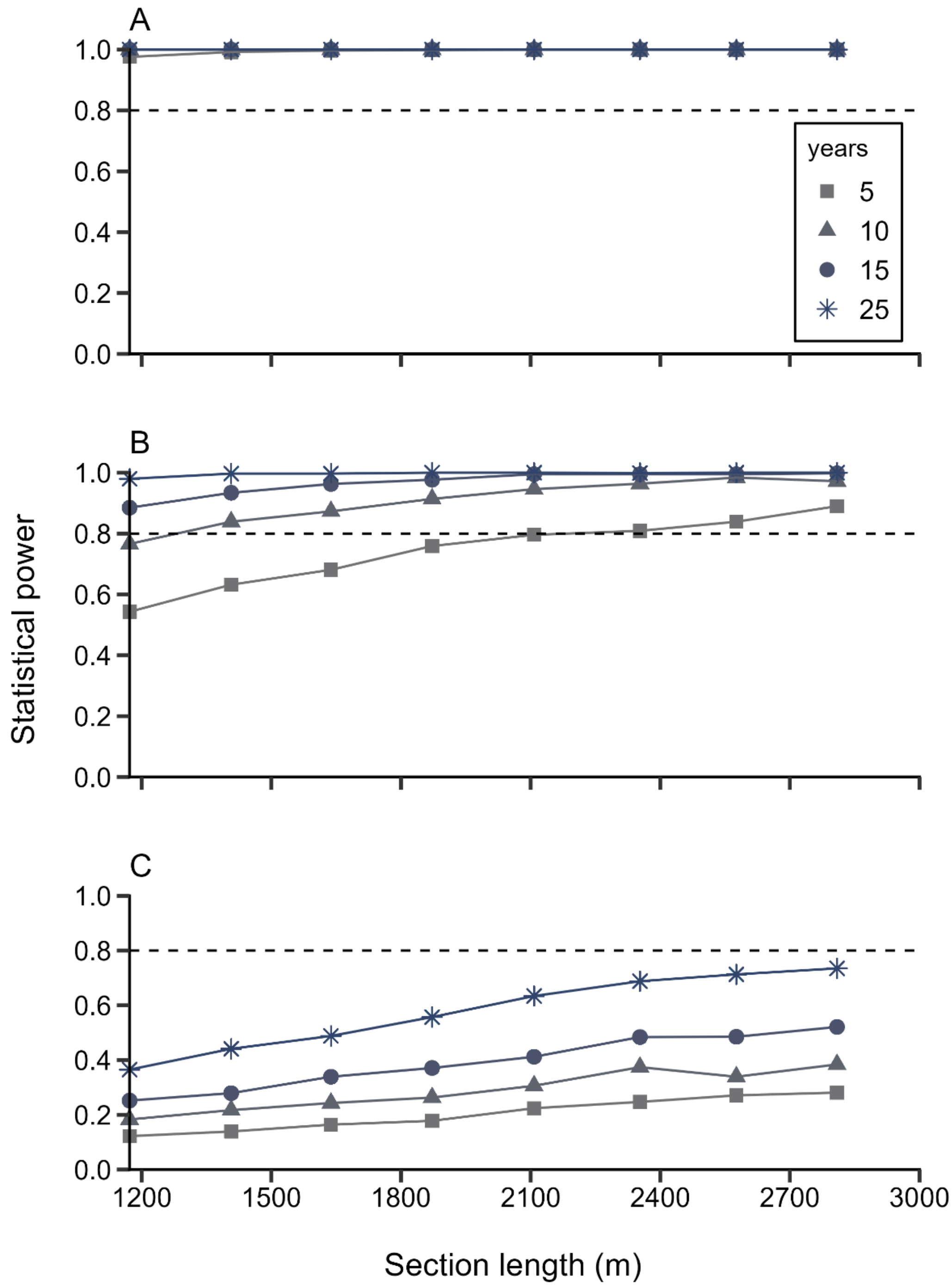

Figure 2.15. Monte Carlo simulation results for the probability of detecting (statistical power) 75% (A), 50% (B) and 25% (C) declines in nonnative trout CPUE over 5- (gold squares), 10- (grey triangles), 15- (brown circles), and 25-year (blue asterisks) periods in the Lamar River. Results represent the proportion of simulated declines that were negative and significant ($P \leq 0.05$).

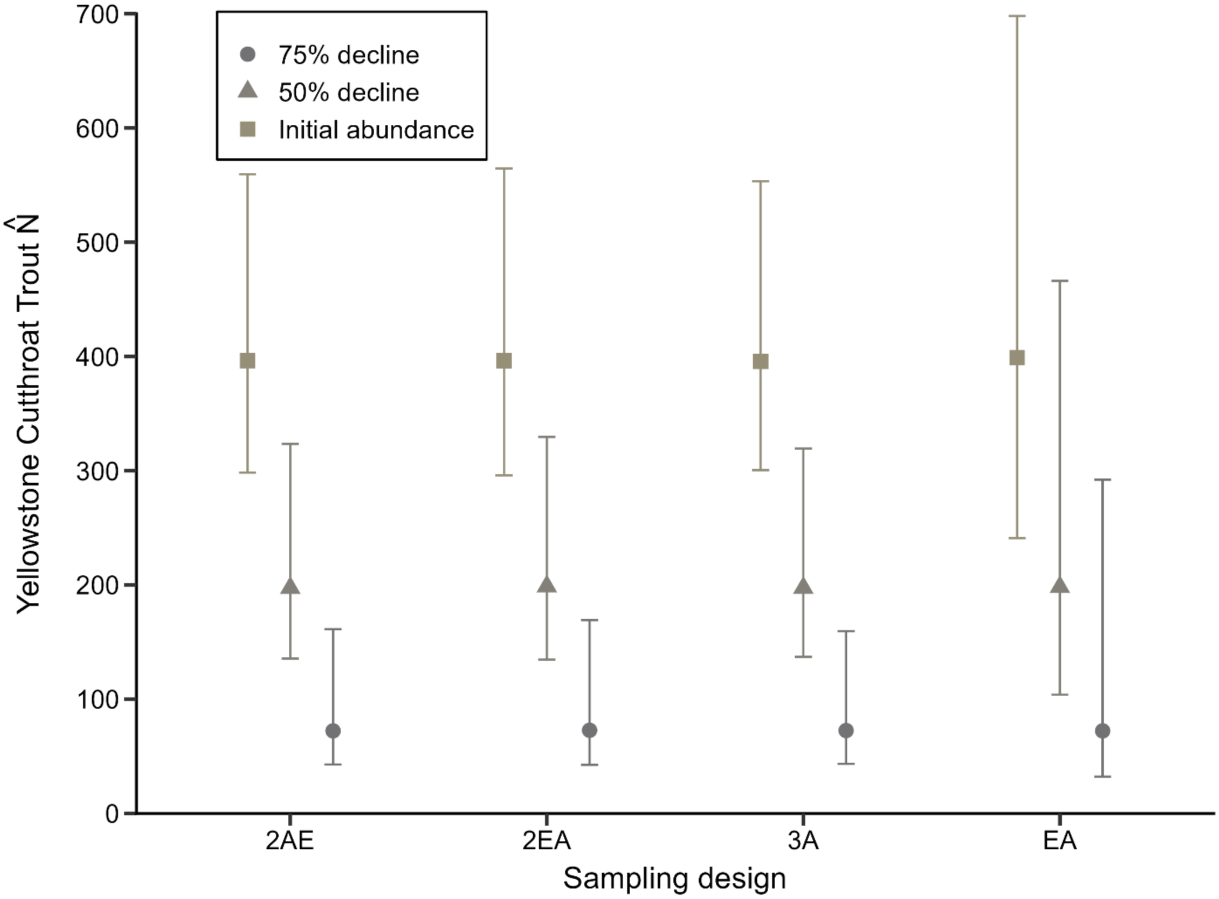

Figure 2.16. Simulated 50% (triangles) and 75% (circles) declines in Yellowstone Cutthroat Trout abundance in the Lamar River across simulated sampling designs when compared to simulations informed by the empirical abundance estimate (squares). 3A represents an all-angling sampling design, 2AE represents two angling events and one electrofishing event, 2EA represents two electrofishing events and one angling event, and EA represents a standard Lincoln-Peterson design with one electrofishing and one angling event. Points (circles and triangles) represent the median abundance estimates from our simulations, and error bars represent the median upper and lower 95% confidence intervals.

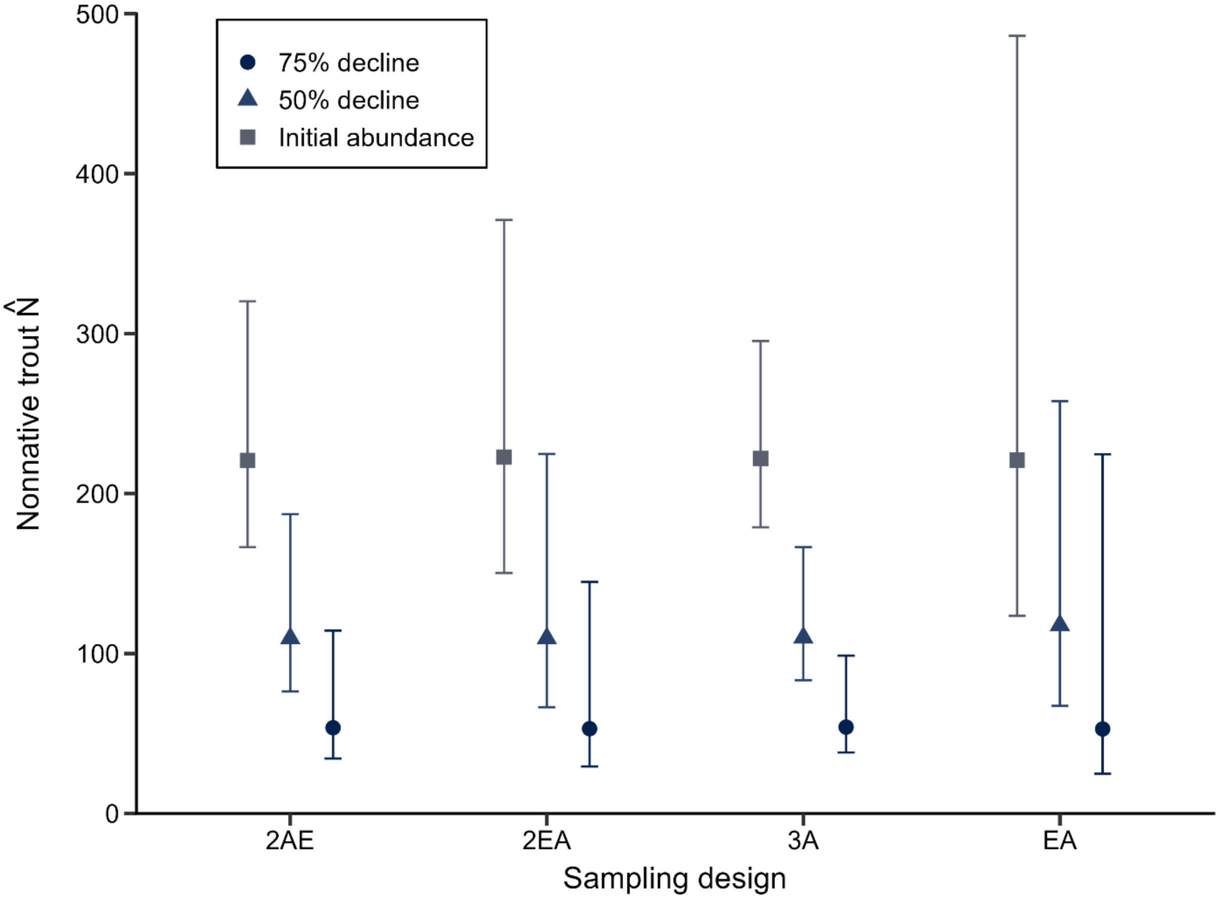

Figure 2.17. Simulated 50% (triangles) and 75% (circles) declines in nonnative trout abundance in the Lamar River across simulated sampling designs when compared to simulations informed by the empirical abundance estimate (squares). 3A represents an all-angling sampling design, 2AE represents two angling events and one electrofishing event, 2EA represents two electrofishing events and one angling event, and EA represents a standard Lincoln-Peterson design with one electrofishing and one angling event. Points (circles and triangles) represent the median abundance estimates from our simulations, and error bars represent the median upper and lower 95% confidence intervals.

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CHAPTER THREE

EVALUATION OF A FIELD-BASED HYBRID IDENTIFICATION KEY: IMPLICATIONS FOR NATIVE AND NONNATIVE TROUT MANAGEMENT IN YELLOWSTONE NATIONAL PARK

Introduction

Successful management of native taxa requires reliable methods for assessing their population status and trends and those of any nonnative taxa that threaten their persistence (Budy et al. 2020; Coggins et al. 2011). Population assessments require accurate estimates of abundance and population dynamic rate functions including survival, growth, and recruitment (Pope et al. 2010), typically estimated using empirical field data. Field identification of target taxa requires the presence of unique, recognizable characteristics (Quist et al. 2009). However, identifying closely related taxa, or hybrid individuals, can be difficult when the taxa share similar phenotypic traits (Haig et al. 2004). Introgressive hybridization further confounds identification because hybrid offspring that backcross with native or nonnative genotypes may exhibit traits similar to parental taxa (Haig et al. 2004; Eastham and Nicholls 2005), potentially limiting the likelihood of correctly identifying individuals. When native and nonnative taxa hybridize, incorrect identification may generate biased estimates of key parameters of interest leading to erroneous management decisions (Baumsteiger et al. 2005).

Nonnative Rainbow Trout (RBT) *Oncorhynchus mykiss* occur sympatrically throughout much of the native range of Yellowstone Rocky Mountain Cutthroat Trout (YCT) *O. virginalis* (Endicott et al. 2016) resulting in the extirpation of many YCT populations because of introgressive hybridization (Allendorf and Leary 1988). Management plans designed to ensure the long-term persistence of YCT populations therefore often specify identification, maintenance, and monitoring of genetically unaltered YCT populations and restoration of genetically altered YCT populations through the eradication or suppression of RBT and YCT × RBT hybrids (Al-Chokhachy et al. 2014; Meyer et al. 2017b; Kovach et al. 2018).

Genetic determination and monitoring of unaltered YCT populations necessitate tissue sample collection in the field and subsequent lab analyses. Genetic analyses are becoming more affordable to conduct, and genetic methodologies are becoming increasingly advanced (e.g., genotyping), resulting in high resolution genetic data and confident assessments of population genetic statuses (Hohenlohe et al. 2011; Campbell et al. 2014; Camak et al 2021). However, selective removal or early detection of nonnative taxa requires rapid differentiation of taxa in the field. For example, selective removal of nonnative RBT and YCT × RBT hybrids (hereafter "hybrids") by mechanical means (e.g., electrofishing, angling, trapping) is often used to mitigate the loss of native Cutthroat Trout genotypes in streams where hybridized populations exist (Al-Chokhachy et al. 2014; Meyer et al. 2017b; Kovach et al. 2018). Resource constraints often preclude agencies from conducting multiple site visits to confirm the genetic status of individuals. Therefore, time- and cost-effective management requires differentiation between YCT and hybrid individuals without the use of genetic methods.

Phenotypic traits are often used to differentiate between native and nonnative trout in the field. Historically, morphological traits (morphometrics) such as scale counts, fin ray counts, and the presence or absence of basibranchial teeth have been used for field identification of Cutthroat Trout and RBT (Behnke 1988; Behnke 2002). Recent studies have identified meristic traits (e.g., coloration and spotting patterns) that can be used reliably to rapidly differentiate taxa in the field (Meyer et al. 2017a; Heim et al. 2020). These meristic traits are also used to identify individuals to species during long-term monitoring efforts, allowing determination of changes in the prevalence of hybridized individuals over time and across geographic areas (Kovach et al. 2018). However, the hybrid status of an individual often affects how reliable these traits are for field identification. For example, first generation hybrids (the hybrid offspring of two parental genotypes; F_1) often exhibit traits unique to both parental taxa such as a throat slashes from YCT and white leading edges on the pelvic and anal fins from RBT. Introgressive hybridization is often prevalent in hybridized populations, confounding the difficulty of accurately identifying those traits; multiple generation hybrids (F_2+) and back-crossed hybrid individuals (backcrosses to either parental species; BC) may exhibit traits from both taxa. Moreover, the valid use of these traits (e.g., white fin tips and head spot counts) is typically limited to hybridized individuals with a high proportion of RBT admixture (> 0.20) or trout > 70 mm (Meyer 2017a; Heim 2020). Identifying age-0 individuals using phenotypic traits may also be limited because of the lack of expression of these traits at short total lengths (Martinez 1984; Seiler et al. 2009).

A standardized identification key was developed to help the National Park Service differentiate between YCT and hybrids in the Lamar River watershed (Heim et al. 2020). Two meristic traits were identified for differentiating between Yellowstone Cutthroat Trout and hybrids with 97% accuracy: (1) the presence of white leading edges on the pelvic and anal fins and (2) the presence of ≥ 6 head spots on the top of the head (Heim et al. 2020). The key is currently used to differentiate among YCT, RBT, and CTX during suppression electrofishing and abundance monitoring efforts throughout the watershed. The National Park Service defines measurable outcomes for the success of management actions in the watershed, including outcomes for specific genetic statuses (e.g., F_1 hybrids) and age groups of trout (Koel et al. 2010); therefore, identifying limitations of this key based on the genetic status and length of individuals is paramount for successful conservation of YCT in the watershed. Our objective was to use genetic data to assess the accuracy of our field identifications and evaluate how the genetic status and length of trout affected our ability to accurately identify the genetic status of individual trout (Heim et al. 2020).

Questions

In this study, we ask: (1) What proportion of trout were correctly identified to taxon using the morphometric traits identified by Heim et al. (2020)? (2) What proportion of trout were correctly identified as native (YCT) and nonnative (RBT and hybrids) using the same traits? (3) At what length are the morphometric traits visible? (4) How does the rate of correct identification differ among individuals with different hybrid statuses (e.g., F_1, F_2, backcross [BC], RBT, and YCT)?

Methods

Study Area

This study was conducted in the lower Lamar River watershed of Yellowstone National Park. The National Park Service is attempting to curtail the spread of invasive RBT in the lower Lamar River watershed to reduce the threat of RBT and hybrid dispersal to the upper watershed (Ertel et al. 2017). We conducted this study in Slough Creek, a prominent tributary to the lower

Lamar River, and Buffalo Creek, a tributary to Slough Creek in the lower Lamar River watershed.

<u>Sample Collection</u>

We collected fish from a 4.7-km section of Slough Creek and six evenly spaced, 200-m sites in Buffalo Creek by electrofishing. Each fish collected in Slough and Buffalo creeks was identified (following suggestion from Heim [2019]) as a YCT, RBT, hybrid with white leading edges on the pelvic fins (CTX-W), hybrid with ≥ 6 head spots (CTX-H; Figure 3.1), hybrid with both white fin tips and ≥ 6 head spots(CTX-WH), or hybrid exhibiting other traits suggesting introgression such as prominent pink coloration along the lateral line (CTX-S). Total length (mm) of each fish was measured and a tissue sample was collected from each individual by removing a 4 – 9-mm^2 portion of the anal fin with scissors and storing it in a 1.5-ml vial filled with 200-proof ethanol. We attempted to include 30 individuals from each of the three phenotypes: YCT, RBT, and hybrids. Putative hybrid trout were further stratified for sampling by the morphometric traits used to identify them (CTX-W; CTX-H; CTX-WH; CTX-S). We identified trout captured in Buffalo Creek to phenotype group using the same methods, but because these collections targeted juvenile trout, tissue samples were stratified by site and age class (ages 0, 1, and 2+) using a length-frequency histogram. We fit an N-mixture model to the length-frequency histogram to delineate age-classes (Puchany 2019; Figure 3.2). In total, 74 tissue samples from Slough Creek and 67 tissue samples from Buffalo Creek (n = 141) were sent to the University of Wyoming for genetic analysis. Genetic analysis followed genotyping-by-sequencing methods described by Mandeville et al. (2019). Trout were identified as YCT, RBT,

F_1 hybrids, F_2 hybrids, BC YCT hybrids, BC RBT hybrids, and "other" based on the proportion of YCT and interspecific ancestry estimated during genetic analysis (Figure 3.3).

Analysis

First, we calculated the proportions of trout < and ≥ 100 mm that were identified correctly in the field. For this analysis, we defined an observation as "correct" if we correctly classified a trout as native or nonnative. For example, if we identified a genetically determined RBT as a hybrid, we deemed the identification to be correct in terms of native versus nonnative. We also calculated (separately) the proportion of correct and incorrect observations that included pure RBT identified as hybrids, and vice versa.

We fit a GLM to determine how the accuracy of our field identifications differed among genetic statuses including pure YCT and RBT, F_1 and F_2 hybrids, BC YCT, and BC RBT, and hybrid individuals with indistinguishable levels of introgression (i.e., other). For this model, we defined an incorrect observation as one differing from the actual genetic status of the individual. For example, if we identified a pure RBT as a hybrid, the observation was deemed incorrect. We used a binomial response variable where each identification was modeled as a Bernoulli function nested within the categorical variable of genetic status. Each observation was defined as either a 1 (correct identification) or 0 (incorrect identification). Prediction intervals were calculated using the *ggpredict* function in the *ggeffects* package (Lüdecke et al. 2021).

Results

We identified 124 of 141 (89%) trout correctly as native or nonnative when all lengths were pooled and hybrids and RBT were combined (Table 3.1). For trout ≥ 100 mm, 117 of 121 trout (98%) were identified correctly whereas 6 of 20 trout (30%) < 100 mm were identified correctly (Table 3.1). We correctly identified all hybrid and Rainbow Trout (CTX-H, CTX-WH, CTX-S, and RBT phenotypes) ≥ 100 mm as nonnative except for one fish with white leading edges on the pelvic fins (CTX-W), which was a YCT. We identified 90 of 121 (74%) of trout ≥ 100 mm correctly and 4 of 20 (20%) of trout < 100 mm correctly (Table 3.2) when attempting to differentiate among YCT, RBT, and CTX. Thirteen RBT were identified as CTX-W, 11 BC RBT were identified as RBT, one BC YCT was identified as a RBT, one BC YCT was identified as YCT, one F_1 hybrid was identified as YCT, two F_2 hybrids were identified as RBT, one "other" hybrid was identified as a RBT, and one YCT was identified as a CTX-H; Table 3.3). Our GLM with genetic status as an explanatory variable suggested that only F_1 hybrids and YCT were identified with > 70% certainty whereas RBT were often identified as F2, BC RBT, BC YCT, and "other" hybrids and vice versa (Figure 3.4).

Discussion

Accurate field identification is essential for conservation and management of species. Field identification is used for suppression and monitoring of nonnative RBT and YCT × RBT hybrids to ensure the long-term persistence of native YCT. We had variable success in differentiating among trout taxa, but the key developed by Heim et al. (2020) led to high rates of accuracy for its intended purpose of differentiating individuals with and without RBT ancestry.

Differentiating Between Individuals ≥ 100 mm with and without RBT Ancestry

Our rate of success was high (98%) when differentiating between native and nonnative trout > 100 mm and corroborates the validity of the key developed by Heim et al. (2020). Regardless, understanding potential error sources may reduce the potential for subjective approaches to trout taxa identification (Heim 2019) and reduce errors. Photos of the F_1 identified as a YCT show a prominent pink stripe along the lateral line but a lack of head spotting and white fin tips (Figure 3.5). Based on our criteria for field identification, this fish should have been classified as a CTX-S (hybrids exhibiting other traits suggesting introgression such as pink coloration along the lateral line). Subjective differences among data collectors in considering characteristics other than those specified by Heim et al. (2020) may have caused this error. However, these cases were infrequent during our study, and in the three occurrences in which trout were classified as CTX-S they were in fact hybrids. Back-crossed YCT often exhibited features more closely resembling YCT, and the BC YCT identified as a YCT did not show any visual signs of hybridization and its proportion RBT admixture was low (0.16; Figure 3.6). Error rates for identification of individuals with low proportions of RBT admixture (< 0.20) are often high (Meyer et al. 2017a; Heim et al. 2020). Therefore, detection of hybrids with low levels of RBT ancestry using field identification protocols may be difficult if not impossible.

Differentiating Between Individuals < 100 mm with and without RBT Ancestry

We had low success (30%) differentiating between native and nonnative trout < 100 mm. For example, hybrid and RBT trout in this length class were often misclassified as YCT because of the lack of white fin tips and presence of < 6 head spots. These traits may have been undetectable under field conditions, were less defined than in trout < 100 mm, or had not been expressed yet (Martinez 1984). Meyer et al. (2017) suggested that these traits may be expressed first in fry, but our results do not corroborate this suggestion. However, all trout < 100 mm were collected in Buffalo Creek, and rates of phenotypic variation among YCT in other watersheds may be different. We also lacked trout ranging from 63 to 97 mm, with only one individual > 62 mm (98 mm). Trout were collected in mid-August, and all were assumed to be age 0 apart from the 98-mm individual. The minimum length used for the key developed by Heim et al. (2020) was 72 mm; we may have missed an important growth period in which white fin tips and head spotting may be expressed. However, YCT growth is typically slow in high-elevation streams (Hildebrand and Kershner 2004; Uthe et al. 2016), and we observed trout in Slough Creek ranging from 39 to 75 mm (Chapter 2) in October. Therefore, we would not expect substantial additional growth to occur until the following Spring.

Differentiating Between RBT and CTX

Interestingly, we had low success when attempting to differentiate between RBT and hybrids. The absence of a throat slash on many of the hybrids identified as RBT indicates that throat slashes were either uncommon or too faint to identify. Conversely, rates of faint throat slashes on RBT vary from 16.0 in the Lamar River watershed (Heim et al. 2020) to 50.3% in the Snake River watershed (Meyer et al. 2017a), which could explain our misidentification of several RBT as hybrids. Subjectivity in differentiating between a faint slash and abnormal coloration under the jaw may have also compounded this error (Weigel et al. 2002).

Additional investigation of techniques for identifying trout < 100 mm such as inspection in a more controlled environment (e.g., the lab) or with a better backdrop may be warranted. Emerging artificial-intelligence technologies may also aid in differentiating between YCT and hybrids. For example, artificial intelligence is being used in creative smartphone applications (iCatch www.icatch.app/; TroutSpotter, beta) to differentiate taxa and individuals based on phenotypic characteristics. These methods may reveal distinguishable phenotypic characteristics that are overlooked by human observers.

We demonstrated that the existing key has a high rate of success when used to differentiate between native and nonnative trout ≥ 100 mm in length. The key is therefore useful for traditional electrofishing or angling monitoring and suppression efforts in the Lamar River watershed; trout often recruit to angling and electrofishing gears at lengths > 120 mm (National Park Service, unpublished data). Conversely, inference from targeted, age-0 trout sampling may be limited without genetic data. For nonnative trout suppression to achieve the greatest reduction of RBT ancestry among a population or mixed-stock aggregation, a more aggressive approach may be warranted, with the removal of any trout suggesting any sign of hybridization whatsoever to ensure the greatest reduction of RBT alleles from the management area. We did not directly attempt to identify trout by their hybrid status (e.g., F_1, F_2, backcrosses) nor would such differentiation be useful for suppression or monitoring without further investigation. Management goals related to specific genotypes (e.g., F_1) should rely on genetic data collection to ensure accurate results.

Tables and Figures

Table 3.1. Proportion of correct identifications of native and nonnative trout by length group and combined when differentiating among trout with and without Rainbow Trout ancestry in the Lamar River, Slough Creek, and Buffalo Creek, Yellowstone National Park in 2021.

Trout lengths	Total observations	Correct IDs	Incorrect IDs	Proportion correct
≥ 100 mm	121	118	3	0.97
< 100 mm	20	6	14	0.30
All lengths combined	141	124	17	0.89

Table 3.2. Sample sizes and proportions of correct and incorrect identifications among all trout phenotypes. Phenotypes identified represent YCT (Yellowstone Cutthroat Trout), RBT (Rainbow Trout), CTX-W (hybrids with white leading edges on the pelvic fins), CTX-H (hybrids with ≥ 6 head spots), CTX-WH (hybrids with both head spots and white fin tips), or CTX-S (hybrids exhibiting other traits suggesting introgression such as prominent pink coloration along the lateral line). Trout were collected in the Lamar River, Slough Creek, and Buffalo Creek in 2021.

Phenotype identified in field	Total observations	Correct IDs	Incorrect IDs	Proportion correct
≥ 100 mm				
YCT	28	26	2	0.93
RBT	22	7	15	0.68
CTX-W	11	11	0	1.00
CTX-H	16	15	1	0.94
CTX-WH	41	28	13	0.68
CTX-S	3	3	0	1.00
Total	121	90	35	0.74
< 100 mm				
YCT	15	1	14	0.07
CTX-W	4	3	1	0.25
CTX-WH	1	0	1	0
Total	20	4	16	0.20

Table 3.3. Relative proportions (%) of field determinations by hybrid statuses (BC = backcrossed; F1 = first generation; F2 = second generation). Field identifications represent YCT (Yellowstone Cutthroat Trout), RBT (Rainbow Trout), CTX-W (hybrids with white leading edges on the pelvic fins), CTX-H (hybrids with ≥ 6 head spots), CTX-WH (hybrids with both head spots and white fin tips), or CTX-S (hybrids exhibiting other traits suggesting introgression such as prominent pink coloration along the lateral line).

	Genetic Status						
Field identification	Yellowstone Cutthroat Trout N = 28	BC Yellowstone Cutthroat Trout N = 8	Rainbow Trout N = 25	BC Rainbow Trout N = 33	F_1 hybrids N = 30	F_2 hybrids N = 9	"Other" hybrids N = 8
			≥ 100 mm				
YCT	0.96	0.17	0	0	0.04	0	0
RBT	0	0.17	0.33	0.41	0	0.29	0
CTX-W	0	0	0	0.15	0.26	0	0.17
CTX-H	0.04	0.66	0	0.03	0.3	0.14	0.17
CTX-WH		0	0.67	0.41	0.36	0.29	0.66
CTX-S	0	0	0	0	0.04	0.28	0
			< 100 mm				
YCT	1.0	1.0	0.5	0.67	0.67	1.0	1.0
RBT	0	0	0	0	0	0	0
CTX-W	0	0	0.25	0.33	0.33	0	0
CTX-H	0	0	0	0	0	0	0
CTX-WH	0	0	0.25	0	0	0	0
CTX-S	0	0	0	0	0	0	0

Figure 3.1. Example of the pictures taken of fish during sampling in Slough Creek, Yellowstone National Park in 2021. This trout was correctly identified as a “CTX-H”—a hybrid with 6 or more head spots but no white edges on the anal or pectoral fins. Genetic analysis confirmed this fish was a back-crossed Yellowstone Cutthroat Trout.

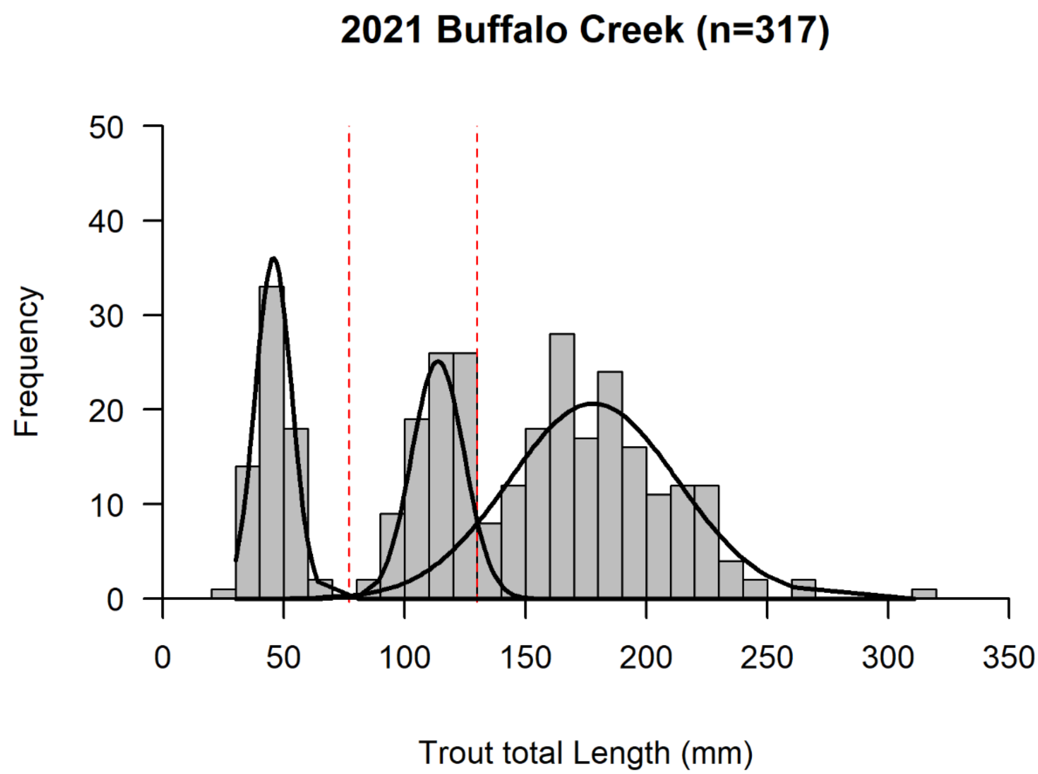

Figure 3.2. Length-frequency histogram of trout captured in lower Buffalo Creek by electrofishing. Age classes (ages 0, 1, and 2+) are defined by gaussian curves using a N-mixture model. Lengths are binned by 10-mm length intervals. Red lines indicate length cutoffs for age classes. Figure adapted from Puchany (2019).

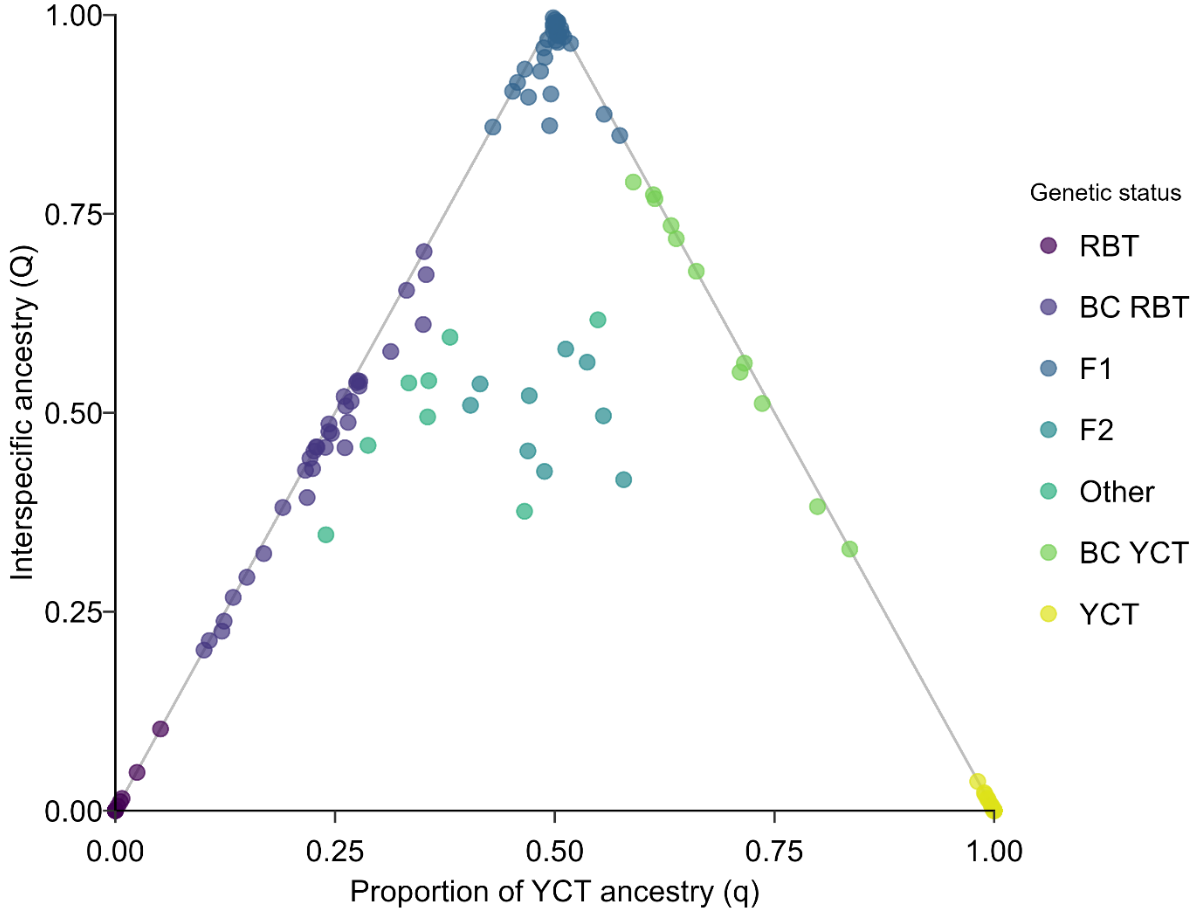

Figure 3.3. Hybrid status of individuals genotyped during our study. Rainbow Trout (RBT), BC (backcrossed) RBT, F_1 (first generation hybrids), F_2 (second generation hybrids), other (individuals with varying levels of introgression), BC Yellowstone Cutthroat Trout (YCT), and YCT are represented.

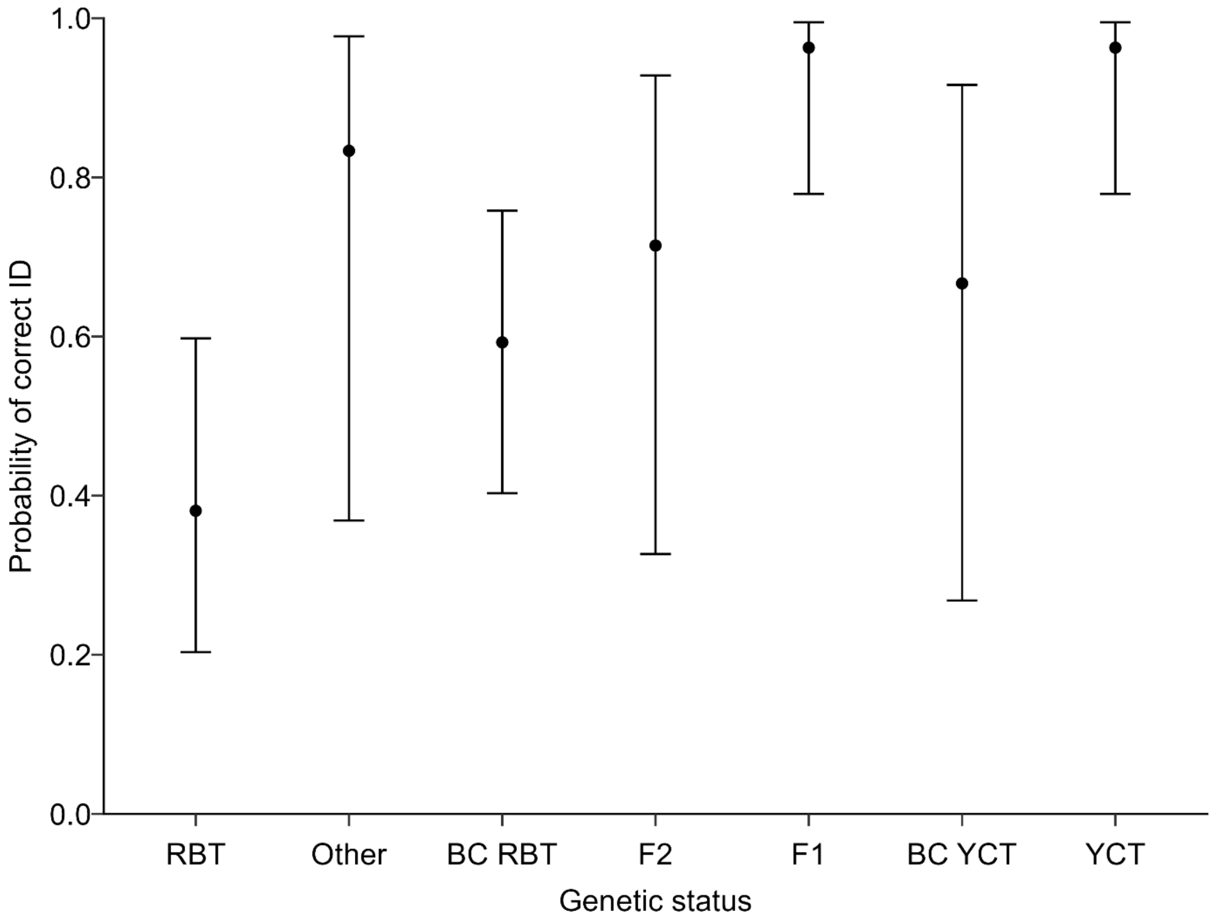

Figure 3.4. Predicted probabilities of correctly identifying trout > 100 mm of different genetic statuses. Genotypes represented include Rainbow Trout (RBT), BC (backcrossed) RBT, F_1 (first generation hybrids), F_2 (second generation hybrids), other (individuals with varying levels of introgression), BC Yellowstone Cutthroat Trout (YCT), and non-hybridized YCT. Error bars represent 95% prediction intervals.

Figure 3.5. A first-generation hybrid that was incorrectly identified as a Yellowstone Cutthroat Trout because of the lack of both white leading edges on the anal and pelvic fins and head spots.

Figure 3.6. A back-crossed Yellowstone Cutthroat Trout that was incorrectly identified as a Yellowstone Cutthroat Trout because of the lack of both white leading edges on the anal and pelvic fins and head spots.

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CHAPTER FOUR

CONCLUSION: MONITORING AND MANAGEMENT RECOMMENDATIONS

Recommendations

Juvenile Sampling

Juvenile sampling (trout < 100 mm) could be used to monitor the genetic status of distinct populations, but catch-per-unit-effort (CPUE) or abundance should only be estimated if genetic data are collected concurrently. Genetic analyses can be used to characterize the frequency of Rainbow Trout alleles among a population or classify the proportion of individuals in a population with Rainbow Trout ancestry. For example, targeted juvenile sampling in lower Buffalo Creek could be implemented to evaluate the immediate effect of the rotenone treatment. High frequencies of Rainbow Trout alleles would indicate recolonization of the system by existing Rainbow and hybrid trout following the treatment, indicating a need for further suppression of established nonnative trout populations. Conversely, a reduction in the frequency of Rainbow Trout alleles or individuals with RBT ancestry would indicate a positive effect. If future juvenile trout surveys are conducted, efforts should be focused on low-velocity habitats with adequate cover (boulders and large woody debris) adjacent to the main flow. These methodologies may also be useful elsewhere to determine the strength of early year classes or assess distinct populations of Yellowstone Cutthroat Trout of high conservation value (Cegelski et al. 2006; Uthe et al. 2016; Al-Chokhachy et al. 2019; Heim et al. 2020).

Adult Sampling

Taxa Identification. Monitoring should focus on classifying an individual trout as either a YCT or as an individual with RBT ancestry when using only phenotypic traits. Classifying trout based on morphological characteristics will reduce error and subjectivity in the field (Heim 2019). We recommend training all technicians to use this identification scheme in the Lamar River watershed. Individual fish with any amount of RBT ancestry should be combined into one group (e.g., "RBT-AN" or "nonnative") for abundance or catch-per-unit-effort estimates. Any performance metrics that include a specific hybrid status (i.e., F_1) would require genetic methods. We also agree with Heim (2019) in recommending that costly genetic analysis of juvenile trout be reserved for specific questions or management actions focused on distinct populations rather than for mixed-stock aggregations in main-stem locations.

Snorkeling. We demonstrated the feasibility of snorkeling for monitoring trout abundance in Slough Creek; however, variability in environmental conditions should be considered. Snorkeling may only be useful in pools < 2 m deep, limiting the inference space of any monitoring. Therefore, we do not recommend using snorkeling as a monitoring method in Slough Creek or the Lamar River.

CPUE. Estimating CPUE by electrofishing may be useful for detecting declines in CPUE of > 50%. We recommend a minimum section length of 3,600 m in Slough Creek and 2,200 m in the Lamar River for CPUE surveys based on the power analysis for detecting trends (Chapter 2). Transporting electrofishing gear to and from the sampling locations was time consuming, but after gear is in place, the entire sections can be sampled with little additional effort. Sampling three long reaches may be an alternative strategy to reduce error by increasing replication (Dauwalter et al. 2009) and would allow for estimation of inter-site variability. With less than three years of data at a single site, we were unable to capture year-to-year variation of the mixed-stock aggregations in the Lamar River and Slough Creek (Dauwalter et al 2009); this variation can be estimated in the future with ≥ 3 years of data.

Mark Recapture. A dual-gear approach is recommended for estimating Yellowstone Cutthroat and nonnative trout abundances by mark-recapture methods in both streams. We recommend conducting angling before electrofishing to increase the number of tagged fish in the system. In the Lamar River, an all-angling approach may also be implemented, but analysis of these data should explore potential heterogeneity in capture probabilities among individuals or size classes. We also recommend using multiple lines of evidence to determine the efficacy of eradication and suppression efforts in the lower Lamar River watershed. For example, absolute abundance and CPUE can be calculated simultaneously. Catch-per-unit-effort does not allow for inference of capture probabilities, making it difficult to determine the potential sources of bias such as environmental conditions, crew experience, and operator error. However, multiple years of data would allow for comparisons between abundance and CPUE estimates, elucidating the relationship between the estimators (Simonson et al. 2022).

Alternatives

Timing

We conducted electrofishing surveys in late summer and early autumn. However, conducting electrofishing surveys in the spring, before runoff, may increase the probability of capturing Rainbow and hybrid trout when they are migrating into the Lamar River and Slough Creek to spawn (Heim et al. 2020). Conversely, electrofishing efficiency may be higher in late autumn because of low discharge levels. Night electrofishing may also increase electrofishing effectiveness (Temple and Pearsons 2000).

Electrofisher Configuration and Settings

Alternative electrofishing methods or less conservative electrofisher settings (e.g., higher voltages, duty cycles, and frequencies) may be warranted in Slough Creek to increase efficiency (Kolz 1989). For example, throwable-anode configurations, rather than the boom-mounted anodes we used, have been shown to reduce fright bias (Monahan 1991; Ensign et al. 2002). This method allows operators to target specific habitats (i.e., pools) before trout observe the approaching boat (Matt McCormack, Montana Fish, Wildlife, and Parks, personal communication).

Calculating Relative Taxa Proportion or the Ratio of Nonnative to Native Trout

Calculating the relative proportions of each taxon (Yellowstone Cutthroat Trout and nonnative trout) could be an alternative to absolute abundance and CPUE. We observed agreement in the proportion of nonnative trout sampled during electrofishing and snorkeling surveys in Slough Creek (Chapter 2). The binomial data structure could be used to estimate uncertainty around the proportion estimate. Estimates of relative taxa proportions or ratios of native to nonnative fish could be estimated in conjunction with absolute abundance and CPUE, providing multiple lines of evidence. This “multiple estimator” approach could be used to evaluate the relationship between the different estimators over time.

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