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GENETIC RESCUE OF ISOLATED CUTTHROAT TROUT

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Genetic rescue of isolated cutthroat trout

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ABSTRACT

Anthropogenic habitat destruction has isolated innumerable populations that now face increased extinction risk due to demographic and genetic factors. Although often the best strategy, restoring connectivity can be challenging or even harmful. Such is the case for westslope cutthroat trout (*Oncorhynchus clarkii lewisi*; WCT) in the Missouri River basin, which are limited to completely isolated populations. Nonnative species threaten WCT in connected watersheds and barrier removal could be detrimental. My dissertation examines trade-offs and strategies for the management of isolated WCT. I first examined how nonnative trout species and climate change influence the distribution of WCT using a multispecies, dynamic occupancy model parameterized with 21,917 surveys collected over 30 years. I predicted that the future distribution of WCT will decline by 16%, primarily due to warming water increasing the distributions of harmful nonnative species. I next asked whether genetic metrics indicated that isolated WCT populations are at risk of inbreeding depression. I found very low effective population sizes ($N_e < 25$) in two of five WCT populations, suggesting risks of inbreeding depression could be high. A promising conservation strategy is to restore gene flow into small populations, which can increase vital rates and, ultimately, persistence probability (i.e., genetic rescue). To examine genetic rescue as a conservation strategy for WCT, we first conducted a literature review to examine what aspects of genetic rescue remain uncertain, including the duration and magnitude of genetic rescue and when gene flow may reduce fitness. Finally, we conducted an experimental test of genetic rescue in four isolated WCT populations. In the two smallest populations, we found that F1 hybrids had a 71% and 379% increase in fitness relative to residents, suggesting genetic rescue occurred. However, in the two larger populations, we found minimal evidence for genetic rescue. Overall, this research demonstrates that isolation likely poses risk to WCT, but removing barriers could pose a far greater risk owing to increased interactions with nonnative trout species. These results provide further evidence that when restoring connectivity is not an option genetic rescue is a powerful conservation strategy for at-risk populations of diverse taxa.

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CHAPTER 1: Introduction and Overview

Freshwater ecosystems contain a considerable portion of global biodiversity yet are among the most threatened ecosystems on earth (Dudgeon et al. 2006, Tickner et al. 2020). Key anthropogenic threats for freshwater ecosystems include climate change, invasive species, habitat loss, and habitat fragmentation (Su et al. 2021). Importantly, the dendritic nature of stream networks exposes them to severe human-driven habitat fragmentation through the construction of water withdrawals, culverts, and dams (Fagan 2002, Gido et al. 2015). Habitat fragmentation threatens populations by preventing critical life-history stages that require movement to other water bodies and by reducing or eliminating demographic and genetic connectivity, often forcing populations to persist in complete isolation (Brauer and Beheregaray 2020).

Small, isolated populations face increased extinction risk in part due to genetic factors (Soulé and Mills 1998). Small populations tend to have reduced effective population sizes, and thus lose genetic variation at a rapid rate. This is concerning because genetic variation determines a population's ability to adapt to environmental changes (Kardos et al. 2021). Additionally, the frequency of inbreeding increases in small populations, which can expose deleterious recessive alleles (Charlesworth and Willis 2009), and, in turn, reduce vital rates and potentially population growth rate (Bozzuto et al. 2019). Innumerable isolated populations are likely at risk from genetic factors (Ralls et al. 2018).

A promising conservation strategy for small, isolated populations is to mediate gene flow to alleviate genetic problems (Tallmon et al. 2004, Whiteley et al. 2015b). Gene flow can increase genetic variation, and allow for adaptive responses to environmental change (Bell and Gonzalez 2009, Gonzalez et al. 2012), potentially increasing long-term persistence. Additionally, gene flow can alleviate inbreeding depression, leading to increased vital rates, population growth rate, and, ultimately, persistence probability (i.e., genetic rescue). Attempting genetic rescue is a promising conservation strategy and has contributed to several successful conservation efforts (Madsen et al. 1999, Hogg et al. 2006, Johnson et al. 2010, Weeks et al. 2017). However, assisting gene flow to promote genetic rescue remains rare as a conservation strategy (Frankham et al. 2017). Some have argued that we need a paradigm shift in the genetic management of small populations away from keeping populations in isolation to maintain genetic uniqueness toward

more widespread genetic rescue attempts (Frankham et al. 2017, Ralls et al. 2018). Despite the potential importance of genetic rescue in conservation, the use of genetic rescue is likely limited by several uncertainties. A major concern is that gene flow will instead reduce the fitness of hybrids, termed outbreeding depression (Edmands 2007). Further, genetic rescue has rarely been examined in freshwater ecosystems, inhibiting its effective implementation.

Genetic rescue is often considered a stop-gap measure to avert extinction in the short term, and genetic rescue will likely be temporary unless the habitat constraints that initially caused inbreeding depression are removed. Ideally, populations should be reconnected to restore natural gene flow (Whiteley et al. 2015b). However, barrier removal can be infeasible or impossible, and managers must weigh the severity of different threats to effectively allocate limited funds. Further, removing barriers may sometimes expose species to detrimental species interactions (Novinger and Rahel 2003). Management of isolated populations thus requires a detailed understanding of the multiple competing threats that an at-risk species faces.

Effective management of isolated populations also requires identification of the populations that are most at risk, and genetic population assessments can offer a powerful and cost-effective way to do so (Schwartz et al. 2007). Effective population size (N_e) can be considered the gold standard for genetic monitoring as it strongly influences the rate that genetic variation is lost, the efficacy of natural selection, and the degree that inbreeding accumulates in a population (Charlesworth 2009). Populations with a N_e lower than 50 are likely to face short-term risks from inbreeding (Jamieson and Allendorf 2012), and thus could be targets for genetic rescue attempts. However, N_e is notoriously difficult to estimate in age-structure populations (Waples et al. 2014). Detailed examinations of N_e not only indicate whether the target population is at risk but allow for the calculation of ratios with other parameters that are more readily estimated (e.g., effective number of breeders), thus helping to provide approximations of N_e for populations with less detailed monitoring efforts.

The trade-offs between managing populations in isolation versus increasing connectivity are exemplified by westslope cutthroat trout (*Oncorhynchus clarkii lewisi*; WCT) in Montana. WCT is a species of concern in Montana, and now occupies a small fraction of their historical range (Shepard et al. 1997, 2005). Nonnative trout have been extensively stocked throughout the range of WCT (Whiteley et al. 2019) and pose a significant threat through competition and hybridization (Allendorf and Leary 1988, Peterson et al. 2004). In particular, nonnative rainbow

trout (*O. mykiss*) readily hybridize with WCT, which can lead to outbreeding depression and genomic extinction of populations (Muhlfeld et al. 2009, Kovach et al. 2016b, Muhlfeld et al. 2017). Further, climate change is likely to increase hybridization between rainbow trout and WCT (Muhlfeld et al. 2014). The threats from invasive trout are considered to be severe enough that managers not only avoid reconnecting many isolated WCT populations but have been installing barriers to intentionally isolate WCT from nonnative species (Hilderbrand and Kershner 2000, Novinger and Rahel 2003). Isolated WCT populations have significantly reduced genetic variation (Carim et al. 2016, Kovach et al. 2021) and, concerningly, non-hybridized WCT in the Missouri River basin are almost all in complete isolation (Kovach et al. 2021). However, the threats of isolation for WCT, and freshwater fishes more generally, remain poorly understood.

My dissertation examines the competing threats to WCT in connected versus isolated populations and the trade-offs managers face while mitigating these threats. Note that throughout my dissertation, I use the first-person plural 'we' due to the highly collaborative nature of my research. We first examined the joint effects of climate change and invasive species on the distributions of native WCT in Montana. Next, we provide a detailed summary of two important evolutionary parameters - the effective number of breeders (N_b) and the effective population size (N_e) – in five small, isolated WCT populations to examine the genetic risks that these populations face. My final two chapters examine genetic rescue. We wrote a review/perspective that examined the remaining uncertainties surrounding genetic rescue and provided recommendations for how to best monitor genetic rescue to guide and promote consistent research to help advance our understanding of this potentially powerful conservation strategy. Finally, we conducted an experimental test of genetic rescue in multiple isolated WCT populations in the Missouri River basin of Montana.

In Chapter 2, we examined how climate change and invasive trout species, including brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), and rainbow trout, influence the distributions of native westslope cutthroat trout and bull trout (*Salvelinus confluentus*) (Bell et al. 2021a). Using 21,917 surveys collected over 30 years, we quantified the impacts of climate change on the past and future distributions of these five interacting native and invasive trout species throughout the northern Rocky Mountains of Montana. We found that the occupancy of native bull trout and cutthroat trout declined by 18% and 6%, respectively (1993-2018), and was

predicted to decrease by an additional 39% and 16% by 2080. However, reasons for these occupancy reductions markedly differed between species: climate-driven increases in water temperature and decreases in summer streamflow likely caused declines of bull trout, while climate-induced expansion of invasive species largely drove declines of cutthroat trout. Our results demonstrate that climate change can impact ecologically similar, co-occurring native species through distinct pathways, necessitating species-specific management actions. For WCT, we add support that the primary conservation threat is interactions with invasive species.

In Chapter 3, we estimated the effective number of breeders (N_b) and effective population size (N_e) and examine the factors driving their variation in five isolated populations of WCT. We report very low N_b (minimum of 2.4) and N_e (minimum of 9) in several populations, suggesting an immediate risk of inbreeding depression. Low ratios of N_b and N_e to N_c were largely explained by the largest fish dominating reproduction, creating a high variance in reproductive success. We also found that high variation in key life-history traits (e.g., variance in reproductive success, generation length, age at maturity, and adult life span) among populations explains differing ratios among N_b, N_e, and N_c, highlighting that caution should be taken when applying these ratios to derive parameters in other populations. Overall, the low effective sizes we report suggest that many WCT populations would likely benefit from genetic rescue attempts.

In Chapter 4, we reviewed the remaining uncertainties in predicting outcomes of genetic rescue to promote and direct future research and to hasten progress toward implementing this potentially powerful conservation strategy across a larger range of taxa. We additionally provide criteria for the evaluation of genetic rescue to promote consistency across studies (Bell et al. 2019). We identified an increase in population growth rate with evidence for a contribution from gene flow (i.e., controlling for environmental change) as the best support for verifying that genetic rescue occurred. On the other hand, an increase in heterozygosity or migrant ancestry without reference to an expectation under neutral gene flow provides the weakest evidence, as both of these metrics should initially increase irrespective of the outcome of gene flow on population persistence. We also identified several outstanding questions about genetic rescue including 1) How will the magnitude of genetic rescue vary across diverse scenarios? 2) How many generations will genetic rescue persist? 3) When and how often will genetic rescue increase, decrease, or have no influence on population growth rate? 4) How often will small, isolated populations have unique local adaptations, and how can the risks of genetic swamping

be minimized? 5) How should populations and individuals be selected for translocations? 6) Under what conditions will severe outbreeding depression occur? Addressing these questions using consistent monitoring and evaluation methods will help managers confidently implement genetic rescue, potentially making it a powerful and often inexpensive tool for decreasing extinction risk.

In Chapter 5, we conducted an experimental test of genetic rescue in isolated WCT to help address uncertainties discussed in Chapter 4 and to determine if genetic rescue is a valuable conservation strategy for WCT. We translocated 6-8 mature fish into four isolated recipient populations that spanned a gradient of inbreeding risk and carefully monitored the genetic and demographic impact of the translocations for five years. The two smallest populations had substantially increased genetic variation (39% and 215%) and increased survival from zygote to maturity (71% and 379% for hybrids compared to residents), suggesting that genetic rescue likely occurred despite high uncertainty in some estimates. The mid-sized population had a smaller increase in genetic variation and minimal effects of gene flow on fitness, and the largest population had a complete translocation failure, suggesting limited effects of gene flow or potentially outbreeding depression. We did not find clear evidence for an increase in population growth rate owing to gene flow in any population, which is considered the strongest evidence for genetic rescue. The increase in vital rates without an increase in population growth could be due to the unique ecology of cutthroat trout compared to previous study organisms, namely high fecundity and high variation in vital rates and population growth. Overall, despite evidence for genetic rescue in the smallest populations, the genetic benefits of translocations may be smaller under this translocation scenario than those reported in some previous studies, highlighting that effective, broadscale implementation of genetic rescue will require examination of diverse translocation scenarios and taxa.

My research has several implications for the management of WCT. First, we add support that invasive trout species, primarily rainbow trout, are perhaps the greatest threat to WCT. However, rising stream temperatures were not found to pose a significant risk to WCT in the absence of invasive species. We also add support that many isolated WCT populations are at risk of inbreeding depression, evidenced by low effective population sizes in the smallest streams. Augmenting gene flow can increase vital rates in WCT and potentially persistence in the smallest populations. Additionally, low levels of gene flow can result in massive increases in genetic

variation, which can lead to increased evolutionary potential for future change. Our results also have implications for barrier installation projects. We found that the amount of habitat required to maintain a N_e of 50, a common guideline for short-term inbreeding risks (Jamieson and Allendorf 2012), can be as little as 1.5 km of good habitat, but may be much higher in the smallest streams with limited high-quality habitat.

Overall, my dissertation research supports that in areas with strong threats from invasive trout species WCT populations are better managed in isolation. However, the smallest isolated populations likely face heightened extirpation risk from genetic problems and may often benefit from restored gene flow. More generally, we add further support that when reconnecting isolated populations is not an appropriate action genetic rescue is a powerful conservation strategy for diverse taxa.

CHAPTER 2: Climate change and expanding invasive species drive widespread declines of native trout in the northern Rocky Mountains, USA

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Abstract

Climate change and invasive species are major threats to native biodiversity, but few empirical studies have examined their combined effects at large spatial and temporal scales. Using 21,917 surveys collected over 30 years, we quantified the impacts of climate change on the past and future distributions of five interacting native and invasive trout species throughout the northern Rocky Mountains, USA. We found that the occupancy of native bull trout and cutthroat trout declined by 18% and 6%, respectively (1993-2018), and was predicted to decrease by an additional 39% and 16% by 2080. However, reasons for these occupancy reductions markedly differed among species: climate-driven increases in water temperature and decreases in summer flow likely caused declines of bull trout, while climate-induced expansion of invasive species largely drove declines of cutthroat trout. Our results demonstrate that climate change can impact ecologically similar, co-occurring native species through distinct pathways, necessitating species-specific management actions.

Introduction

Climate change and invasive species are leading causes of global biodiversity loss (Clavero and García-Berthou 2005, Urban 2015, Trisos et al. 2020) and will likely interact in complex ways to further threaten native species (Rahel and Olden 2008). Invasive species often have higher tolerances to changing environmental conditions than native species (Bates et al. 2013) and may be favored as climate change proceeds (Rahel 2000, Sorte et al. 2013). Thus, many populations of native species may need to cope with both altered abiotic conditions and biotic interactions under future climate change or become extirpated (Cahill et al. 2013, Ockendon et al. 2014).

Freshwater ecosystems are experiencing an outsized loss of biodiversity (Strayer and Dudgeon 2010, Burkhead 2012, Tickner et al. 2020) and are particularly vulnerable to the combined effects of climate change and invasive species (Sorte et al. 2013, Su et al. 2021). Despite these concerns, few empirical studies have examined the joint impacts of changing abiotic conditions and interactions with invasive species on native freshwater species across broad spatial and temporal scales (Gervais et al. 2020).

Trout—a group of cold-water fishes of enormous ecological and socioeconomic value (Prosek 2013)—are excellent organisms for examining these critical threats to freshwater ecosystems. Like many freshwater species, the distribution, abundance, and phenology of trout are strongly influenced by climatic conditions through species-specific adaptations to water temperature and flow regimes (Wenger et al. 2011, Kovach et al. 2016c), and climate-induced changes in these environmental conditions are predicted to have detrimental effects on many trout populations (Wenger et al. 2011, Comte et al. 2013). Moreover, invasive trout species have been widely introduced for recreational fisheries (Whiteley et al. 2019) and can impact native trout through competition, predation, and hybridization (Shepard et al. 2005, Kovach et al. 2016c). Increasing evidence suggests that climate change may be facilitating the expansion of invasive trout, potentially to the detriment of native trout species (Wenger et al. 2011, Almodóvar et al. 2012, Dauwalter et al. 2020). However, how climate-induced changes in temperature and stream flow interact with invasive species to influence the distribution of native trout across space and through time remains a critical uncertainty for developing effective climate adaptation strategies.

In this study, we use long-term monitoring data to examine how the distributions of native bull trout (*Salvelinus confluentus*) and westslope cutthroat trout (*Oncorhynchus clarkii lewisi*; here referred to as cutthroat trout) have been influenced by climate change and invasive trout species across the northern Rocky Mountains of Montana, USA. This region is well suited to examine these dynamics because it is a stronghold for native trout and spans diverse environmental gradients. Invasive trout species, including brook trout (*S. fontinalis*), brown trout (*Salmo trutta*), and rainbow trout (*O. mykiss*), have been widely introduced for sportfishing from the late 1800s to the early 1970s (Whiteley et al. 2019). Moreover, the region has warmed at nearly twice the rate of the global average over the past century (Pederson et al. 2010), resulting in rising stream temperatures, reductions in summer flow, and increased winter flooding

(Pederson et al. 2011, Isaak et al. 2012, Jones et al. 2017, Martin et al. 2020). Previous distribution modeling using space for time substitution projected a 47% decline in total suitable habitat for native and invasive trout species across the interior western USA (Wenger et al. 2011). However, time-series analyses conducted on smaller spatial scales in the northern Rocky Mountains show that warming temperatures may benefit some invasive trout species (Muhlfeld et al. 2014, Al-Chokhachy et al. 2016). We hypothesized that an increase in the distribution of invasive trout could further imperil native trout species beyond the direct challenges posed by shifting climatic conditions.

To test this hypothesis, we assessed the effects of rising stream temperatures and changing hydrological conditions on the distributions of five interacting trout species (native cutthroat trout and bull trout; and invasive brook trout, brown trout, and rainbow trout) using a multi-species dynamic occupancy model (MacKenzie et al. 2017, Kery and Royle 2020). Dynamic occupancy models allow for the direct modeling of local colonization and extinction processes, which leads to a more accurate characterization of environmental niches and interspecific interactions (Yackulic et al. 2015, Kery and Royle 2020). We parameterized this model with 21,917 fish surveys collected over 30 years (1989-2018; Fig. 2-1). We modeled initial occupancy (1989-1993) and subsequent annual colonization and persistence probabilities (1994-2018) as functions of the presence of invasive species and high-resolution (1 km) estimates of summer stream temperature (Isaak et al. 2017), summer flow (Wenger et al. 2010), and winter flood frequency (Wenger et al. 2010) (fig. S2-1). We then used parameter estimates from the dynamic occupancy model and climate change projections under the A1B emissions scenario (Wenger et al. 2010, Isaak et al. 2017) (similar to the RCP 6.0 emissions scenario) to predict the distribution of all five species across the entire stream network (127,705 km) annually from 1993 to 2080. Species interactions were allowed to evolve in our model because the distributions of invasive species could shift with climate change. Finally, we conducted a sensitivity analysis to identify the main drivers of the distribution shifts for each species. Together, these analyses describe past and future effects of changing climatic conditions and invasive species on native aquatic biota, thereby providing a detailed examination of how climate change acts directly and indirectly to influence aquatic ecosystems.

Results

Local persistence and colonization probabilities

Differences in local persistence and colonization probabilities revealed distinct environmental niches among trout species (Fig. 2-2; table S1). Invasive rainbow trout and brown trout persisted in warmer streams with higher flow, whereas brook trout persisted in streams with cooler temperatures and relatively lower flow (Fig. 2-2a). Native bull trout persisted in colder streams with higher flow (Fig. 2-2a,b). In contrast, native cutthroat trout had high persistence probabilities across a wide range of temperature and flow regimes (Fig. 2-2a,b; fig. S2-2).

Native bull trout and cutthroat trout also differed in their responses to invasive species. The presence of brown trout lowered the local persistence of bull trout (Fig. 2-2c), but this was offset by higher colonization rates (Fig. 2-2f). This suggests that brown trout cause increased habitat turnover for bull trout rather than complete displacement. In contrast, the presence of invasive species, including brook trout and, especially, rainbow trout, substantially decreased local persistence of cutthroat trout (Fig. 2-2c).

Both native trout species, as well as brook trout, generally had low colonization probabilities across all environmental conditions (Fig. 2-2d,e; fig. S2-3). This suggests that once lost, native species (and brook trout) are unlikely to recolonize streams. On the other hand, invasive brown trout and rainbow trout had the highest colonization rates, particularly in streams with moderate to high flow (Fig. 2-2e), suggesting that the distributions of these species are shifting across the landscape.

Past and future shifts in distribution sizes

We detected region-wide declines in the distribution sizes (i.e., the proportion of occupied stream length) of native trout species in the past and predicted continued declines under future projections (Fig. 2-3; table S2-2). The length of occupied habitat for bull trout and cutthroat trout declined by 18% and 6%, respectively, from 1993 to 2018 (Fig. 2-3b) and was predicted to decrease by an additional 39% and 16% by 2080 under the A1B emissions scenario. In contrast, changes in the distributions of invasive species varied from contractions to expansions. The distribution of brook trout declined by 16% in the past and was projected to decrease by an

additional 15% in the future. Brown trout declined slightly in the past (5%), and the size of their overall distribution was projected to remain stable in the future (2% increase). Conversely, the distribution of rainbow trout expanded in the past (6%) and under future projections (10%). These trends suggest that invasive rainbow trout may become more widely distributed than cutthroat trout by the end of the century in the northern Rocky Mountains (Fig. 2-3a).

Examination of trends in occupancy among watersheds revealed considerable spatial variation in distribution shifts. All species underwent both declines and expansions in at least some watersheds over the last 25 years (Fig. 2-4a-e). However, future projections showed less spatial variability (Fig. 2-5a-e), where habitat became consistently less suitable for both native trout species and more suitable for invasive rainbow trout across the majority of watersheds (Fig. 2-5a,b,e). We also found substantial differences in predicted future distribution shifts east and west of the Continental Divide in the Missouri and Columbia River drainages. As of 1993, native trout species were more broadly distributed west of the Continental Divide where abiotic conditions were more hospitable, while all three invasive trout species were more common in the east (fig. S2-1; fig. S2-4). However, future predictions suggest that the distributions of invasive brown trout and rainbow trout will substantially expand west of the Continental Divide (21% and 19%, respectively) but not to the east, while brook trout are predicted to decline less dramatically west of the Continental Divide (11%) than east (17%). Overall, the increase in invasive trout and the decline of native trout is occurring more rapidly west of the Continental Divide, the current stronghold for native trout in the region (fig. S2-4). These results suggest that the more dire environmental conditions in the east may portend future conditions in the west without sufficient conservation action.

As future climate change projections are inherently uncertain, we also examined the sensitivity of our future projections to the rate of climate change. Specifically, we re-estimated future distribution sizes when climate-induced changes in summer stream temperature, summer flow, and winter flood frequency were 50% greater by 2080 than predicted under the A1B emissions scenario, reflecting outcomes under high emissions scenarios (e.g., A2 or RCP 8.5). These more extreme projected changes had little influence on the future distribution sizes of brown trout and rainbow trout, as compared to projections under the A1B emissions scenario (fig. S2-5). In contrast, both native trout species, as well as brook trout, were predicted to experience greater declines under the more extreme climate change scenario, with the

distributions of bull trout and cutthroat trout predicted to decline by 62% and 27%, respectively. These results suggest that the faster climate change proceeds the more native trout will decline and the more invasive trout will be favored.

Drivers of distributions shifts

Sensitivity analyses in which aspects of global change were omitted from future projections revealed that altered abiotic conditions under climate change likely promoted the stability or expansions of invasive brown trout and rainbow trout (Fig. 2-6). Without future increases in stream temperature, the distribution size of brown trout was predicted to decline by 4% from 2018 to 2080, rather than remain stable (Fig. 2-6b,c), and, similarly, the distribution size of rainbow trout was predicted to remain stable rather than increase. Although future reductions in summer flow were predicted to decrease the occupancy of both species, the net effect of changing thermal and hydrological conditions allowed brown trout and rainbow to occupy a greater amount of habitat than if climate change did not occur (Fig. 2-6b,c). In contrast, climate change negatively affected brook trout; without increasing summer temperatures, brook trout would have only declined by 4%, less than one-third of the predicted decline in the full model.

The factors responsible for distribution declines differed markedly for the two native trout species. The decline in bull trout occupancy was primarily explained by reductions in summer flow and increases in summer stream temperature, not interactions with invasive species (Fig. 2-6d). Without climate-induced changes in flow and stream temperature, bull trout were predicted to undergo much smaller declines of 26% and 19% by 2080, and without changes in any abiotic conditions, bull trout were predicted to decline by only 7%. Conversely, the future distribution size of bull trout was predicted to be similar with or without invasive species in the region.

In strong contrast to bull trout, invasive species had substantial adverse effects on the future occupancy of cutthroat trout (Fig. 2-6e). Without invasive species, cutthroat trout were predicted to occupy 26% more habitat in 2080 than in 2018 despite rapid changes in stream temperatures and flow. Notably, the removal of rainbow trout alone was predicted to allow cutthroat trout to occupy 15% more habitat in 2080 rather than decline. The climate-induced reduction in suitable habitat was smaller for cutthroat trout than bull trout. Without rising stream

temperatures, the distribution size of cutthroat was predicted to undergo a decline of 6%, but this was at least in part due to reduced interactions with invasive species, which are tracking changing abiotic conditions upstream into cutthroat trout habitat (fig. S2-6). However, cutthroat trout were predicted to occupy the most habitat if no invasive species or climate change occurred, clearly demonstrating the joint impacts of these stressors on future distributions.

Discussion

Interactions between climate change and invasive species are a key uncertainty in future projections of biodiversity change (Sorte et al. 2013). Using long-term monitoring data spanning diverse freshwater ecosystems, we show that past and projected future declines of two native trout species were driven by climate-induced reductions of suitable habitat and expansion of invasive species. However, the relative impacts of these threats differed markedly among ecologically similar, co-occurring native species, demonstrating that species-specific climate adaptation strategies may be needed for conservation of freshwater biodiversity.

We found that declines in bull trout distributions were primarily driven by climate-induced increases in water temperatures and decreases in summer flow. These changing abiotic conditions reduced the distribution of bull trout by 18% from 1993 to 2018 and are predicted to cause an additional 39% decline by 2080. Bull trout are habitat specialists that require cold, connected, high-quality, and complex riverine habitats for persistence (Rieman and McIntyre 1993), and the loss of these critical habitats due to climate change has contributed to their decline (Eby et al. 2014, Kovach et al. 2017, LeMoine et al. 2020). In contrast, declines in cutthroat trout were primarily driven by negative interactions with invasive brook trout and, especially, rainbow trout. Brook trout can outcompete cutthroat trout (Peterson et al. 2004), while climate-induced expansions of rainbow trout lead to hybridization and genomic extinction of cutthroat trout (Muhlfeld et al. 2014, 2017). Surprisingly, in the absence of invasive species, our projections suggest that cutthroat trout could occupy more habitat at the end of the century than at present despite rapid climate change, consistent with a recent physiological study that found cutthroat trout have a higher thermal tolerance than previously documented (Macnaughton et al. 2021).

The distinctive pathways by which climate change threatens native trout species highlights the need for different management and climate adaptation strategies. For example,

conservation efforts for cutthroat trout may often be better aimed at reducing invasive species through intensive suppression and eradication efforts (Al-Chokhachy et al. 2014, Day et al. 2021) and intentional isolation of at-risk populations (Peterson et al. 2008). Conversely, conservation efforts for bull trout could focus on protecting, reconnecting, and restoring critical cold-water habitats across entire riverscapes (Rieman and McIntyre 1993, Armstrong et al. 2021). However, the scope for mitigating climate impacts on bull trout may be more limited since an increasing amount of stream habitat—much of which is in protected areas with minimal human impact (Isaak et al. 2015) —is predicted to exceed their narrow thermal niche as the climate continues to warm. Accounting for species-specific sensitivities to climate change and its interactions with other stressors, such as with invasive species, is a prerequisite for effective climate adaptation planning that could extend beyond freshwater fishes to include a range of other taxa.

Species distribution models are increasingly used to make projections of species' responses to future climate change, but efforts to validate these results with past data are rare (Kovach et al. 2016c). Our results provide empirical evidence that climate change has already had strong ecological impacts on native trout across the northern Rocky Mountains. Smaller-scale studies on occupancy and population dynamics within the region have documented climate-associated declines in native trout (Eby et al. 2014, Al-Chokhachy et al. 2016, Kovach et al. 2017, LeMoine et al. 2020) and increases in invasive brown trout and rainbow trout over time (Muhlfeld et al. 2014, 2017, Al-Chokhachy et al. 2016). We show that these trends have also occurred across a broad and ecologically diverse region, but with considerable spatial variation in occupancy shifts. Although native species distributions increased in some watersheds over the last 25 years, our future projections show region-wide declines through 2080. As status quo management is implicit in our model, this suggests that climate change impacts may soon overwhelm current conservation strategies unless more proactive and innovative measures are implemented.

Several previous bioclimatic studies have projected substantial declines in both native and invasive trout distributions (Rieman et al. 2007, Wenger et al. 2011, Almodóvar et al. 2012). For example, another broad-scale study in the interior western USA (which encompasses our study region) projected dramatic declines in both native cutthroat trout (58%) and invasive brook trout (77%), brown trout (48%), and rainbow trout (35%) by 2080 under the A1B emissions

scenario. In contrast, we predict smaller declines in cutthroat trout (16%) and brook trout (15%) and, importantly, increases in the distributions of invasive brown trout (2%) and rainbow trout (10%), with more pronounced increases west of the Continental Divide (21% and 19%, respectively). The disparity between these findings could be due to several factors. First, ecological conditions in the broader region examined by (Wenger et al. 2011) could differ from those in the northern Rocky Mountains, which contains a substantial amount of protected cold-water habitats. Second, the previous analysis used air temperature as a surrogate for stream temperature to estimate changes in thermally suitable habitat (Wenger et al. 2011), which may have overestimated the amount of future habitat losses. The latter possibility emphasizes that species-range projections, including those herein, should be adaptively updated as downscaled climate models are developed and future climate-change simulations are updated. Finally, our use of an extensive temporal dataset in a multi-species dynamic occupancy modeling framework likely improves future predictions of species distributions compared to models based on a single time-period (Clement et al. 2016). Occupancy models that use space-for-time substitution assume that species are in equilibrium with the environment, which is unrealistic for species experiencing range shifts (Yackulic et al. 2015). This highlights the importance of broad-scale and long-term datasets for understanding the effects of climate change and other anthropogenic stressors on freshwater biodiversity.

A major strength of our modeling approach was our ability to account for interactions among multiple native and invasive trout species under changing climatic conditions. However, other invasive fishes that we did not consider may pose additional threats to native trout persistence. For example, invasive lake trout (*S. namaycush*) have caused declines in bull trout and cutthroat trout populations inhabiting lake ecosystems (Kovach et al. 2017), emphasizing that invasive species negatively influence bull trout in some habitats. Looking forward, smallmouth bass (*Micropterus dolomieu*) have been expanding and impacting native salmonids (*Salmonidae*) in some rivers, a pattern that is predicted to continue under future climate change (Carey et al. 2011, Rubenson and Olden 2020). While our model may partially account for these additional interactions via watershed level random effects, more research is needed to understand how climate change will affect the community structure of entire aquatic ecosystems for climate adaptation planning and mitigation.

Our results add to a growing body of evidence that climate change threatens freshwater biodiversity by altering both abiotic conditions (Comte and Olden 2017) and biotic interactions (Ockendon et al. 2014). Globally, over one-third of freshwater fishes are predicted to be threatened by future climate-induced changes in water temperature and flow in at least half of their range (Barbarossa et al. 2021). Compounding this threat, many invasive species may be 'poised to prosper' and outperform native species in aquatic ecosystems under future climate change (Sorte et al. 2013), thereby further homogenizing freshwater biodiversity (Rahel 2000, Villéger et al. 2011). We add to this body of research by demonstrating that the relative threats of direct and indirect climate impacts can differ substantially for ecologically and phylogenetically similar native species. Progressive climate adaptation strategies will be essential to reverse declines in native species and prevent further homogenization of freshwater ecosystems in the face of rapid environmental change.

Methods

Study region and delineation of stream segments

Our study area encompasses the Rocky Mountains of Montana, USA. This region is a stronghold for native trout species and spans large thermal and hydrological gradients (fig. S2-1). We restricted the analysis to streams and rivers with available environmental data. Further, our study did not include lakes or the potential impacts of invasive species and climate change in lake ecosystems. Our study area included 127,705 km of stream in 39 subbasins (HUC 8). The study area was primarily within two major river drainages, the Columbia River and Missouri River basins.

We divided the regional stream network into biologically significant stream segments. Stream networks are comprised of linear sections of stream that merge with other streams at confluences. These stream confluences are often associated with changes in environmental conditions (Benda et al. 2004, Kiffney et al. 2006), and are also natural locations to begin fish surveys. Confluence to confluence stream segments are thus a meaningful spatial scale to study ecological processes (Kanno et al. 2015) while accounting for variation in detection probability.

We used the National Hydrography Dataset (NHD) to delineate confluence to confluence sections of stream. Stream segments were then created based on several additional criteria. First,

we merged stream sections of the same stream order (a metric of stream size based on contributing tributaries) until the length was ≥ 2 km or the stream order changed. Second, as larger streams and rivers are minimally influenced by confluences with smaller streams (Kiffney et al. 2006) and survey distance is generally greater in larger streams, we scaled the stream order used to determine the terminus of a stream segment based on the size of the focal stream. The downstream terminus of stream segments in second through fourth-order streams were confluences with streams that were one order lower (e.g., a third-order stream segment ends at its confluence with a second-order stream), and the downstream terminus of fifth and higher-order stream segments was their confluences with streams that were one or two orders lower (e.g., a fifth-order stream ends at its confluence with a third or fourth order stream). Third, sections of stream that crossed permanent fish movement barriers such as waterfalls and dams (Montana Fish, Wildlife & Parks MFISH database) were used to break stream segments. Finally, we excluded above barrier drainages that only contained a single first-order stream because colonization of these stream segments is impossible, and stream segments less than 50 m were deemed too small and removed from the analysis. This resulted in 39,638 stream segments with a median length of 2.6 km (*IQR* = 2.1 km).

Fish surveys

We used electrofishing data from 1989 to 2018, which covers the years with the most extensive sampling and starts well after the stocking of nonnative trout species ended (see below for stocking details), providing 21,917 surveys (Montana Fish, Wildlife & Parks MFISH database). We included all stream segments with at least one survey in our occupancy model (4,633 stream segments covering 21,874 km). We simplified surveys to detections or non-detections for each species. Detections were inferred from any survey in which at least one individual of the focal species was captured, regardless of the life-stage. Non-detections were inferred from surveys that failed to detect any fish or detected a salmonid species but not the focal species. False-positive detections were unlikely because visual identification of trout is reliable, except for hybrids between rainbow trout and westslope cutthroat trout. Any fish visually identified as a hybrid between these species was considered a rainbow trout because conservation efforts in Montana prioritize non-hybridized cutthroat trout. Hybrids between brook trout and bull trout are less common but were likewise considered to be brook trout in this analysis.

Covariates

Initial occupancy, colonization, and persistence probability were all modeled as a function of summer stream temperature and flow, which are key limiting factors for all trout species throughout their native and invasive ranges and are often considered 'master variables' in freshwater ecology (Wenger et al. 2010, Kovach et al. 2016c, Isaak et al. 2017). Additionally, we included winter flood frequencies in all biological models because fall spawning trout (including bull trout, brook trout, and brown trout) can be negatively influenced by winter flooding (Wenger et al. 2011). We limited abiotic covariates to these three well-supported factors to avoid oversaturating the model because directly modeling colonization and persistence probability requires a large amount of temporal data. We obtained spatially explicit summer stream temperature predictions from the NorWeST database (Isaak et al. 2017). Mean summer flow and winter flood frequency (number of winter days in the top 5% of annual flows) were acquired from the Western U.S. Stream Flow Metric Dataset (Wenger et al. 2010). These stream temperature and flow metrics were available both during an initial baseline period (1977-2002 and 1993-2011 for flow and temperature, respectively) and in two future periods under the A1B emissions scenario (2040s and 2080s) (Wenger et al. 2010, Isaak et al. 2017). We predicted annual stream temperature and flow metrics using separate linear regressions for the two available periods: the middle of the initial period (1987 and 2002 for flow and temperature, respectively) to 2040 and 2040 to 2080. Linear regressions were fit separately for each stream segment, and temperature and flow were predicted in each stream segment and every year from 1989 to 2080. Thus, the climatic covariates were both spatially and temporally explicit. We obtained covariates for each stream segment using ArcGIS (ESRI 2015), and, since these covariates had a spatial resolution of 1 km, covariate values were averaged for stream segments greater than 1 km.

Extensive fish stocking records (1924–1980; Montana Fish, Wildlife & Parks MFISH database) were used to estimate a spatially explicit index of stocking intensity for all invasive species. Specifically, stocking intensity was derived for each stream segment using the following equation:

$$stocking\ intensity = \sum_{1}^{\#\ Locations} \#\ Stocked * e^{-0.05 * Distance}$$

Where *# Locations* is the number of locations within a connected watershed where stocking has occurred, *# Stocked* is the total number of fish stocked at a location across all years, 0.05 is the constant decay rate for straying fish, and *Distance* is the distance to each stocking site in km (Bennett et al. 2010, Muhlfeld et al. 2017). Stream distances were calculated using the National Hydrography Dataset.

We standardized all continuous covariates (i.e., mean = 0, sd = 1) to improve model convergence. Additionally, we transformed stocking intensity, flow, and stream length because these covariates have a strong right skew. Transformations included the cube root of stocking intensity, the square root of stream length, and the natural logarithm of flow. Pairwise correlations of the covariates used in our analysis were all below 0.7 (table S3), suggesting multicollinearity was not a substantial issue (Dormann et al. 2013).

Additionally, stream segments were designated to be impossible to occupy or colonize if they were located in a stream where the focal species has never been detected, either because it is outside of their native range (bull trout never colonized the Missouri River basin) or above a complete stream barrier (Montana Fish, Wildlife & Parks MFISH database). We therefore accounted for habitat fragmentation and its interaction with climate change by not allowing for upstream colonization above natural and anthropogenic barriers (Herrera-R et al. 2020).

Analyses

We used extensive survey data and microclimatic predictions to parameterize a Bayesian multi-species dynamic occupancy model (MacKenzie et al. 2003, Kery and Royle 2020). Dynamic occupancy models account for imperfect detection and directly model local colonization and extinction processes (MacKenzie et al. 2003, 2017). Dynamic occupancy models have closed periods in which multiple surveys are used to model detection probability and open periods in which local colonization and extinction occurs. The open period extended from February 20 to December 14 (298 days) to capture the entire life history of each species, but the majority of surveys (72%) were conducted from July 1 to September 30 (91 days). Further, the range of sampling dates for a given site was much shorter (*median* = 29 days). Due to the long open period, ‘occupied’ habitat is better interpreted as habitat that is used by the species, rather than habitat that sustains a year-round population.

In a dynamic occupancy model, z_{it} is the latent state representing the true, unobserved occupancy of a stream segment *i* during time *t*. The occupancy at the first period (z_{i1}) is determined by the initial occupancy probability (ψ_{i1}). For all subsequent time steps, z_{it+1} is conditional on occupancy in the previous time step. Sites that were occupied remain occupied based on the persistence probability (ϕ_{it}), and sites that were vacant become occupied by the colonization probability (γ_{it}). The observed occupancy status for site *i* at time *t* during survey *j* (y_{itj}) is conditional on the latent occupancy status and dependent on the detection probability (p_{itj}).

$$z_{i1} \sim Bernoulli(\psi_{i1})$$
$$z_{it+1}|z_{it} \sim Bernoulli(z_{it}\phi_{it} + (1 - z_{it})\gamma_{it})$$
$$y_{itj}|z_{it} \sim Bernoulli(z_{it}p_{itj})$$

Occupancy, colonization, and persistence probabilities were all modeled using generalized linear models with Bernoulli distributions and logit links and using similar sets of covariates because they are influenced by similar processes. We included summer stream temperature, summer flow, and winter flood frequency as covariates in all three of these biological models. Temperature was included as a quadratic in all models because, as ectotherms, trout have a suitable thermal range that dictates where they can occupy, persist, and colonize. Stream length was also included as a covariate in all initial occupancy models because longer stream segments have a higher probability of occupancy, and stocking intensity was included in the initial occupancy models for invasive species.

To account for species interactions, we included the occupancy of invasive species as a covariate in all biological models for native species. Models of native and invasive species were fit simultaneously, allowing for the predicted distribution of invasive species in the previous time step to be used as a covariate for native species models while fully accounting for uncertainty in the invasive species distribution. For westslope cutthroat trout, we included the presence of all three invasive species as covariates (Shepard et al. 2005, Muhlfeld et al. 2017), and for bull trout, we included brown trout and brook trout (Rieman et al. 2006, Kovach et al. 2017).

We included random effects for subbasin (HUC 8; i.e., mid-sized river drainages) in all biological models, which accounted for spatial autocorrelation and the effects of other environmental processes not directly incorporated in the models. As an example of the model

structure, the colonization probability for bull trout in stream segment *i* at year *t* (γ_{it}) was modeled as a function of abiotic covariates (*temperature*$_{it}$, *flow*$_{it}$, *and floods*$_{it}$), the presence of invasive species in the previous year (*brook*$_{it-1}$, *brown*$_{it-1}$), a random effect for the subbasin using a zero-mean Normal distribution with variance σ^2_{HUC}, and an indicator for whether the stream segment was in their possible range (*range limit*$_i$*;* 1 if within the species range and 0 if outside):

$$\gamma_{it} \sim inverse\ logit(\beta_0 + \beta_1 * temperature_{it} + \beta_2 * temperature_{it}^2 + \beta_3 * flow_{it} + \beta_4 * floods_{it} + \beta_5 * brook_{it-1} + \beta_6 * brown_{it-1} + \beta_{HUC_i}) * range\ limit_i$$

$$\beta_{HUC_i} \sim norm(0, \sigma^2_{HUC})$$

We modeled detection probability as a function of stream order and year (table S2-4). Although electrofishing has high individual capture probabilities (*median = 0.*6; fig. S2-7), accounting for species-level detection probability was necessary because surveys may fail to detect fish when densities are low and when usage varies spatially and by season. We estimated separate intercepts and slopes for four groups of stream orders (1-2, 3-4, 5-6, 7-8). Stream order likely influences detection probability because alternate electrofishing methods are used in streams of different sizes and fish abundance can vary with stream size. We included a linear effect for the survey year to account for possible temporal changes in detection probability which could bias trends in occupancy (Tingley and Beissinger 2009).

All models were analyzed in a Bayesian framework in the program JAGs (Plummer 2003) called from the programming language R (R Core Team 2018) using the rjags and jagsUI packages (Plummer 2018, Kellner 2019). We used a burn-in of 15,000 iterations, ran 10,000 additional iterations, thinned the chains by 25, and included five chains. Priors were set on the logit scale using a normal distribution with a standard deviation of 1,000, truncated between -5 and 5 for all covariates. We used a uniform distribution from 0 to 10 for the standard deviations of the random effects for subbasins. These priors typically provided an acceptable range for all parameters, but in the few cases that posterior distributions were visually determined to be constricted, we changed the priors to -7 to 7 on the logit scale. The priors for the intercepts of detection probability were constrained to be greater than 0.12 (-2 on the logit scale), because values less than this would indicate extremely minimal usage that is of low biological and management interest. Additionally, the prior for the quadratic term of stream temperature was constrained to be less than 0 because thermal niches are not U-shaped.

Model convergence and assessment

The model converged well based on visual inspection of Markov chains and $\hat{R}$ values that were less than 1.1 for all estimated parameters (Gelman and Rubin 1992). To assess the performance of our model, we calculated the area under the receiver operating characteristic curve (AUC), predictive accuracy (i.e., the proportion of correctly assigned detections), and goodness-of-fit based on posterior predictive checks (Kery and Royle 2020) for all stream segments within the species range limits (table S2-5; fig. S2-8). The goodness-of-fit test suggested that the model fit the data well (supplementary text; table S2-5). AUC values were moderate for brook trout (0.74) and good to excellent for all other trout species (0.83-0.92), and predictive accuracy ranged from 0.66 to 0.85. AUC estimates and predictive accuracy were comparable to, and slightly exceeded, those from previous occupancy models in the region (Wenger et al. 2011, LeMoine et al. 2020). When we included all stream segments in the study area, including those outside of the focal species range limits, AUC (0.78-0.95) and predictive accuracy (0.69-0.89) increased (table S2-5).

Past and future occupancy predictions and sensitivity analysis

We used the parameter estimates from the dynamic occupancy model to predict the occupancy of all species in all stream segments within the study region (127,705 km) for each year from 1993 to 2080. Occupancy was calculated separately for 200 Bayesian iterations to incorporate uncertainty in the parameter estimates. We then summarized the spatiotemporal predictions of occupancy in several ways. We calculated the proportion of total stream length occupied for each species separately for each year. This was done for the entire region, and also grouped by stream order, subbasin (HUC 8; mid-sized river drainages), and east and west of the Continental Divide in the Missouri and Columbia River drainages (HUC 2; i.e., major river drainages). Although some surveys were available in the Saskatchewan River drainage, we did not separately examine occupancy dynamics in this basin due to the very small sample size. We estimated temporal trends in occupancy by subtracting the 1993 from 2018 predictions to obtain past trends and subtracting 2018 from 2080 predictions to estimate future trends. All of these estimates were calculated separately for each of the 200 iterations to incorporate uncertainty and allow for calculation of the mean and 95% credible intervals.

Future projections used the A1B emissions scenario and a mean of 10 global climate models that have the strongest association with key aspects of climate in western USA (Wenger et al. 2011, Hamlet et al. 2013, Isaak et al. 2017) (Supplementary text). Although the CMIP3 (including A1B) simulations have now been replaced by CMIP5, CMIP3 and CMIP5 have produced similar ecological projections (Wright et al. 2016), and the A1B is a middle-of-the-road emissions scenario, similar to RCP 6.0. The A1B emissions scenario thus provides a reasonable baseline to examine in future shifts in occupancy. We then conducted a sensitivity analysis to determine how a 50% greater change in abiotic variables by 2080 than under the A1B emissions scenario would influence future occupancy projections, reflecting a high emissions scenario, such as the SRES A2 and RCP 8.5.

We also conducted additional sensitivity analyses to determine which abiotic and biotic factors were the main drivers of distribution shifts. We re-estimated future (2080) occupancy using the parameter estimates from the dynamic occupancy model but while omitting different aspects of global change. To account for climate change, we re-estimated future (2080) occupancy while holding one abiotic variable (e.g., stream temperature, summer flow, and winter floods) at its 2018 values. To account for invasive species presence, we re-estimated future occupancy while each invasive species was separately removed from the landscape (i.e., the presence was set to 0 for all stream segments and years). We also re-estimated occupancy when all climatic variables were held at their 2018 values, all invasive species were omitted, and the combination of both to examine the relative influence of abiotic versus biotic factors on distribution shifts. As with the full model, we used parameter estimates from 200 iterations from JAGs to incorporate uncertainty. We then calculated the percent change in occupancy from 2018 (based on the full model which provides our best estimate of current occupancy) to 2080 for each of the sensitivity models.

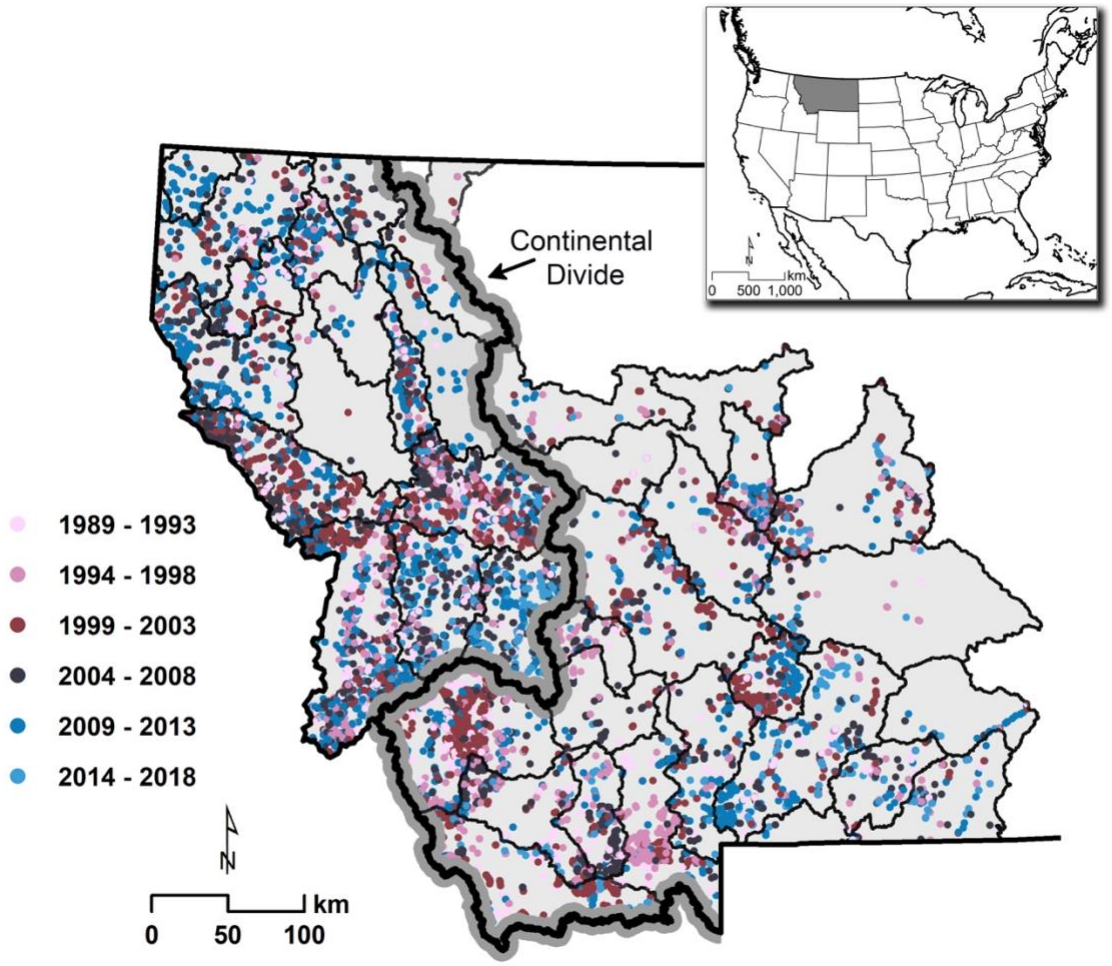

Figure 2-1. Fish surveys used to characterize trout distribution shifts in the northern Rocky Mountains of Montana, USA. Fish surveys collected between 1989 and 2018 (21,917 surveys) grouped by 5-year periods. The Continental Divide separates two major river drainages, the Columbia and Missouri River drainages, which have considerably different environmental conditions.

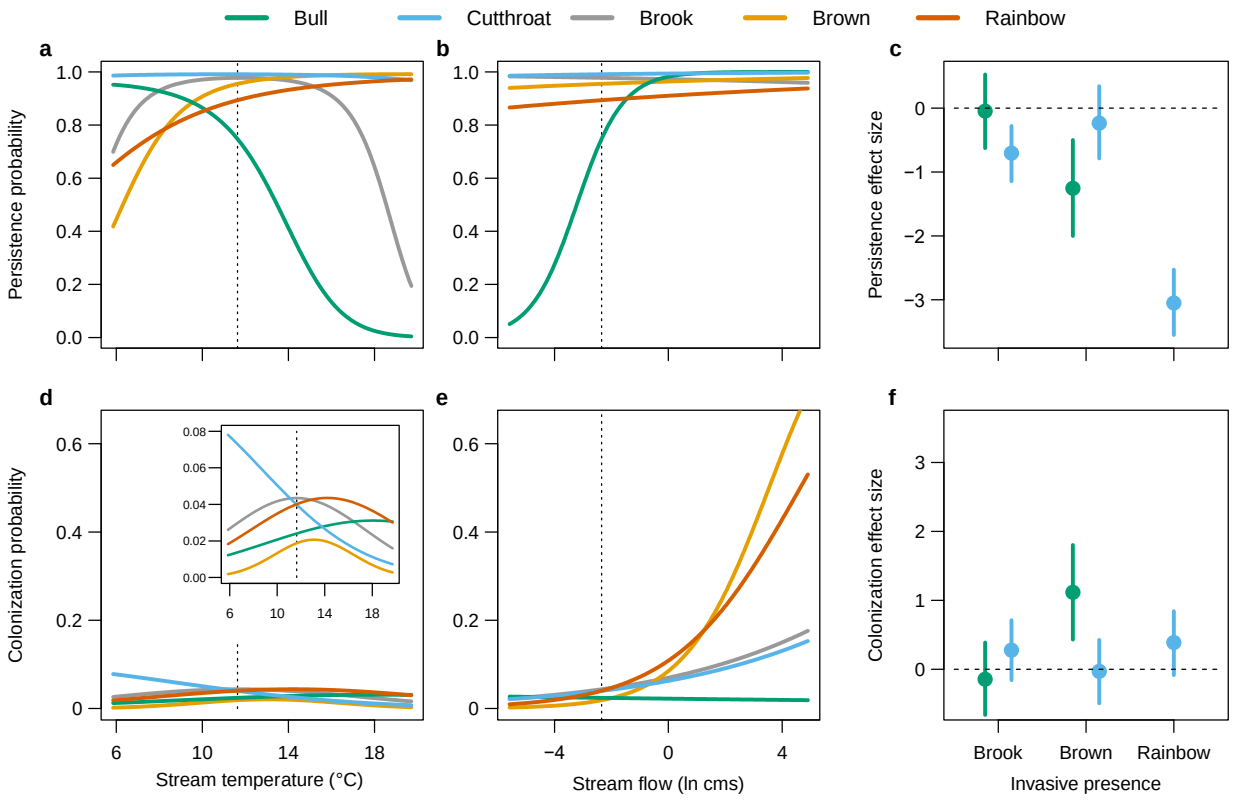

Figure 2-2. Abiotic and biotic factors influencing local persistence and colonization probabilities. The effects of summer stream temperature, summer stream flow, and the presence of invasive trout on annual, local persistence (**a-c**) and colonization (**d-f**) probabilities. The effects of summer stream temperature and summer flow are shown while all other covariates are held at their mean and excluding biotic interactions. Effect sizes of invasive species on native trout persistence (**c**) and colonization (**f**) probabilities are shown on the logit scale with bars representing 95% credible intervals. Black, vertical, dotted lines represent the mean stream temperature and flow (**a,b,d,e**). The inset in panel **d** shows the same trends with an expanded y-axis.

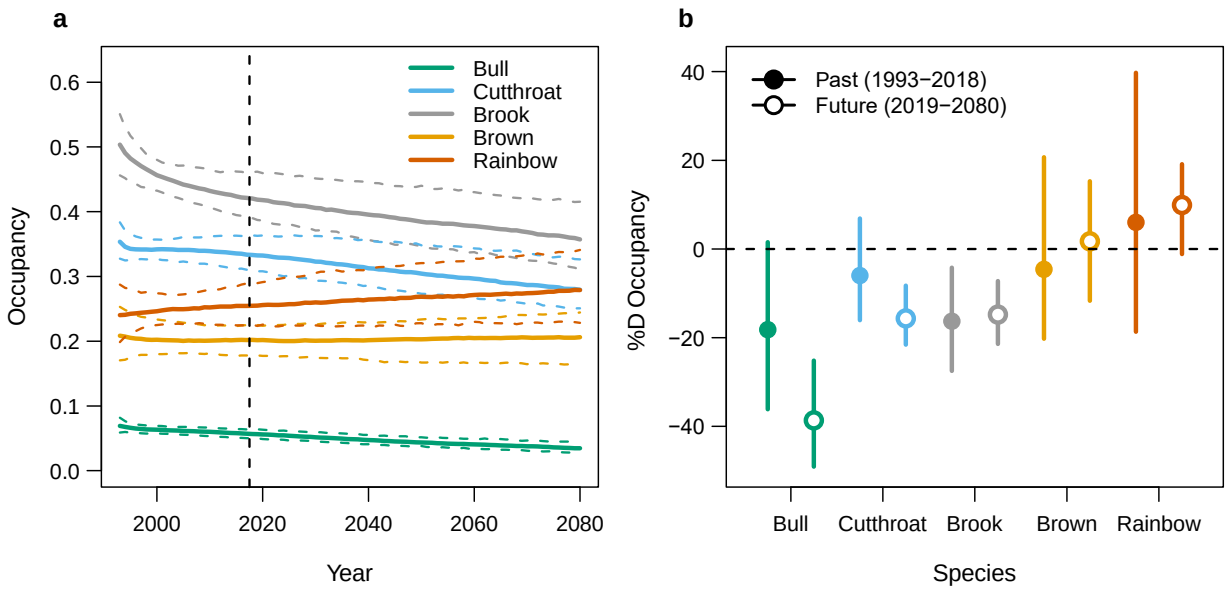

Figure 2-3. Past and future trends in the proportion of occupied stream length across the Rocky Mountains of Montana, USA. (**a**) Trends in the predicted proportion of stream length occupied (i.e., occupancy) from 1993 to 2080. The vertical dashed line indicates the final year of past predictions (2018). (**b**) Past and future estimated percent changes in occupancy (note that the periods are different lengths). 95% credible intervals are indicated by dashed, colored lines (**a**) and solid, colored bars (**b**). Climate change projections assume the A1B emission scenario.

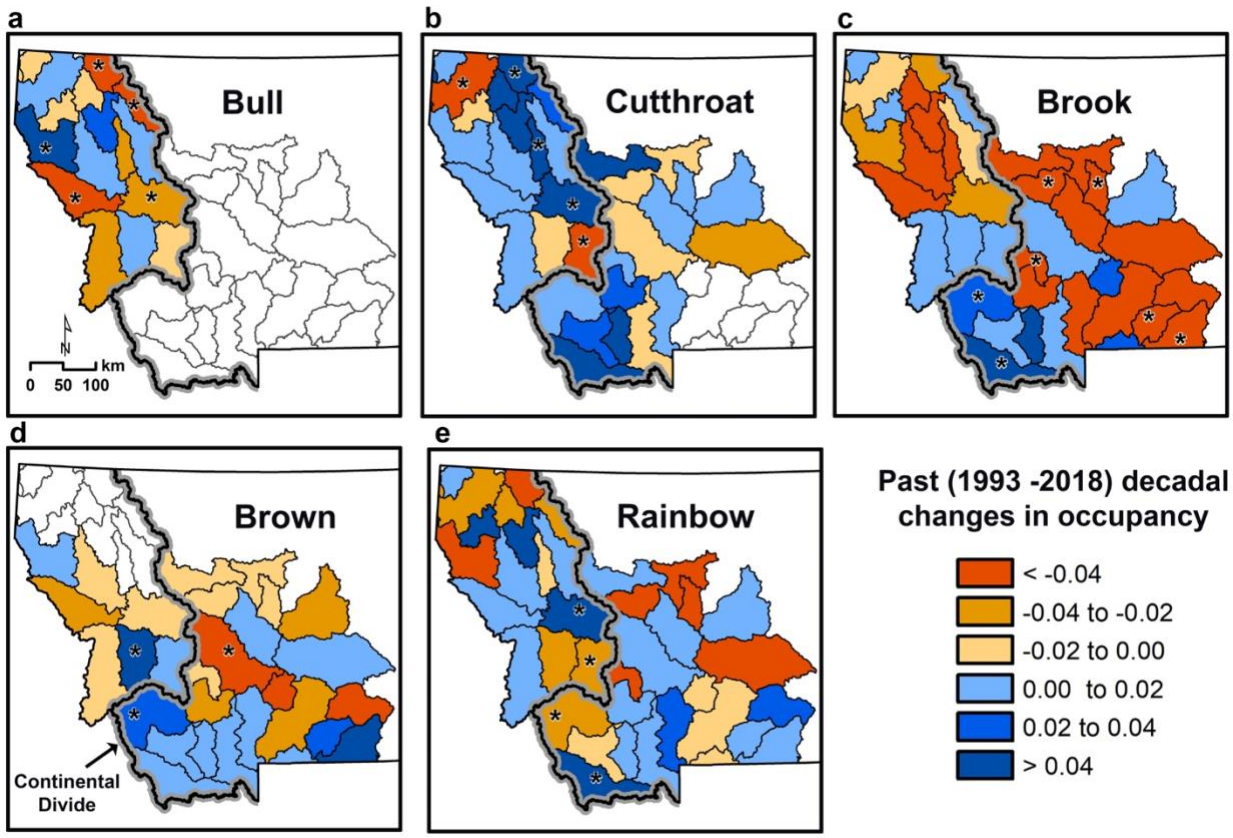

Figure 2-4. Spatial variation in past shifts in the proportion of occupied stream length across the Rocky Mountains of Montana, USA. Past (1993-2018) decadal changes in the proportion of occupied stream length by subbasin (HUC 8). Asterisks indicate 95% credible intervals that do not overlap zero.

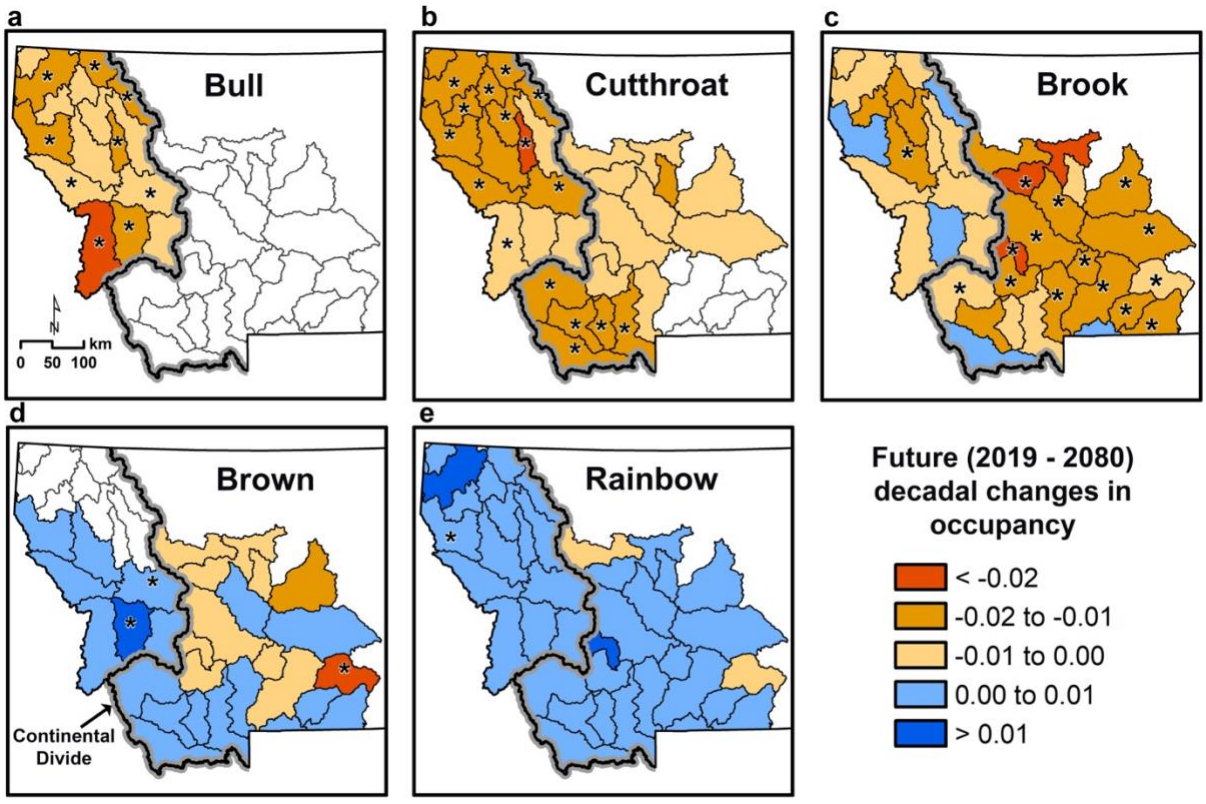

Figure 2-5. Spatial variation in predicted future shifts in the proportion of occupied stream length across the Rocky Mountains of Montana, USA. Predicted future (2019-2080) decadal changes in the proportion of occupied stream length by subbasin (HUC 8). Asterisks indicate 95% credible intervals that do not overlap zero.

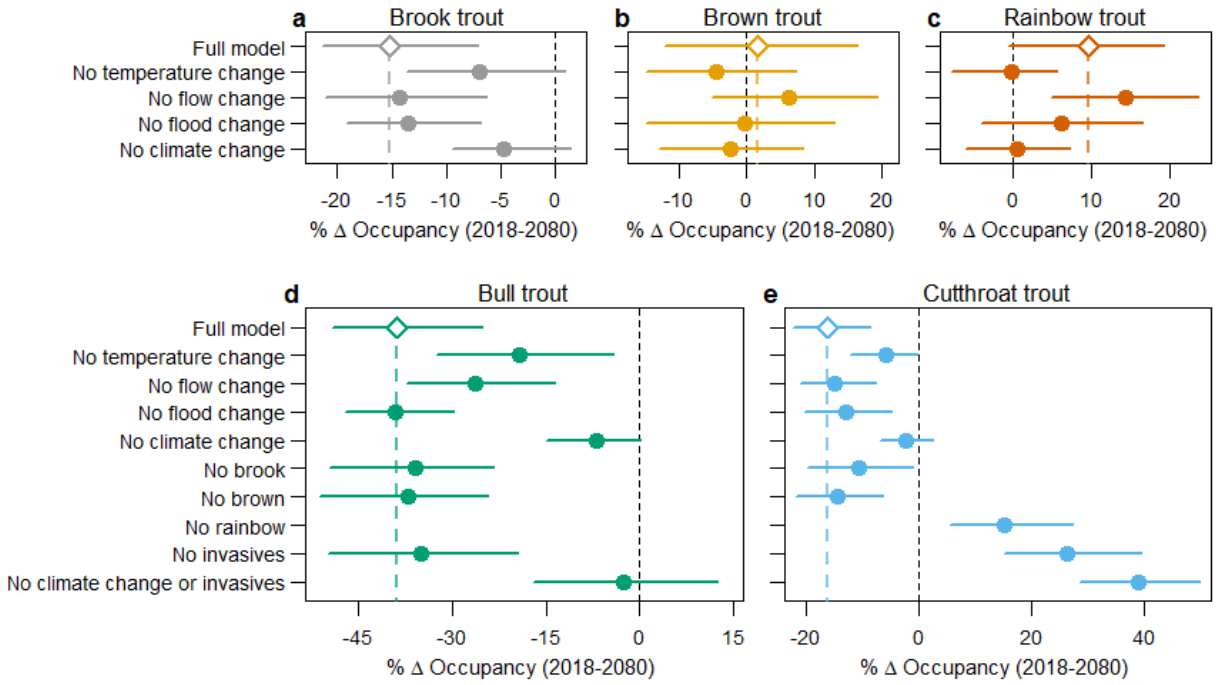

Figure 2-6. Sensitivity analysis depicting how omitting aspects of climate change and invasive species presence influences predicted occupancy in 2080. (**a-e**) The % change in predicted occupancy from 2018 (based on the full model) to 2080 when an element of global change was omitted. To omit elements of global change, we held abiotic variables constant at their 2018 values and assumed that invasive species were completely absent in the region. In addition to omitting single elements of global change, we also tested the effect of no climate change (i.e., no change in summer flow, winter flood frequency, or summer stream temperature), no invasive species (i.e., all of the invasive species were omitted), and the combination of no climate change and no invasive species. The diamonds and colored dashed lines represent the % change for the full model (i.e., including climate change and invasive species). Horizontal, colored bars are 95% credible intervals. The black dashed lines are included as a reference at no change in occupancy.

CHAPTER 3: Demographic drivers of small effective population sizes in isolated trout populations

Abstract

Human-driven habitat degradation and fragmentation of freshwater ecosystems have resulted in heightened extirpation risks for many populations, in part due to the increased influence of genetic drift in small populations. Perhaps the most informative metric for monitoring small, isolates is the effective population size (N_e), but N_e is challenging to estimate. The effective number of breeders (N_b) is a promising, related metric for genetic monitoring that can provide information about habitat availability, adult census size (N_c), and N_e, but N_b is more difficult to interpret. Thus, detailed analyses of N_b and N_e can provide valuable insight into whether a population is at risk and also how less intensive monitoring efforts can be used to interpret N_b in other populations. Here, we conducted a detailed analysis of N_b, N_e, and N_c and the factors driving their variation in five isolated populations of westslope cutthroat trout (WCT). WCT in the Missouri River basin of Montana are now limited to a patchwork of completely isolated populations. We report very low N_b (a minimum of 4) and N_e (minimum of 9) in several populations, suggesting an immediate risk of inbreeding depression. Low N_b and N_e to N_c ratios were largely explained by the largest fish dominating reproduction, creating high variance in reproductive success. We also find that high variation in life-history traits among populations (e.g., generation length varied from 3.3 to 10.3) drives differing ratios among N_b, N_e, and N_c, highlighting that caution should be taken when using these ratios to derive parameters in other populations. Overall, our results suggest that many WCT populations could likely benefit from receiving gene flow and highlight that intensive monitoring of N_b and N_e could provide valuable information for conserving a range of freshwater organisms that are threatened by isolation.

Introduction

Anthropogenic stream fragmentation poses one of the greatest threats to freshwater biodiversity (Brauer and Beheregaray 2020). Humans have extensively fragmented freshwater ecosystems by constructing barriers (e.g., dams, water diversions, and culverts) and contributing to habitat degradation that reduces connectivity (e.g., climate-induced temperature increases and drought) (Gido et al. 2015). A major consequence is that many populations are now completely isolated in small habitat patches and face increased extirpation risk due in part to genetic factors (Soulé and

Mills 1998). Importantly, small, isolated populations often have increased levels of inbreeding, lower genetic variation and adaptive potential, and higher rates of genetic drift, which ultimately reduces their persistence probability (Kardos et al. 2021, Willi et al. 2022). A crucial role of management will be to determine which small, isolated populations are at immediate risk.

The effective population size (N_e) is perhaps the most important parameter for the genetic monitoring of small, isolated populations. N_e strongly influences the loss of genetic variation, the rate of inbreeding, and the efficacy of natural selection (Charlesworth 2009). As a general conservation guideline, a contemporary $N_e < 50$ indicates that a population could be at high risk of inbreeding depression (Jamieson and Allendorf 2012). However, N_e is very difficult to estimate in age-structured populations (Waples et al. 2014), which is the case for many species of concern.

A promising metric related to N_e is the effective number of breeders that gives rise to a cohort (N_b), which can provide valuable information about both genetic and demographic threats to a population. N_b can be estimated by applying single-sample genetic estimators to a cohort (Waples 2005) and is thus more easily estimated than N_e. N_b can be extrapolated from N_e using two to three basic life-history traits (Waples et al. 2013), which aids with the interpretation of how N_b relates to genetic threats. However, estimation of life-history traits requires detailed demographic data, and applying estimates for other populations may have low accuracy in taxa with high variation in life-history across populations. N_b can also correlate with the adult population size (N_c) and may offer a cheaper alternative for demographic monitoring (Yates et al. 2017, Luikart et al. 2021). However, for some populations and taxa, N_b can be more closely related to spawning habitat quality and quantity and show little correlation with N_c (Whiteley et al. 2010, 2015a). Due to the difficulty in its interpretation, detailed examinations of N_b and its relationship to N_e and N_c can provide valuable information about how N_b can be incorporated into conservation efforts.

Although studies rarely have enough ecological data to obtain demographic estimates of N_e (Waples 2005), incorporating demography and life-history data into analyses of N_b and N_e has several advantages. Several life history parameters are needed for either estimating N_e or extrapolating N_e from N_b, including variance in reproductive success, generation length, age at maturity, and adult life span. Further, in contrast to genetic estimators, demographic estimates of N_e do not require conformity to Hardy-Weinberg proportions, and may thus provide the only

option in some cases (e.g., for populations with high gene flow). Finally, demographic estimators allow for examination of the factors that influence N_b, N_e, and their ratios with N_c (Serbezov et al. 2012). For example, variance in reproductive success is the main factor that reduces N_b and N_e below N_c, and understanding what factors drive variance in reproductive success can provide insight into why some populations have very low N_b and N_e. Overall, detailed examinations of N_b and N_e based on both demographic and genetic data will help determine whether populations are at immediate risk, and also provide insight into how these parameters can be interpreted for less intensive monitoring efforts.

We provide the first detailed examination of N_b and N_e for westslope cutthroat trout (*Oncorhynchus clarkii lewisi*). Westslope cutthroat trout (WCT) is a taxon of concern throughout much of their range and are listed in Canada under the Species at Risk Act. Invasive trout species, which were extensively introduced in Montana from the late 1800s through the 1980s (Whiteley et al. 2019), pose a serious threat to WCT (Muhlfeld et al. 2014, Kovach et al. 2016a, Bell et al. 2021a), with the biggest concern coming from hybridization with non-native rainbow trout (*O. mykiss*). These threats are the most pressing in the Missouri River drainage, which has the lowest genetic variation of any basin within the subspecies range (Drinan et al. 2011). Further, all of the remaining populations of Missouri WCT are isolated, often with very low genetic variation (Kovach et al. 2021). Understanding genetic threats to these populations is crucial for conservation efforts.

We estimate N_c, N_b, and N_e, and examined factors that drive variation in their ratios using a combination of genetic data and detailed demographic data in five completely isolated populations of WCT in the Missouri River drainage of Montana. We address four related questions: 1) How much does variance in annual reproductive success (V_k) vary among individuals in a population and what demographic factors cause variation in V_k? 2) How much do life-history traits that are important for driving variation in N_b and N_e differ among populations? 3) What are the estimates of N_b in small, isolated populations, and does the ratio of N_b to N_c vary across populations? and 4) What is the N_e in these populations and how does it relate to N_c and N_b?

Methods

Study populations and sampling procedures

We intensively monitored five WCT populations from 2017 to 2021. The monitored populations were all located on the east of the Continental Divide in the Missouri River basin in Montana (Figure 3-1). All populations were completely isolated and had low heterozygosity compared to other WCT populations in Montana (Kovach et al. 2021). This monitoring effort was part of an experimental test of genetic rescue (Chapter 5), but the detailed dataset allowed for additional analyses. We sampled all populations using backpack electrofishing annually from 2017 to 2021. We sampled the entirety or near entirety of the inhabited stream for Gold Run Creek, Hall Creek, and Staubach Creek. We sampled roughly half of the occupied stream length of Crawford Creek (0.4 km) and NF Little Belt Creek (1.2 km). For all captured fish, we measured body length and clipped a small piece of the upper caudal fin to provide tissue for genetic analyses. Fish were anesthetized using eugenol. We additionally inserted Passive Integrated Transponder (PIT) tags into the body cavity of all fish over 70 mm at the study sites on the first capture of the fish and scanned all fish for previous PIT tags. PIT tags have minimal influence on growth and survival of trout (O'Donnell and Letcher 2017).

We returned to the streams to perform a recapture one to two weeks after fish were marked in 2017 and 2018 to allow for the estimation of individual detection probability. During the recapture event, fish were scanned for a PIT tag and visually examined for a fin clip to determine if they were previously captured. Fish that had not been previously captured underwent the same sampling protocol as fish captured in the initial stream visit. Resampling was limited to 8 to 12 randomly sampled 40-meter sections. Half of the sections we selected had below-average fish counts, and half had above-average fish counts. This helped ensure that we included a range of densities and difficulties of electrofishing.

Bioinformatics and genotyping

We genotyped all captured fish using a previously established GTseq panel (Campbell et al. 2015), which included 373 SNPs and a sex ID marker. We additionally genotyped fish from Hall Creek, Staubach Creek, and Gold Run Creek using a previously established RAD-Capture (Ali et al. 2016) SNP panel. To increase read depth in individuals with low DNA concentrations, we included some individuals on multiple sequencing lanes/runs and then combined reads. Genotype

error rates were 0.016% for GTseq (83 duplicated individuals) and 0.1% for RAD-Capture (53 duplicated individuals).

We tested for conformity to Hardy-Weinberg and Linkage Equilibrium expectations for each population. We limited tests to fish sampled in 2017 as this was before the pulse of age-1 hybrids, which would cause large deviations from both HW and LD. HW expectations were tested in the R program *pegas* (Paradis 2010) and used Chi-squared tests and exact tests (Guo 1992). We examined LD using chi-squared tests in the *genetics* package in R (Warnes et al. 2021). Markers in which the chi-squared test was significant ($P < 0.001$) in 2 populations were removed in several analyses.

We removed loci that had greater than 40% missing genotypes for both RAD-Capture and GTseq. Further, individuals with 75% missingness on GTseq and 50% on RAD-Capture were not genotyped for that panel, but could still be retained for further analyses if the individual was successfully genotyped on the other panel. We were more stringent with RAD-Capture as the error rates were higher for individuals with low read depth using that method. For GTseq, we removed 14 markers that did not conform to HW proportions in two or more populations (p-value <= 0.01), both with the same direction of F_{IS}. We also inspected loci that significantly deviated from HW proportions in one population, but found no clear signs that the markers had problems.

To avoid close physical linkage and multiple SNPs on the same bait, we thinned markers so that only one SNP was selected for every 10,000 base pairs. To reduce additional linkage, we removed markers that had a mean $r > 0.5$ across at least 2 populations. Finally, we found a block of rainbow trout ancestry in Hall Creek that covered roughly half of chromosome 6. We only selected one marker on this block. When deciding which markers to retain, we favored markers on the GTseq panel as genotypes were available for all populations, and we chose the loci with the high average allele frequency across populations. We retained 825 SNPs (229 GTseq and 596 RAD-Capture) before filtering for linkage and 554 SNPs (199 GTseq and 355 RAD-Capture) after filtering for linkage.

Individual identification and pedigree construction

PIT tags allowed for individual identification of most fish, but we used genotype data to identify individuals that were too small to initially PIT tag (e.g., < 70 mm) or that shed their PIT tags. PIT shed rates are very low but do occur in larger females during spawning. We used the *dupGenotype* function from the R package *StrataG* (Archer et al. 2017) to identify duplicate genetic samples, which uses pairwise comparisons of all individuals in a population to calculate the proportion of identical genotypes across loci. We used a 99% percent match as a threshold to call the same individual, which typically provided adequate power.

Admixture following a pulse of gene flow can cause a deviation from Hardy-Weinberg expectations for a generation, which is required as an assumption in many parentage analyses. We thus used a combination of exclusion-based parentage, which does not require HW proportions, and maximum likelihood-based sibship and parentage, which has higher power but assumes populations are close to HW proportions. Exclusion-based methods also lack an implicit expectation of LD, allowing for more markers to be used in the analysis.

Potential parents for a cohort were allowed from all sampling years, but potential parents were omitted based on being an unreasonable length to have produced offspring in the cohort of interest. Length cutoffs for parents were informed by growth modeling (described below) and previous estimates of size at maturity (Downs et al. 1997). Offspring for a cohort were determined based on being age-1 at time t+1, or being age-2 at time t+2. Age-1 and age-2 fish were determined based on visual inspection of length-frequency histograms separately for each sex, and we used length at age-2 of known fish to help verify length cutoffs for age-2.

Exclusions were based on both offspring and the parent having opposite homozygote genotypes and on both parents being homozygous for the same allele while the offspring was heterozygous (Cockburn et al. 2021). Additionally, we used full-likelihood joint sibship and parentage estimation in *Colony2* allowing for polygamy in both males and females and for inbreeding, which relaxes HW assumptions (Wang and Santure 2009, Jones and Wang 2010, Wang 2012). We ran *Colony2* separately for each population and each cohort.

To combine results, we used *Colony2* results to determine resident x resident crosses. When *Colony2* determined two potential parents as having similar probabilities of being the true

parent, we checked if exclusion had identified either of these parents as the top parent. For F1 hybrid fish, we used exclusion-based parentage results due to non-conformity with HW proportions for these fish.

Demographic modeling

Detailed demographic modeling allowed for the construction of life tables, which are the basis of much theory on N_e. The parameters of primary interest were age-specific survival and reproductive output, which allow for estimation of generation length and variance in lifetime reproductive success. However, a series of demographic models were required to estimate these parameters and construct life tables, including individual detection probability, individual growth rate, survival by length, and annual reproductive output by length.

Individual detection probability was needed to estimate both abundance (described below) and reproductive output since detection probabilities of less than 1 lead to biased estimation of demographic parameters (Kery and Royle 2020). We used the within-year recaptures from 2017 and 2018 to estimate detection probability as the proportion of fish captured on the recapture event that were marked earlier in the same year. This was done using generalized linear models (GLM) with a logit link and a Bernoulli distribution. We estimated detection probability separately for every stream and for different size classes, including fish < 120 mm (juveniles), ≥ 120 and < 150 mm (sub-adults), ≥ 150 and < 180 (smaller adults), and ≥ 180 (large adults).

Individual annual growth was modeled to estimate body lengths for years in which fish were not caught. For each population, growth was modeled using a GLM with a normal distribution and a log link. The log link prevented negative growth and helped to account for the non-linear relationship between fish length and growth. Growth was modeled as a function length (quadratic) in the previous year, which was estimated separately for both sexes. We used parameter estimates from this GLM to estimate length in all years in which the fish of interest was not captured. Importantly, this allowed us to estimate the parental length in the year that reproduction occurred. As growth is equal to size at time t minus size at size t -1, these values can be rearranged to estimate lengths in both future and previous time steps. We additionally used our growth model to estimate length-at-age. Separately for each population, we simulated

length at age for fish starting at age-1 through age-15 (2 years older than the maximum identified age of WCT in the region; Janowicz et al. 2018). We used the mean and standard deviation of age-1 length to specify a normal distribution for each population, and simulated growth for 100,000 individuals. We then calculated the mean length at age from the simulated output.

We estimated survival at age (s_x) using a multistate CJS (Kery and Royle 2020). The models allowed for uncertainty in the length class into which an individual fell during years in which it was not captured and corrected for individual detection probability. This allowed for unbiased estimates of stage-specific survival. Specifically, sex-specific survival was dependent on length-based size categories (age-1, and age-2+ < 130 mm, >= 130mm & < 170 mm, >= 170mm & < 210 mm, and >= 210 mm), and transition probabilities were also calculated between length categories, while accounting for category-specific detection probability (Kery and Royle 2020). The state transition probabilities were:

$$
\begin{array}{c}
 \\
a1_t \\
a2_{(<130)t} \\
a2_{(\geq 130\ \&\ <170)t} \\
a2_{(\geq 170\ \&<210)t} \\
a2_{(\geq 210)t} \\
dead_t
\end{array}
\begin{bmatrix}
a1_{t+1} & a2_{(<130)t+1} & a2_{(\geq 130\ \&\ <170)t+1} & a2_{(\geq 170\ \&<210)t+1} & a2_{(\geq 210)t+1} & dead_{t+1} \\
0 & phi_1 * \alpha_{12} & phi_1 * \alpha_{13} & phi_1 * \alpha_{14} & 0 & 1 - phi_1 \\
0 & phi_2 * \alpha_{22} & phi_2 * \alpha_{23} & phi_2 * \alpha_{24} & phi_2 * \alpha_{25} & 1 - phi_2 \\
0 & 0 & phi_3 * \alpha_{33} & phi_3 * \alpha_{34} & phi_3 * \alpha_{35} & 1 - phi_3 \\
0 & 0 & 0 & phi_4 * \alpha_{44} & phi_4 * \alpha_{45} & 1 - phi_4 \\
0 & 0 & 0 & 0 & phi_5 & 1 - phi_5 \\
0 & 0 & 0 & 0 & 0 & 1
\end{bmatrix}
$$

with rows representing the starting state (*t*) and the columns the ending state (*t+1*).

We used pedigree results to model a parent's annual number of offspring recruiting to age-1 as a function of body length. We separately modeled whether or not an individual produced any offspring that survived to age-1 (recruited) and the annual number of recruiting offspring given successful reproduction, akin to a zero-hurdle model (Zuur et al. 2009). Both of these processes contribute to Poisson overdispersion in reproductive success. The probability of reproduction was modeled using a generalized linear model with a Bernoulli distribution and a logit link. We used parental length at the time the cohort was produced as an independent variable because length increases the probability of maturity.

Given an individual produced recruiting offspring, the annual number of recruiting offspring was modeled using a binomial mixture model that used a latent, unobserved, estimate of true family size in which detection probability had been accounted for. The latent true number of offspring was the dependent variable in a GLM with a zero-truncated negative binomial distribution and a log link. This allowed us to obtain estimates of annual reproductive success with low bias and increased precision. Binomial-mixture models are commonly used to estimate abundance using multiple surveys within a 'closed' period to estimate and account for imperfect detection. Here, we resampled families on two occasions separated by one year. As mortality occurred over this period, we used Bayesian estimates of age-1 to age-2 survival ($surival_{1\text{-}2}$) and age-specific detection probability (p) as fixed variables in the model:

$$offspring\ counts_{it} \sim Binomial(p_{age(i)} * survival_{age(i)},\ \#\ offpsring_{it})$$

Using estimates from 2 years was important because age-1 fish have lower detection probabilities (p = 0.12 to 0.48) than age-2 fish (p = 0.34 to 0.77), and there were multiple instances in which a family was first detected at age-2. We first ran this model without covariates to allow for the incorporation of both sampled parents and parents that were imputed in *Colony2* to ensure we included all offspring in the model. We then ran a second model with only known parents and with parental length at the time of the cohort production as an explanatory variable.

All demographic models described above were analyzed using Bayesian inference in the program JAGS (Plummer 2003) in the R program *jagsui* (Kellner 2019). Models were run with a burn-in of 50,000 iterations, 50,000 additional iterations, and five chains. This resulted in successful convergence of all models based on $\hat{R}$ values less than 1.1 and visual inspection of MCMC chains.

Life history parameters and life table construction

We used the demographic models (described above) to construct population-specific life tables, including age-specific survival (s_x) and reproduction (b_x). We estimated age-specific survival by simulating age of death for 100,000 fish starting at age-1 in the multistate survival model. The number of individuals that died at each age was converted to age-1 specific survival probabilities. Age-specific reproductive output was estimated by inputting the mean length at age into both the model for the probability of reproduction and the number of offspring produced,

given reproduction. These two components of reproduction were then multiplied together to get a total estimate of age-specific reproductive success. Both of these parameters were calculated separately for each sex and in each population.

We additionally calculated several life-history parameters that were important for calculating or interpreting N_b and N_e estimates. Maximum age (ω) was determined as the age at which 99% of individuals had died. Age at maturity (α) was the first age in which 10% of individuals were mature based on our length estimates and length-based probability of maturity from Downs et al (1997). Life tables were then analyzed in *AgeNe* (Waples et al. 2011), which provides estimates of lifetime variance in reproductive success and generation length (described further below).

Number of mature adults (N_c)

We estimated abundance as the number of detected fish in a given year divided by the detection probability. This was done separately for each size class and population. The summation of age-2+ (age-2 and older) size-class provides an estimate of the total age-2+ abundance. However, not all age-2+ fish are sexually mature, and the timing of our sampling did not permit us to determine if fish were mature. However, a previous study in the same region developed a logistic equation for the probability of maturity by length (Downs et al. 1997). We used this logistic equation to randomly sample whether fish were mature based on their length. We repeated this 1000 times to incorporate uncertainty into our N_c estimates.

Effective number of breeders (N_b) and effective population size (N_e)

We estimated the effective number of breeders using two methods: a genetic-based estimation using linkage disequilibrium ($N_{b(gen)}$) (Waples and Do 2008) and a demographic-based estimation ($N_{b(dem)}$). Genetic-based estimators are widely used but assume HW equilibrium and that the primary or only source of LD comes from genetic drift, and are thus more sensitive to gene flow. For the genetic estimator, we used the program *LDNe*. We selected monogamy because our parentage analysis suggested that polygamy is common across years but is uncommon within a cohort. Nevertheless, low levels of polygamy could bias our estimates high because *LDNe*

estimates using monogamy are higher than those assuming polygamy. We only included loci with a minor allele frequency of 0.02 or higher within the population (Waples and Do 2010). We provide estimates of $N_{e(gen)}$ for all populations despite the low level of gene flow except for Staubach Creek, which had significantly higher gene flow than other populations. We thus only estimate $N_{e(gen)}$ in Staubach Creek before gene flow, in the 2015 and 2016 cohorts. To prevent admixture LD, we removed hybrids from our samples. Note that these estimates will have a small bias because 1-2 families were removed from the sample.

For the demographic estimation of annual N_b ($\widehat{N}_{b(dem)}$), we first estimated sex-specific annual N_b based on the variance in annual reproductive success (V_k), where individual reproductive success (k_i) was the latent true estimate from the binomial mixture model described above. Using females as an example (Hedrick 2000):

$$N_{bf} = \frac{N_f \bar{k}_f - 1}{\bar{k}_f - 1 + {V_{kf}}/{\bar{k}}}$$

where $\bar{k}_f$ and $\bar{k}$ are the mean numbers of progeny produced in a given year for females and all adults, respectively, and the variance in reproductive success was calculated as (Crow and Kimura 1970):

$$V_{kf} = \frac{\sum({k_i}^2)}{N_f} - \left(\frac{\sum k_i}{N_f}\right)^2$$

We then used sex-specific estimates of N_b to adjust for skew in the sex ratio, and estimated an overall N_b using the equation (Crow and Kimura 1970):

$$N_b = \frac{4N_{bf}N_{bm}}{N_{bf} + N_{bm}}$$

We only included individuals that produced offspring that recruited to age-1 because omitting parents with no offspring does not influence inbreeding N_e or N_b (Waples and Waples 2011).

We used our life tables and the program *AgeNe* to estimate the ratio of N_b to N_e (Waples et al. 2011). *AgeNe* provides estimates for N_e for age-structured populations (Felsenstein 1971, Hill 1972, Waples et al. 2011). These estimates can be highly accurate, and have been used to

ground truth other methods to estimate N_e (Waples et al. 2014). However, directly estimating N_e from *AgeNe* is dependent on age-1 abundance, and sensitive to variation in these estimates. On the other hand, the ratio of N_b to N_e estimated from *AgeNe* is not dependent on abundance estimates. As age-1 abundance is highly uncertainty and variable in WCT, we used estimates of N_b/N_e rather than N_e, and then used N_b/N_e to convert our estimates of N_b to N_e.

Finally, we used estimates of N_e and generation length to predict the loss of genetic variation in each population by 2100 (e.g., Pero et al. 2022). Specifically, we used the recursive equation:

$$h_{t+1} = \left(1 - \frac{1}{2N_e}\right) h_t$$

where *t* is time in generations. We then converted generations to years using estimated generation lengths for each population. We used the genetic based estimate of N_e for this projection.

Results

Variation in life history traits

Annual reproductive output was strongly influenced by adult body length. The probability of contributing offspring to a cohort that recruit to age-1 had a strong positive relationship with length in all populations and for both males and females (Figure 3-2). Previous research found that WCT have a 50% probability of reaching sexual maturity (based on the presence of mature gametes) at 135 mm for males and 156 mm for females (Downs et al. 1997). However, fish were not predicted to have a 50% chance of successfully reproducing in a given year until they reached 201 mm for males and 198 mm for females, on average (Figure 3-2). Additionally, given that a fish does reproduce in a given year, the number of offspring produced was positively associated with length in all population and for both females and males with the exception of males in Hall Creek, which had no relationship between length and reproductive output (Figure 3-3). These differences in length based reproductive output translate to differences in age-based reproductive output (see Table 3-1 for example life tables), which helps to explain why variance in annual and life-time reproductive success is high.

Life history parameters derived from demographic models and life-tables varied considerably across populations (Table 3-2). Variance in lifetime reproductive success was far greater than expected under a Poisson process (2), and ranged from 22.3 in Staubach Creek to 50.1 in Crawford Creek. Age at first maturity estimates ranged from 2 for males in Crawford Creek, NF Little Belt Creek, and Staubach Creek, to 4 for females Gold Run Creek and Hall Creek. Maximum age estimates ranged from 4 for males in NF Little Belt Creek to 13+ for males and females in Gold Run Creek. Finally, generation lengths ranged from 3.3 years in NF Little Belt Creek to 10.3 years in Gold Run Creek. Overall, these life history traits that are important for determining N_b and N_e all had at least a twofold difference across populations.

N_b estimates and N_b to N_c ratios

Demographic estimates of N_b ($\widehat{N}_{b(dem)}$) ranged from 2.4 in Hall Creek during 2018 to 169.8 in NF Little Belt creek during 2016 (Figure 3-4). Similarly, genetic estimates of N_b ($\widehat{N}_{b(gen)}$) ranged widely from 2.6 in Hall during 2017 to 115 in NF Little Belt during 2016. Notably, the harmonic mean N_b of Hall Creek was very low, with estimates of 3.6 and 5.3 for $N_{b(dem)}$ and $N_{b(gen)}$, respectively. $N_{b(dem)}$ and $N_{b(gen)}$ were highly correlated (r = 0.96) but $N_{b(dem)}$ estimates were generally larger than $N_{b(Gen)}$ (Figure 3-4).

The correlation between N_b and N_c within populations ranged from 0 to 0.99 for $N_{b(gen)}$ based estimates and -0.90 to 0.91 for $N_{b(dem)}$. Although correlations between 3-4 points are prone to spurious relationships, this nevertheless suggests that N_b is unlikely to closely track N_c in all populations. The ratio of N_b to N_c ranged from 0.04 in Hall for the 2017 cohort to 0.24 in Crawford for the 2018 cohort based on $N_{b(gen)}$ (Table 3-3). Similarly, N_b to N_c ratio ranged from 0.05 in Hall for the 2018 cohort to 0.34 in NF Little Belt for the 2017 cohort based on $N_{b(dem)}$. Across all populations and years, mean N_b/N_c was 0.13 for $N_{b(Gen)}$ based estimates and 0.18 for $N_{b(dem)}$ for based estimates.

N_e estimates, N_b/N_e, and N_e/N_c

N_b/N_e estimates from *AgeNe* ranged from 0.38 to 0.61 (Table 3-2). On average, N_b was predicted to be roughly half of N_e ($\overline{N_b/N_e} = 0.54$). N_e estimates derived from N_b/N_e varied by an order of magnitude despite similar habitat patch sizes (Table 3-3, Table S3-4). Hall Creek, the population at the small extreme, had an $\widehat{N}_{e(gen)}$ of 9 and an $\widehat{N}_{e(dem)}$ of 14 in Hall Creek, while NF Little

Belt Creek, the population at the large extreme, had an $\widehat{N}_{e(gen)}$ of 108 and an $\widehat{N}_{e(dem)}$ of 246 (Table 3-3). Of the five populations, four had an $N_{e(gen)} < 50$ and three of those four had an $N_{e(dem)} < 50$.

N_e was correlated with N_c across populations ($r = 0.70$). However, populations with similar N_c had vastly different N_e. For example, Gold Run Creek had a slightly larger N_c than NF Little Belt Creek, but had an N_e of 140% or 203% lower than NF Little Belt Creek based on $N_{b(gen)}$ and $N_{b(dem)}$, respectively. N_e/N_c ranged from 0.11 to 0.40 or 0.19 to 0.74 for estimates based on $N_{b(gen)}$ and $N_{b(dem)}$, respectively.

The rate of loss of genetic variation per year is based both on N_e and the generation length. Hall and Staubach are expected to have large declines in heterozygosity by 2100 (54% and 42%, respectively). Although Gold Run has less than half of the N_e of Little Belt, the longer generation length led to a similar, small loss of heterozygosity for both streams of 9% and 11% by 2100, respectively (Fig. 3-5).

Discussion

Innumerable small, isolated populations face increased extirpation risk due to genetic factors (Frankham et al. 2017), which is especially concerning for freshwater ecosystems due to extensive habitat fragmentation (Gido et al. 2015). Our detailed analysis of using both genetic and demographic data shows that isolated westslope cutthroat trout (WCT) populations often had very low effective population sizes (N_e), consistent with a previous study that provided less precise estimates of N_e (Carim et al. 2016). At least three of the five study populations likely had an $N_e < 50$, indicating potential immediate threats from inbreeding (Jamieson and Allendorf 2012). This is also consistent with extremely high genetic divergence and very low genetic variation recently reported in isolated WCT populations throughout the Missouri drainage in Montana (Kovach et al. 2021). Further, our study populations are representative of the range of heterozygosity of WCT in the Missouri River drainage, suggesting that many of these populations could have very low N_e.

Both N_b and N_e were considerably lower than the number of mature adults (N_c). This appears to be primarily driven by high variance in reproductive success across length and age. Importantly, we show that the size and age of sexual maturity may be substantially smaller than the size and age at which fish begin to reproduce (Downs et al. 1997, Carim et al. 2021).

Although our N_b/N_c and N_e/N_c ratios are not as low as many previous estimates (Frankham 1995, Palstra and Fraser 2012), this finding emphasizes that apparently healthy densities in these isolated populations could belie the genetic threats that the populations face. We also note that the method in which N_e and N_e are calculated can dramatically change the N_b/N_c and N_e/N_c in these populations, further emphasizing the difficulty with interpreting these ratios.

There was substantial variation in N_b, N_e, and N_c ratios among populations. These differences could be due to several factors. Importantly, WCT, and salmonids in general, have considerable life history variation (Carim et al. 2017). We document variation in life-history traits among nearby populations, including differing generation length, age-at-maturity, maximum age, and variance in life-time reproductive success. This life-history variation likely underlies variation in the in ratios among the tested parameters (e.g., Waples et al. 2013). Due to the high variability, applying these ratios to other, even nearby, populations is unlikely to provide precise approximations of unmeasured parameters. Nevertheless, we report mean N_b/N_e of 0.54, N_b/N_c of 0.15-0.19, and N_e/N_c of 0.26-0.34, which could allow for useful conversions between parameters for isolated WCT populations when other information is not available.

Despite difficulty in its interpretation, the effective number of breeders (N_b) has been suggested to be a useful metric for the monitoring of freshwater ecosystems (Waples and Do 2010, Whiteley et al. 2015a). N_b could be particularly useful for the monitoring of small isolated populations. N_b estimates are the more precise in small populations (Waples and Do 2010, Luikart et al. 2021), and sampling for the entirety of an inhabited stream reach will often be possible simply because of the decrease in time and effort required. Additionally, isolated populations will have little or no gene flow, which can cause bias for genetic estimators (Waples and England 2011, Whiteley et al. 2017). Further, N_b is much easier to estimate than N_e for species with overlapping generations, while still likely capturing the rate of genetic drift and degree of inbreeding in a stream, and also potentially providing valuable information on the amount of breeding habitat a population contains (Whiteley et al. 2015a). Overall, N_b could be one of the most cost-effective ways to determine that a population is at high risk of genetic problems, and the same sampling efforts can readily produce H_e estimates, which could together provide complementary pieces of information for conservation.

Estimates of N_b and N_e will be particularly informative for determining when to attempt genetic rescue – an increase in persistence probability owing to gene flow (Whiteley et al. 2015b,

Ralls et al. 2018, Bell et al. 2019). Genetic rescue has been documented in several at-risk species, but the strategy is highly under-utilized (Ralls et al. 2018) and has rarely been attempted in freshwater fishes (but see Robinson et al. 2017, Kronenberger et al. 2018). The low N_b and N_e in our study populations further support that many WCT populations could benefit from human mediated gene flow (Kovach et al. 2021).

Intentional isolation by installing barriers is an important strategy to protect cutthroat trout from harmful effects of invasive trout species that has been used for decades. An important consideration is to determine how much habitat is needed for cutthroat trout populations to persist (Hilderbrand and Kershner 2000, Novinger and Rahel 2003, Peterson et al. 2008, Fausch et al. 2009). Our results provided further evidence that populations with two to three habitable kilometers of stream can face risks from inbreeding. However, similar stream lengths support vastly differing fish densities. For example, Hall Creek and Gold Run Creek had slightly more habitable kilometers of stream, both streams were first order with no tributaries, and Hall had a larger drainage area, yet Gold Run Creek had considerably larger N_b and N_e (4 to 9 times greater depending on the estimator). Care must be taken to ensure sufficient habitat is available for barrier construction projects, and N_b and N_e offer promising metrics to help determine the minimum amount of habitat that should be isolated.

Countless freshwater populations and species face increased extinction risk due to being isolated in small habitat patches. N_e is perhaps the important indicator of which populations are likely to be the most vulnerable to genetic problems. However, due to the difficulty in estimating N_e, N_b may provide a cost effective wet powerful monitoring alternative. Taxa specific examinations of N_b, N_e and N_c will provide valuable insight into how N_b can be interpreted and used to guide conservation actions.

Table 3-1. Example life tables for Staubach Creek and Gold Run Creek. The remainder of the population-specific life tables are provided in Table S3-1, Table S3-2, and Table S3-3. s_x is age-specific annual survival probability, l_x is the probability of surviving to age x, and b_x is the age-specific number of offspring that survive to age-1.

Stream	Age (x)	$s_{x(\text{male})}$	$l_{x(\text{male})}$	$b_{x(\text{male})}$	$s_{x(\text{female})}$	$l_{x(\text{female})}$	$b_{x(\text{female})}$
Staubach	1	0.63	1.00	0.0	0.68	1.00	0.0
	2	0.53	0.63	0.9	0.59	0.68	0.0
	3	0.53	0.33	4.5	0.55	0.40	2.1
	4	0.47	0.18	10.3	0.43	0.22	8.1
	5	0.43	0.08	16.1	0.39	0.09	15.7
	6	0.43	0.04	21.0	0.37	0.04	22.3
	7	0.00	0.02	24.9	0.00	0.01	27.5
Gold Run	1	0.64	1.00	0.0	0.62	1.00	0.0
	2	0.85	0.64	0.0	0.86	0.62	0.0
	3	0.76	0.54	0.0	0.83	0.53	0.0
	4	0.75	0.41	0.1	0.81	0.44	0.0
	5	0.75	0.31	0.3	0.81	0.36	0.1
	6	0.75	0.23	0.7	0.81	0.29	0.3
	7	0.75	0.17	1.3	0.80	0.24	0.6
	8	0.75	0.13	2.1	0.79	0.19	1.0
	9	0.75	0.10	3.0	0.79	0.15	1.5
	10	0.74	0.07	4.1	0.79	0.12	2.1
	11	0.74	0.05	5.1	0.79	0.09	2.6
	12	0.75	0.04	6.2	0.78	0.07	3.2
	13	0.00	0.03	7.2	0.00	0.06	3.8

Table 3-2. Life-history parameters, including age at first maturity, maximum age, generation length, and the variance in life-time reproductive success shown for each population and the mean across all populations.

Population	Age at maturity (female)	Age at maturity (male)	Maximum age (female)	Maximum age (male)	Generation Length	Life-time variance in reproductive success
Crawford	3	2	7	6	4.9	50.1
Gold Run	4	3	13	13	10.3	36.3
Hall	4	3	7	8	5.8	49.6
Little Belt	3	2	5	4	3.3	39.5
Staubach	3	2	7	7	4.4	22.3
Mean	3.4	2.4	7.8	7.6	5.7	39.6

Table 3-3. Estimates of the census size ($\widetilde{N}_c$), effective number of breeders ($\widetilde{N}_b$), the effective population size (N_e) and the ratios between these parameters. $\widetilde{N}_b$ and N_e include both demographic (dem) and genetic (gen) estimators.

Population	N_b/N_e	$\widetilde{N}_{b(gen)}$	$\widetilde{N}_{b(dem)}$	$N_{e(gen)}$	$N_{e(dem)}$	$\widetilde{N}_c$	$N_{e(gen)}/\widetilde{N}_c$	$N_{e(dem)}/\widetilde{N}_c$
Crawford	0.54	15	9	28	17	90	0.31	0.19
Gold Run	0.61	28	49	45	81	411	0.11	0.20
Hall	0.38	4	5	9	14	63	0.15	0.22
Little Belt	0.55	59	135	108	246	334	0.32	0.74
Staubach	0.64	11	10	18	16	44	0.40	0.36
Mean	0.54	23	42	42	75	188	0.26	0.34

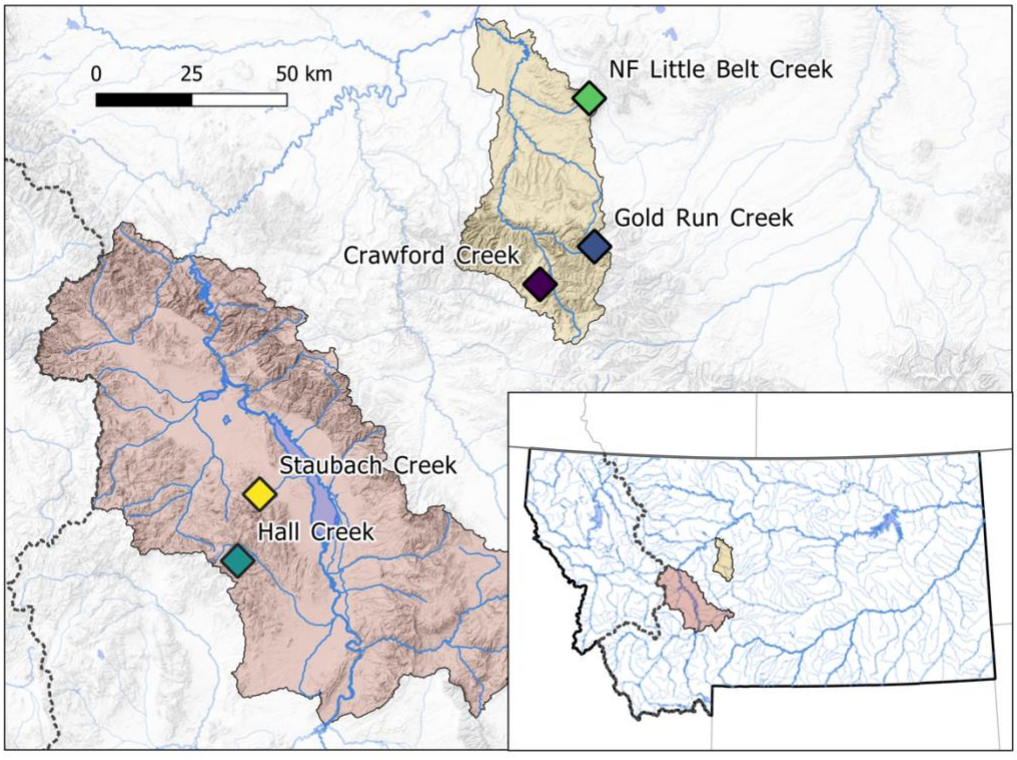

Figure 3-1. The five study populations located on the east of Continental Divide (dashed line) in Montana. The red polygon indicates the Upper Missouri Drainage (HUC 8) and the orange polygon indicates the Belt Drainage. The inset map shows Montana.

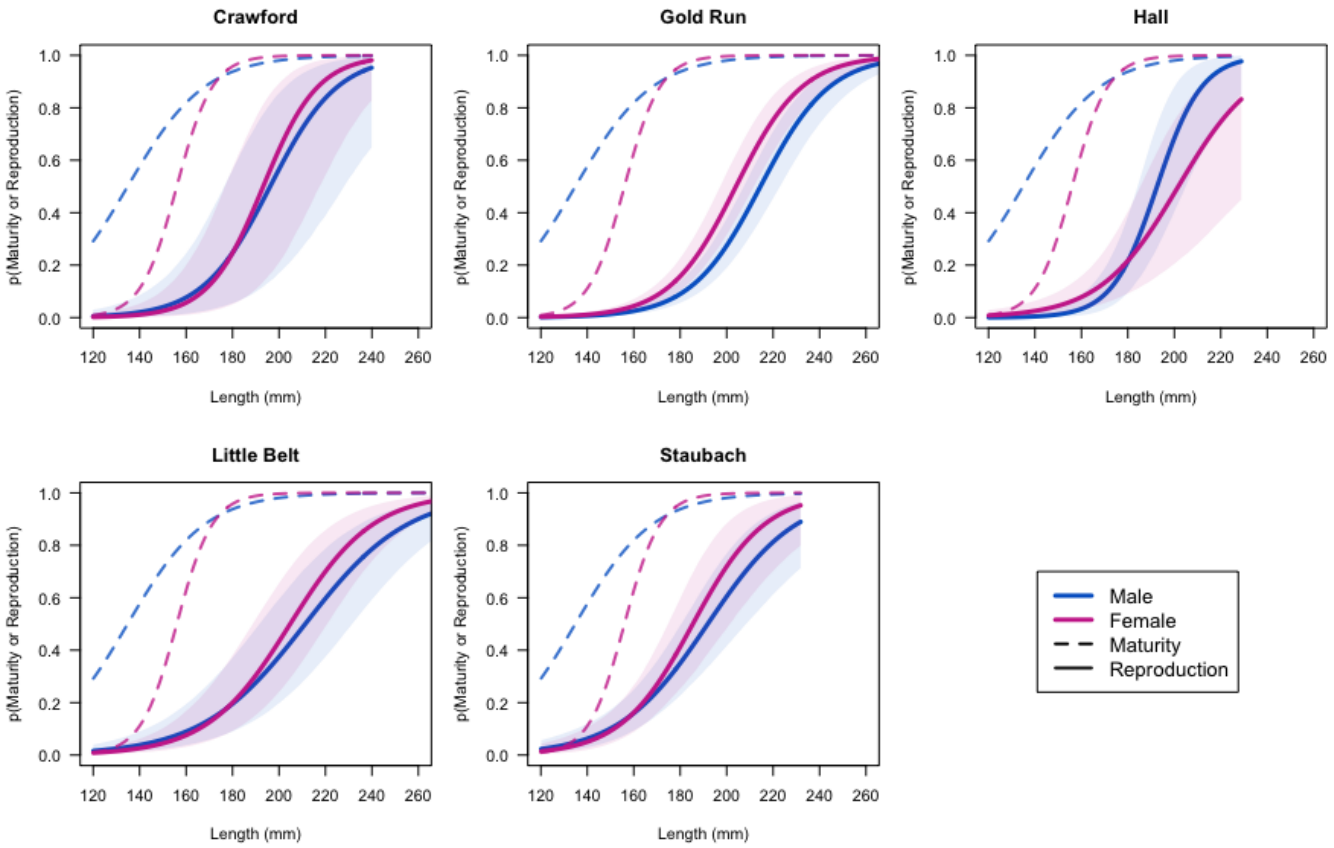

Figure 3-2. Pedigree-based probability of sexual maturity and producing offspring that recruit to age-1 in a given year as a function of parental length (mm; solid lines) shown separately by sex. Dashed lines represent the probability of maturity based on the presence of mature gametes from nearby populations (Downs et al. 1997). Confidence bands are 95% credible intervals.

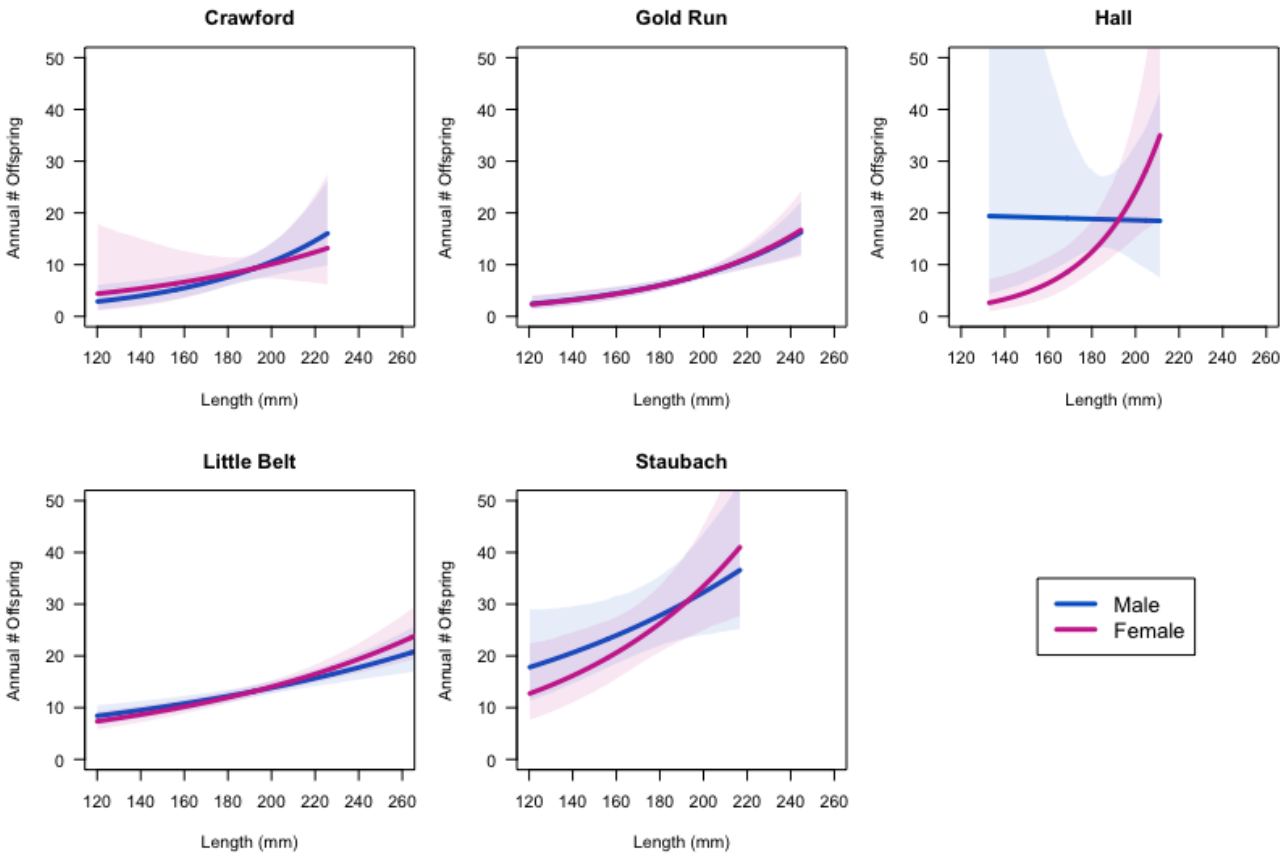

Figure 3-3. The annual number of offspring produced that recruit to age-1 (given that reproduction occurred) as a function of parental length (mm) shown separately by sex. Confidence bands are 95% credible intervals.

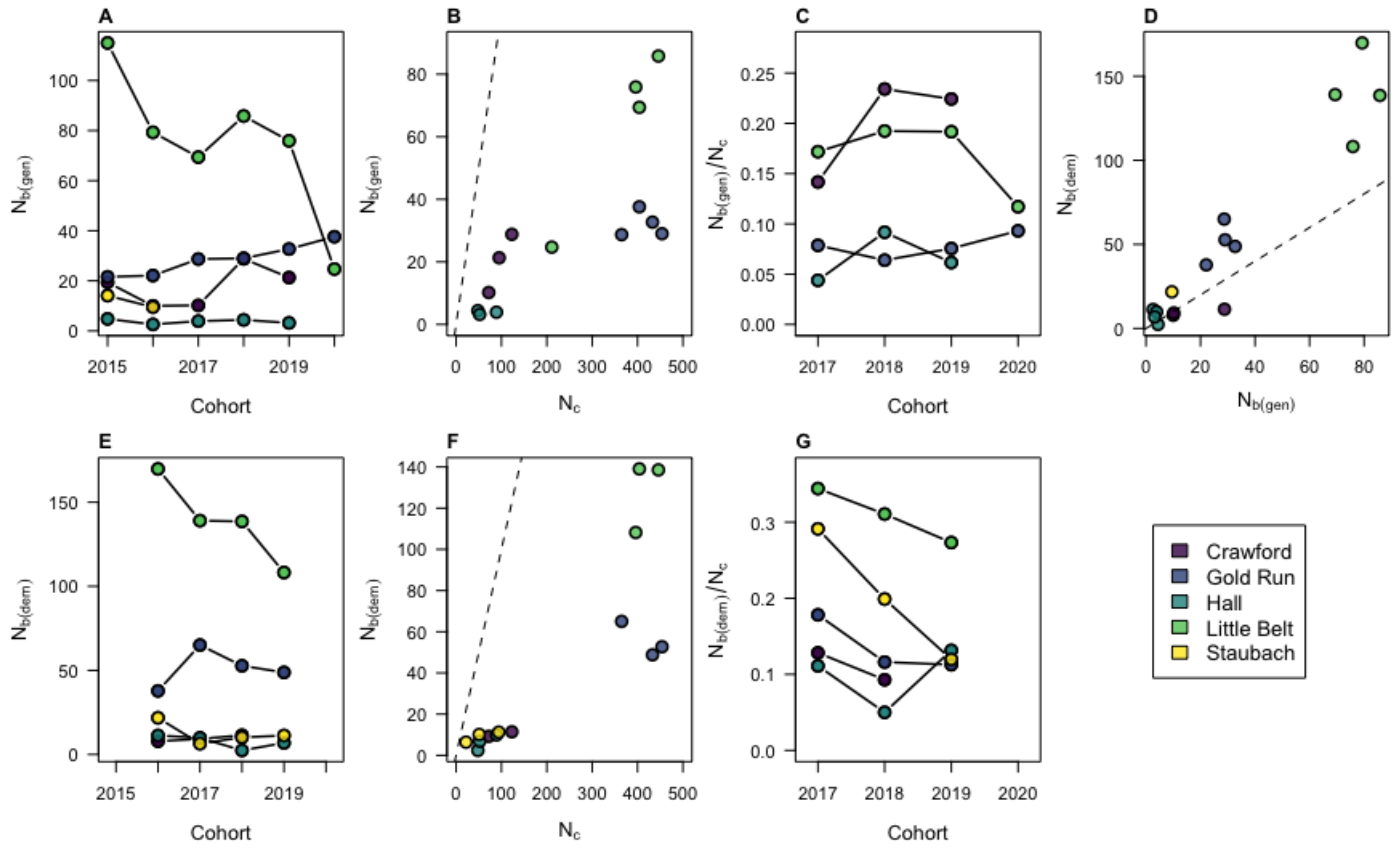

Figure 3-4. Relationships between the effective number of breeders (N_b), the census population size (N_c), and cohort year. The effective number of breeders includes genetic estimates (gen, A-D) demographic estimates (dem, D-G). Dashed lines represent a 1:1 ratio between parameters.

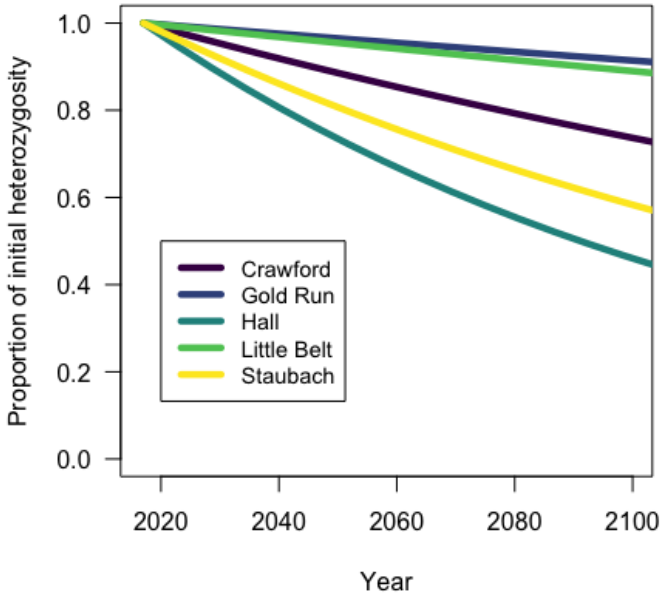

Figure 3-5. Predicted loss of genetic variation (proportion of initial observed heterozygosity, H_o) through 2100.

CHAPTER 4: The exciting potential and remaining uncertainties of genetic rescue

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Abstract

Restoring gene flow into small, isolated populations can alleviate genetic load and decrease extinction risk (i.e., genetic rescue), yet augmented gene flow is rarely implemented. Due to this discrepancy between opportunity and action, a recent call was made for widespread genetic rescue attempts. However, several aspects of augmenting gene flow are poorly understood including the magnitude and duration of beneficial effects and when deleterious effects are likely to occur. We discuss the remaining uncertainties of genetic rescue in order to promote and direct future research and to hasten progress toward implementing this potentially powerful conservation strategy on a broader scale.

The promise of genetic rescue and calls for a paradigm shift

Restoring gene flow is a promising strategy to combat the global threat of human-driven population declines and extinctions. Habitat destruction and fragmentation have isolated many small populations (Haddad et al. 2015), and interactions between demographic and genetic factors can drive these populations toward extinction (Soulé and Mills 1998). Over the last two decades, researchers have provided strong evidence that restoring gene flow into these small, isolated populations can alleviate **genetic load** (see Glossary) and increase persistence probability (Tallmon et al. 2004, Frankham 2015, Whiteley et al. 2015b), termed **genetic rescue** (Tallmon et al. 2004). Evidence for genetic rescue has now been documented across a wide range of taxa including plants (Newman and Tallmon 2001), invertebrates (Hufbauer et al. 2015), fish (Fitzpatrick et al. 2016, Robinson et al. 2017), birds (Westemeier et al. 1998, Heber et al. 2012), reptiles (Madsen et al. 1999) and mammals (Johnson et al. 2010, Weeks et al. 2017, Hasselgren et al. 2018).

Despite the promise of genetic rescue, augmented gene flow is rarely used as a conservation strategy (Frankham et al. 2017). Recommendations have been made for cautious and limited application of augmented gene flow due to concerns about **outbreeding depression** (Edmands 2007) and **genetic homogenization** (Kolodny et al. 2019). The standard conservation practice is to manage populations in isolation to preserve genetic distinctiveness (Weeks et al. 2016, Ralls et al. 2018). However, genetic distinctiveness can be caused by genetic drift in small, isolated populations, and managing these populations in isolation may increase their extinction risk (Weeks et al. 2016). Recent calls have been made for a paradigm shift in the genetic management of small, isolated populations away from inaction and toward widespread consideration of augmenting gene flow (Weeks et al. 2016, Frankham et al. 2017, Ralls et al. 2018, Chan et al. 2019).

We agree that genetic rescue should be attempted more frequently. Nevertheless, several aspects of genetic rescue are poorly understood. Importantly, the benefits and risks of restoring gene flow need to be better characterized to provide realistic expectations and to enable accurate cost-benefit analyses with competing conservation strategies. Conservation practitioners also need a clearer understanding of how to best implement genetic rescue attempts across a broad range of scenarios in order to maximize the utility of restoring gene flow. Here, we highlight aspects of genetic rescue that remain uncertain. Our goal is to promote and direct additional research that will help transition the conservation community toward widespread genetic rescue attempts.

The definition of genetic rescue

The 'rescue effect' was coined nearly 50 years ago to refer to decreased extinction risk of populations following immigration (Brown and Kodric-Brown 1977). The rescue effect was primarily attributed to the simple addition of immigrants to the population, which decreases **Allee effects** and **demographic stochasticity** (Ingvarsson 2001) (i.e., **demographic rescue**). Genetic rescue was distinguished from demographic rescue after studies provided empirical evidence that the genetic contribution of immigrants can cause a further increase in abundance (Westemeier et al. 1998, Madsen et al. 1999, Ingvarsson 2001).

Genetic rescue was originally defined as "the increase in the probability of a population's survival due to the immigration of genes from another population (Richards 2000)." Several

competing definitions of genetic rescue have since been used. Definitions that reduce the emphasis on extinction risk can cause confusion in how genetic rescue is best evaluated, which in turn may be inhibiting much-needed progress. We contend that genetic rescue is best defined as "a decrease in population extinction probability owing to gene flow, best measured as an increase in population growth rate". This is consistent with the original more theoretical definition (Brown and Kodric-Brown 1977, Richards 2000) but also emphasizes that, in practice, genetic rescue is best measured as an increase in **population growth rate** (Box 1).

Genetic rescue is typically attributed to the masking of deleterious alleles. However, gene flow can also promote adaptation to changing environmental conditions by increasing the variation upon which selection acts. These mechanisms are not mutually exclusive, and will often co-occur in small populations that suffer from both inbreeding depression and mal-adaptation. Genetic rescue overlaps with **evolutionary rescue** when gene flow provides the variation needed for evolution to reverse population declines, which is often the case for small populations (Gonzalez et al. 2012).

The complex reality of genetic rescue

Although genetic rescue is conceptually simple, gene flow has complex influences on individual fitness and population dynamics. These influences depend on the genetic composition and environmental conditions of the recipient and source populations. The maximum potential increase in fitness is determined by the severity of the genetic load in the recipient population, but realized fitness benefits also depend on the introduced genetic material. Migrants introduce both beneficial and deleterious genetic variation. Beneficial effects of gene flow include masking deleterious, recessive alleles and increasing additive genetic variation (Whiteley et al. 2015b). Deleterious effects of gene flow can be caused by a reduction in local adaptation or **genetic incompatibilities** between the source and recipient populations. The net effect of introduced beneficial and deleterious genetic variation determines whether genetic rescue, outbreeding depression or neither occurs.

The fitness effects of gene flow change over time because beneficial and deleterious genetic variation manifest at different time scales. In the first (F1) generation, the maximum number of deleterious, recessive alleles are expected to be masked, often causing **heterosis**. In the second (F2) generation, hybrid fitness declines as the population approaches Hardy-

Weinberg equilibrium (Tallmon et al. 2004), but maternal effects can transfer the fitness benefits of heterosis to F2 progeny (Frankham 2015). As a result, fitness benefits are predicted to be maximal in the F1 and F2 generations and can decline in later generations as genetic load re-accumulates due to inbreeding and genetic drift. In the F2 and later generations, recombination can expose genetic incompatibilities (Frankham et al. 2011, Havird et al. 2016) or form novel beneficial genotypes (Hwang et al. 2011). The fitness effects of gene flow are also influenced by the effective population size and the strength of natural selection, which determine whether novel beneficial alleles and genotypes increase in frequency.

These evolutionary dynamics, of course, play out in an ecological theater. Gene flow can only increase population growth rate when abundance is suppressed below carrying capacity due in part to a high genetic load. Additionally, population growth rate is influenced by environmental conditions. For example, in a deteriorating habitat, abundance may continue to decline despite beneficial effects of gene flow. These complex eco-evolutionary interactions make it difficult to accurately predict how restoring gene flow will influence a population.

Uncertainties surrounding genetic rescue

What is the magnitude of genetic rescue?

Understanding how often gene flow appreciably decreases population extinction risk is critical for informing conservation decisions. As genetic rescue is due to alleviating genetic load, uncertainty about the magnitude of genetic rescue is related to the long-standing debate over how often genetic load is a key contributor to extinction. Substantial evidence now suggests that inbreeding and genetic drift can depress individual fitness (Keller and Waller 2002, Charlesworth and Willis 2009), with strong evidence coming from genetic rescue studies (Frankham 2015). Less is known about how often elevated hybrid fitness will translate into increased population growth rate. Evidence for increased population growth rate following gene flow has been found in laboratory and wild populations (Tallmon et al. 2004, Whiteley et al. 2015b). In wild populations, concurrent habitat improvements and lack of control and replicate populations make it difficult to characterize the contribution of genetic factors to increased population growth rate (Tallmon 2017). Additionally, current genetic rescue attempts have involved severely inbred populations, but many populations with less severe genetic loads could

still benefit from gene flow. In these cases, the magnitude of genetic rescue is not expected to be as large. Better characterization of the magnitude of genetic rescue will increase confidence and interest in conservation applications of restoring gene flow.

What is the duration of genetic rescue?

The duration of genetic rescue is a major outstanding question (Waller 2015). Most studies have been limited to the period when beneficial effects are expected to be maximal (i.e., F1 and F2 generations). A recent meta-analysis provided evidence that increased fitness due to gene flow can persist through, and may even be higher in, the F3 generation (Frankham 2016). However, this meta-analysis was based on a small number of mostly laboratory invertebrate populations (16 of 17 comparisons). These data limitations highlight the lack of long-term studies on genetic rescue. Even if elevated hybrid fitness is primarily limited to the F1 and F2 generations, abundance may still increase if sufficient habitat is available, which in turn would decrease Allee effects and demographic stochasticity. Importantly, genetic rescue is still beneficial in this scenario because it can buy time while further conservation strategies are planned and implemented.

Genetic rescue is expected to be temporary when the same habitat constraints that caused the initial population decline remain present or when habitat is deteriorating (Fig 4-1A; Box 4-2). Unfortunately, habitat constraints are a recurring theme in the limited number of conservation-motivated genetic rescue attempts. For example, the abundance of greater prairie chickens (*Tympanuchus cupido*) initially increased following gene flow (Westemeier et al. 1998), but habitat constraints likely contributed to the subsequent population decline (Bouzat et al. 2009). In another recent example, gene flow was augmented as part of a broader conservation strategy to protect mountain pygmy possums (*Burramys parvus*) (Weeks et al. 2017). Genetic rescue likely contributed to the rapid increase in abundance, and concurrent habitat improvements may allow for abundance to remain elevated. However, climate change is beginning to cause large declines in a key food resource for mountain pygmy possums (Gibson et al. 2018), and continued conservation efforts will be essential for the possums' persistence. Both examples were last-ditch efforts to prevent extinctions in populations that face extreme habitat constraints. Future genetic rescue attempts are likely to include populations where habitat constraints are more easily alleviated and the benefits of gene flow are longer lasting (Fig. 4-1A).

When will outbreeding depression occur?

The limited number of genetic rescue attempts is partly due to concerns over outbreeding depression. Risks of outbreeding depression can be minimized by following current genetic rescue guidelines (Hedrick and Fredrickson 2010, Frankham et al. 2011). These guidelines call for selecting populations that occur in similar habitats and have low **population divergence** to avoid reducing local adaptation and genetic incompatibilities, respectively. A meta-analysis of studies adhering to these guidelines found very limited evidence for outbreeding depression (Frankham et al. 2011). This has led several researchers to assert that outbreeding depression is avoidable and concerns are overstated (Ralls et al. 2018, Chan et al. 2019).

However, current guidelines are mostly based on studies that are limited to the F1 and F2 generations. Delayed onset of outbreeding depression until F3 and later generations has not been well examined and may be a concern in some circumstances. Outbreeding depression may not manifest until later generations because heterosis is temporary and recombination can expose additional genetic incompatibilities over time (Fenster and Galloway 2000). Although concerning, severe genetic incompatibilities are unlikely to occur in closely related populations because they tend to form over long time periods. The onset of outbreeding depression may be further delayed if local adaptations are to extreme, periodic events (e.g., floods or fires). This would delay the manifestation of outbreeding depression until the next extreme event, though this has not been demonstrated to our knowledge. The potential for late onset of outbreeding depression further emphasizes the need for long-term studies on genetic rescue, but should not dissuade genetic rescue attempts that fall within existing guidelines.

Outbreeding depression is less predictable and presents a greater concern when source populations that meet the criteria in the current guidelines are unavailable (Harrisson et al. 2016). This may be common for endangered species with few remaining populations. Evolutionary theory predicts that natural selection tailors populations to their local environment, and gene flow predominantly reduces these local adaptations (Lenormand 2002). For example, migrants had substantially reduced fitness compared to residents in large Atlantic salmon (*Salmo salar*) populations (Mobley et al. 2019). However, small populations that are governed by strong genetic drift are less likely to have fine-scale local adaptations (Leimu and Fischer 2008), especially in changing or stressful environments, and alleviation of genetic load may overpower the deleterious effects of reduced local adaptation (Sexton et al. 2011). A recent study

documented genetic rescue in Trinidadian guppies (*Poecilia reticulata*) despite many generations of divergent selection pressure to high versus low predation (Kronenberger et al. 2018). Trinidadian guppies offer a classic example of adaptive differentiation (Bassar et al. 2010), but local adaptation is generally difficult to identify in wild populations (Hoban et al. 2016). More studies will be required to understand when differences in local adaptation will cause outbreeding depression in small, inbred populations.

Population divergence is less likely to cause outbreeding depression than differences in environmental conditions (Frankham et al. 2011). The extent of population divergence before strong genetic incompatibilities form is highly variable among taxa (Box 4-3), but complete reproductive isolation often takes millions of years (Edmands 2007). The genetic rescue guideline of 500 years of divergence is purposely conservative to minimize risk. However, genetic rescue attempts with greater divergence times are being increasingly considered (e.g., (Harrisson et al. 2016)) and may become common in the future. Researchers need to carefully evaluate what is known about outbreeding depression in their focal species because the extent of local adaptation and the potential for genetic incompatibilities will vary widely among taxa.

When will outbreeding depression increase the probability of population extinction?

Compared to inbreeding depression, even less is known about when outbreeding depression will substantially decrease persistence probability, but outbreeding depression does not appear to be a common contributor to extinction. In the commonly cited example, outbreeding depression resulting from maladaptive birth timing contributed to the extinction of Tatra Mountain ibex (*Capra ibex*) (Templeton et al. 1986). However, immigrants were moved from arid to alpine environments, and are now considered to be different species (*C. nubiana* and *C. aegagrus*). This example should not deter genetic rescue attempts because most conservation practitioners would not consider such a high-risk translocation today. Generally, outbreeding depression is most likely to appreciably depress population growth rate when increases in migrant ancestry are large, either due to high migration rates or substantial reproductive success of migrants and their offspring.

In some cases, populations have recovered from outbreeding depression (Templeton 1986, Hwang et al. 2011, 2016) (Fig. 4-1b). Crosses between marine copepod (*Tigriopus californicus*) populations with known genetic incompatibilities had reduced fitness in the F2

generation but elevated fitness in the F3 generation due to novel beneficial genotypes (Hwang et al. 2011). This has led several researchers to suggest that outbreeding depression is often temporary (Erickson and Fenster 2006, Ralls et al. 2018). However, in small populations, a rebound in abundance following outbreeding depression may be prevented by low efficacy of natural selection. In some cases, even subtle outbreeding depression could tip the scale toward extinction.

Can native ancestry be preserved following genetic rescue?

The potential for loss of evolutionary lineages and genetic homogenization are prominent concerns for restoring gene flow. **Genetic swamping** may eliminate the unique adaptations that made the population of such high conservation value in the first place. Large increases in migrant ancestry appear common and difficult to prevent. High profile genetic rescue studies consistently document large increases in migrant ancestry (Johnson et al. 2010, Adams et al. 2011, Fitzpatrick et al. 2016, Robinson et al. 2017). For example, migrant ancestry reached approximately 70% following translocations into an inbred bighorn sheep population (*Ovis canadensis*) (Hogg et al. 2006). Further, recent simulation work shows that the magnitude of genetic rescue can be strongly associated with loss of native ancestry (Harris et al. 2019). Although the increase in migrant ancestry is a stochastic process and the extent of genomic sweeps will be hard to anticipate, conservation practitioners can influence migrant ancestry by introducing an appropriate number of migrants (see (Hedrick 1995); Box 4-3), or potentially using controlled crosses in a captive environment (Hedrick and Fredrickson 2010, Hedrick and Garcia-Dorado 2016). The challenge facing conservation practitioners is to determine if the consequences of inbreeding depression outweigh the risk of genetic homogenization (Kolodny et al. 2019) and if genetic distinctiveness is the product of unique local adaptations.

Genomics and genetic rescue

The genomic revolution provides new and exciting opportunities to address many of the uncertainties described above (Fitzpatrick and Funk 2019). Understanding the genomic architecture of the genetic load will be valuable for informing expectations about the magnitude and duration of genetic rescue. However, the genomic architecture remains poorly understood (Kardos et al. 2016). The genetic load in small, inbred populations is likely caused by many loci

of varying effect (Charlesworth and Willis 2009, Paige 2010). If the loci underlying the genetic load are also highly variable among populations, the notion that inbred source populations can produce genetic rescue will be reinforced (Heber et al. 2012). This would also imply that specifically tailoring source populations and immigrants to maximize genetic rescue effects would be difficult. Another related uncertainty is whether loci contributing to genetic load contain deleterious alleles that are segregating within a population or have become fixed due to strong genetic drift. If most deleterious alleles are segregating, we expect genetic rescue effects to be ephemeral unless the effective population size increases, subsequently allowing selection to overwhelm genetic drift (Harris et al. 2019). Alternatively, if fixed deleterious alleles are primarily responsible for reduced fitness, gene flow will expose novel genetic variation to selection and the duration of genetic rescue may be greater, particularly if fixation occurred during a period of low effective population size.

Genomic techniques can help identify recipient populations in need of genetic rescue and source populations that will maximize benefits and minimize risks. Genomic approaches allow for precise estimates of inbreeding (Kardos et al. 2016), which is a useful indicator of genetic load in the recipient population (see (Fitzpatrick and Funk 2019)). Genomic techniques can also help researchers to identify loci that have a large contribution to the genetic load. When large effect loci are identified, specifically selecting immigrants or source populations that possess beneficial alleles will be more practical. Similarly, facilitating adaptation to climate change may be improved by targeting specific loci (but see (Kardos and Shafer 2018)). Researchers can also use genomic approaches to identify inversions and other structural differences that may cause outbreeding depression. In addition, researchers can increasingly identify adaptive differentiation among populations (Forester et al. 2018), which will help to minimize the risk of outbreeding depression and also to distinguish between neutral versus adaptive genetic distinctiveness.

Concluding remarks: The path forward for genetic rescue

Evidence for genetic rescue is rapidly accumulating and a transition toward widespread restoration of gene flow is likely warranted. However, further research is needed to address remaining uncertainties and to increase confidence in this promising strategy (see Outstanding Questions). Researchers should take advantage of naturally occurring genetic rescue and outbreeding depression to help reduce this uncertainty (e.g., natural immigration (Vilà et al.

2003, Hedrick et al. 2019), hybrid zones (Muhlfeld et al. 2009), and invasive species (Kolbe et al. 2004)). In addition, academics should continue to collaborate with managers to assist with detailed evaluation of genetic rescue attempts and publish findings (for an excellent example see (Johnson et al. 2010)). When possible, multigenerational genetic rescue experiments should be implemented (Tallmon 2017).

Additionally, deliberate efforts to experimentally examine genetic rescue and outbreeding depression across a wide range of conditions would enhance our ability to refine current guidelines. Although diverse outcrossing scenarios have been explored in the plant literature (e.g., (Fenster and Galloway 2000, Willi and Van Buskirk 2005, Bontrager and Angert 2018)), examining these relationships across diverse taxa would be informative. A more detailed understanding of genetic rescue will help conservation practitioners weigh restoring gene flow as a stop-gap measure against alternative conservation strategies, or better still, to incorporate genetic rescue into broader conservation plans that include restoring, expanding, and reconnecting habitat.

Although uncertainties remain, the extinction crisis is happening now (Ceballos et al. 2017). Genetic rescue should be attempted more aggressively when proposed translocations conform to current guidelines. When translocations do not meet guidelines, potential risks of outbreeding depression and genetic homogenization need to be compared against inaction (Ralls et al. 2018). In these instances, genetic rescue should be attempted with caution because even if severe outbreeding depression is rare, one high profile case may inhibit progress by altering perceptions (Royzman and Rozin 2001). Researchers should strive to improve our understanding of genetic rescue to the point where we can confidently and effectively restore gene flow with minimal monitoring. Once this is achieved, restoring gene flow may become one of the most practical, powerful, and inexpensive tools in conservation biology, potentially decreasing the extinction risk for a vast number of populations.

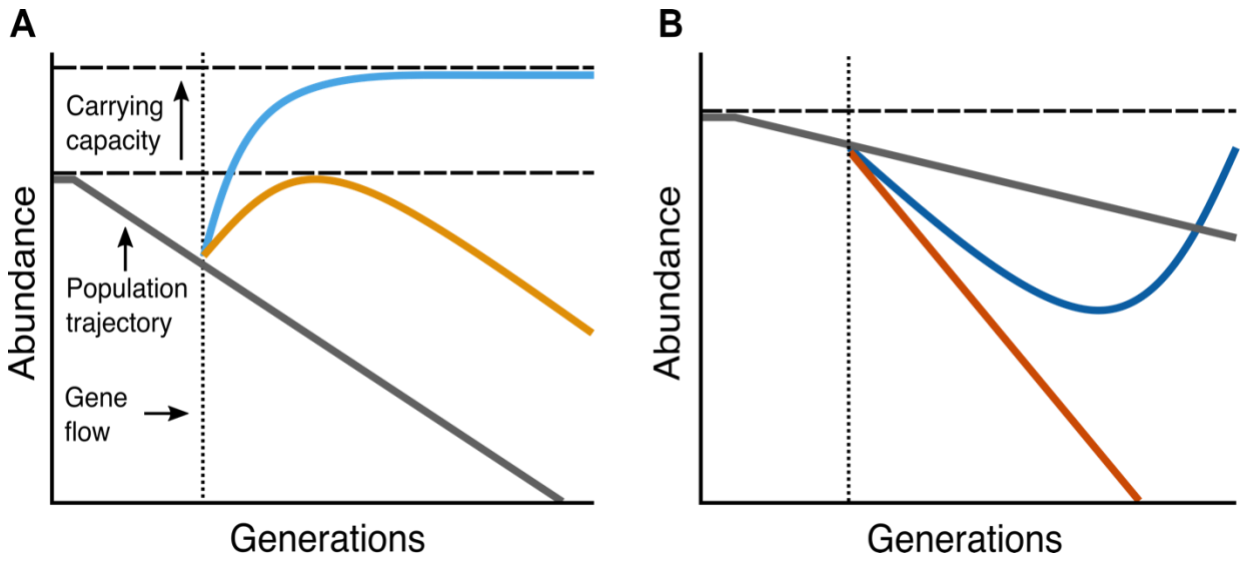


Figure 4-1. Potential trends in abundance following gene flow into a declining population. (A) Genetic rescue will likely be ephemeral (orange line) unless the habitat constraints that caused the initial population decline are removed (light blue line). (B) Severe outbreeding depression may drive populations toward extinction (red line) unless the efficacy of natural selection is sufficient to allow for recovery (dark blue line). The initial population trajectory is represented by the solid grey line, gene flow is represented by the dotted line, and carrying capacity is represented by the dashed line.

Glossary

Allee effects: a positive relationship between population growth rate and density. Allee effects can increase extinction probability in small populations.

Carrying capacity: the maximum number of individuals that a habitat can sustain given no genetic load.

Demographic rescue: a decrease in population extinction probability owing to the demographic contribution of immigrants.

Demographic stochasticity: fluctuations in population size due to random variation in survival and birth rates. Demographic stochasticity can increase extinction probability in small populations.

Evolutionary rescue: a decrease in population extinction probability owing to adaptation to environmental stress from standing genetic variation, de novo mutation, or gene flow.

Genetic homogenization: an increase in genetic similarity of populations due to gene flow. Genetic homogenization can lead to loss of species-level genetic diversity (see (Kolodny et al. 2019)).

Genetic incompatibilities: reduced fitness due to deleterious interactions among loci.

Genetic load: the proportional decrease in fitness between the average genotype in a population and the theoretically fittest genotype (see (Wallace 1991, Hedrick and Garcia-Dorado 2016)). Genetic rescue can alleviate genetic load that is due to inbreeding depression, deleterious alleles that have reached high frequency or fixation by genetic drift, and maladaptation to changing environmental conditions.

Genetic rescue: a decrease in population extinction probability owing to gene flow, best measured as an increase in population growth rate.

Genetic swamping: loss of locally adaptive alleles due to gene flow.

Heterosis: elevated fitness of F1 hybrids relative to their parents (see (Charlesworth and Willis 2009)). Heterosis is due to increased genome-wide heterozygosity following mating between individuals from divergent lineages.

Hybrid: an individual with both migrant and resident ancestry. Here, we are referring to both intraspecific and interspecific hybrids and including first and later generation hybrids.

Inbreeding depression: reduced fitness of offspring with related parents.

Outbreeding depression: reduced fitness of hybrids. Outbreeding depression is typically attributed to maladaptation to local environmental conditions or genetic incompatibilities.

Population divergence: the time since isolation between populations (see (Edmands 2007)).

Population growth rate: change in abundance over time.

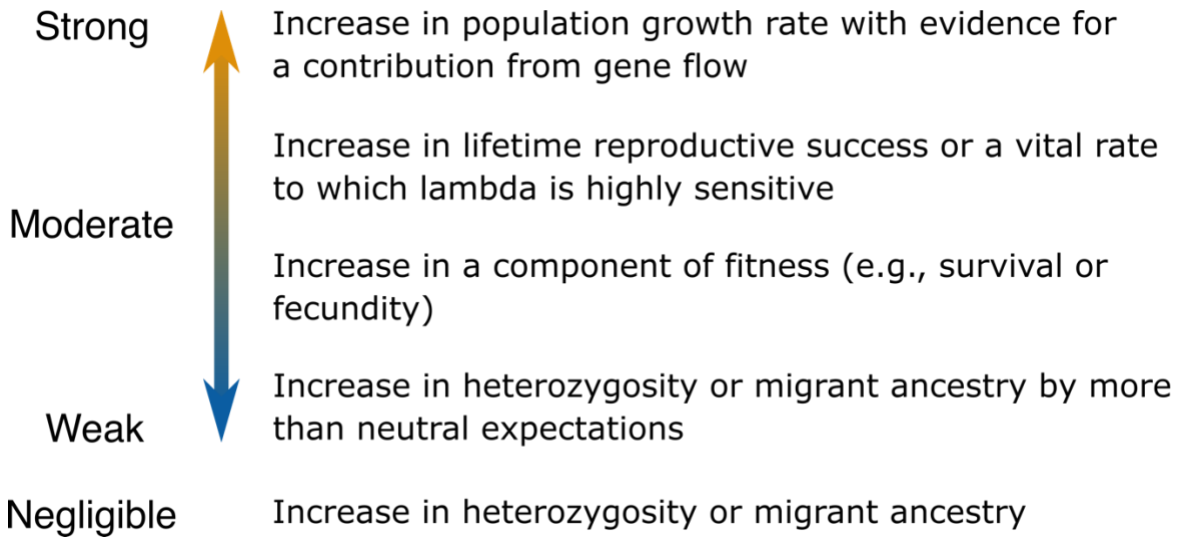
Box 1. Expanding the definition of genetic rescue and providing a framework for its evaluation

The ultimate goal of attempting genetic rescue is to decrease a population's risk of extinction. Whether a population persists or goes extinct is determined primarily by the population growth rate (Shaffer 1981), making population growth rate the critical parameter for conservation. For this reason, we emphasized population growth rate in our previous definition of genetic rescue: "an increase in population growth rate owing to gene flow" (Tallmon et al. 2004, Whiteley et al. 2015b). This definition has received criticism for being overly narrow (Hedrick et al. 2011). Populations cannot expand when habitat is limiting, even when gene flow alleviates genetic load. Additionally, an increase in population growth rate is difficult to measure in wild populations. In order to capture a wider range of beneficial outcomes, we expand our definition of genetic rescue to "a decrease in population extinction probability owing to gene flow, best measured as an increase in population growth rate".

A concern arising from this broader definition is that studies may report genetic rescue based on parameters that are weakly associated with persistence probability. Importantly, an increase in heterozygosity (i.e., decrease in inbreeding) by itself provides very limited evidence for genetic rescue. Increased heterozygosity is associated with future adaptive potential, but resulting demographic responses will typically occur outside of the timeframe of monitoring and conservation objectives. Increased genetic variation is a weak indicator of contemporary extinction risk because gene flow initially increases heterozygosity irrespective of whether genetic rescue or outbreeding depression occurs.

A positive demographic response is needed to infer increased persistence probability in the short-term (Fig. 4-I). An increase in migrant ancestry, beyond expectations under genetic drift alone, provides evidence for elevated fitness of **hybrids** compared to residents (Hedrick et al. 2011), but determining neutral gene flow expectations is difficult in practice. Better evidence for increased persistence probability is an increase in vital rates to which population growth rate

has a high sensitivity (Johnson et al. 2011). The best evidence is an increase in population growth rate due to gene flow. Monitoring should cover multiple generations and focus on the metrics that provide the strongest evidence for evaluating whether genetic rescue occurred given the available resources. Conservation practitioners can follow similar criteria for evaluating genetic rescue attempts, but will often be less concerned with separating the genetic versus demographic contribution of immigrants.

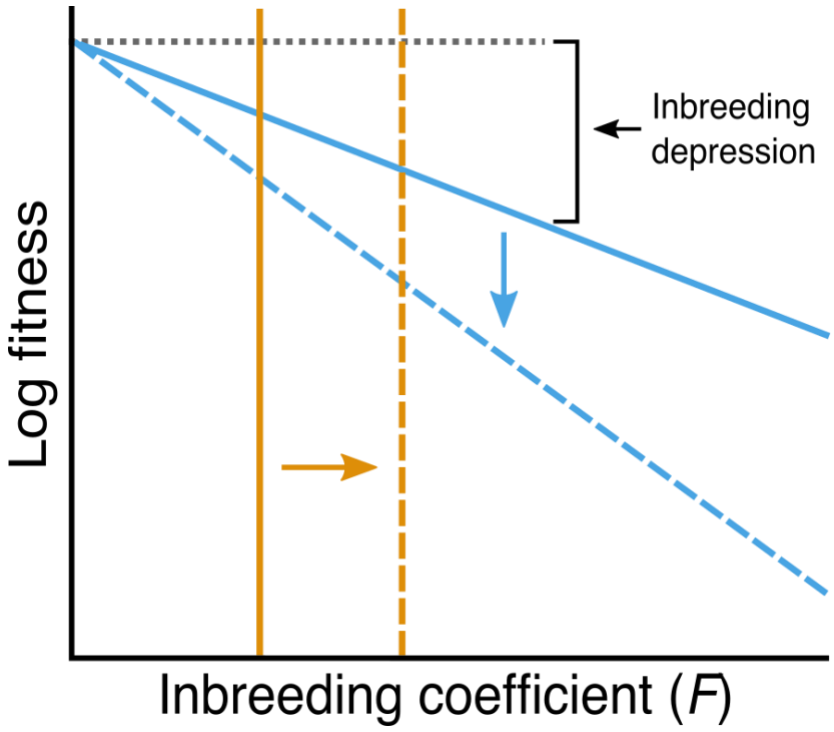


Box 4-1; Figure I. The relative strength of different types of evidence for genetic rescue.

Box 4-2. The mystery on Isle Royale

Isle Royale wolves present perhaps the most detailed example of inbreeding depression contributing to a functional extinction (Hedrick et al. 2019, Robinson et al. 2019), but the influence of gene flow on this extinction is unclear. Isle Royale (on Lake Superior, Michigan, USA) contains a small population of highly inbred wolves (mean census size of 24 (Adams et al. 2011)). In 1997, a single male immigrated to Isle Royale. Due to extremely high fitness, his ancestry constituted 56% of the genomic composition of the population within two generations (Adams et al. 2011). Inbreeding coefficients rapidly increased within the population, which likely contributed to a precipitous decline in abundance. By 2018, only two highly related wolves remained on the island, with the male being both the father of and half-siblings with the female. They produced one inviable offspring (Hedrick et al. 2019) and have shown no further signs of courtship (Mlot 2018).

It is uncertain whether the migrant's arrival forestalled or contributed to the demise of the Isle Royale wolves. If the migrant increased the rate of extinction, it would be the first documentation of a distinct negative effect of gene flow in which a genomic sweep leads to a rapid increase in inbreeding depression (Figure I). Current genetic rescue guidelines would not be relevant for this deleterious effect because individuals with a low risk of outbreeding depression could still cause a genomic sweep. Interestingly, if more than one wolf had immigrated to Isle Royale, inbreeding depression may have been less severe because inbreeding coefficients would have increased less rapidly. Alternatively, additional immigrants may have introduced more deleterious alleles into the population and increased the extent of inbreeding depression for a given inbreeding coefficient. Further research is needed to understand how gene flow into populations with severe habitat constraints can influence the duration of genetic rescue or potentially increase extinction risk. A wolf reintroduction program was recently announced (Mlot 2018) and translocations began in 2018, which will allow researchers to observe the process unfold again.

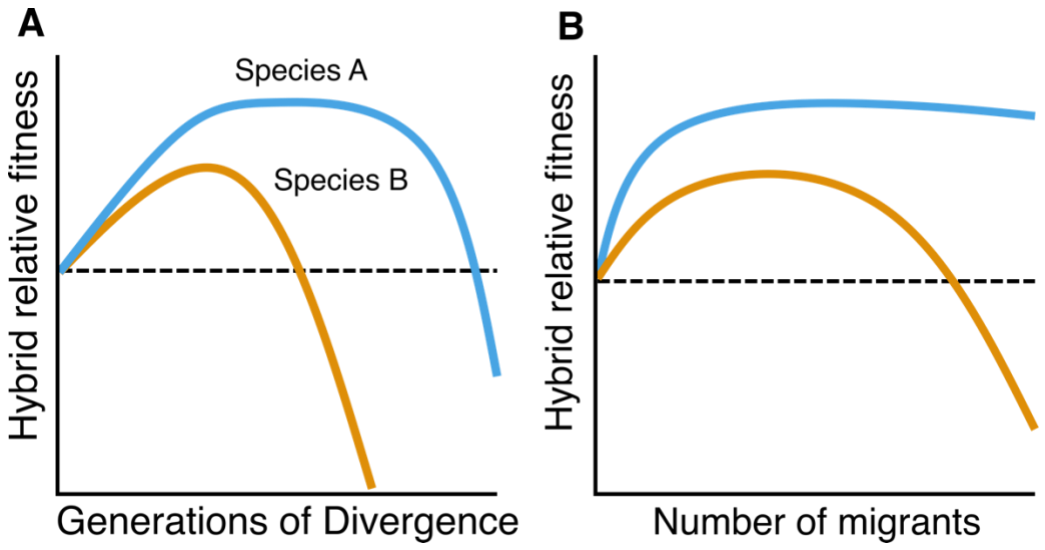


Box 4-2; Figure I. Can Gene Flow Increase Inbreeding Depression? Blue lines represent the relationship between inbreeding coefficients and fitness, and orange lines represent the mean inbreeding coefficient of a population. Gene flow may be able to increase inbreeding depression by causing a genomic sweep that increases the mean inbreeding coefficient in a population (orange dashed line), that introduces novel deleterious alleles that increase the severity of inbreeding depression for a given inbreeding coefficient (blue dashed line), or both. Unbroken lines represent the pre-gene flow and dashed lines represent the post-gene flow conditions. Inbreeding depression is the reduction in fitness of an inbred individual relative to a non-inbred individual (dotted grey line). The intersection of blue and orange lines represents the mean inbreeding depression of individuals in a population.

Box 4-3. Intermediate optima in population divergence and number of migrants

Intermediate amounts of population divergence and immigration rates should result in the strongest genetic rescue effects. Populations with low divergence may minimize rescue effects because they will often share the majority of the loci underlying their genetic load. On the other hand, high divergence may lead to outbreeding depression (Willi and Van Buskirk 2005, Kovach et al. 2016a). Making matters more complicated, the relationship between population divergence and rescue effects is taxon-specific and is also influenced by demographic history and the extent of local adaptation within the species (Figure 4-IA). These complexities make it difficult to predict the amount of population divergence that will have high risks of outbreeding depression. Attempts to identify optimally divergent source populations can be difficult, risky, and often unnecessary. However, more detailed considerations are necessary for cases where few, divergent source populations exist, especially for species with fine-scale local adaptations.

Likewise, intermediate immigration rates will typically result in the greatest rescue effects (Hedrick and Fredrickson 2010). Moving too few individuals may limit rescue effects and potentially accelerate the re-accumulation of genetic load (See Box 4-2). On the other hand, moving too many individuals may result in the loss of genetic distinctiveness and can potentially make outbreeding depression more likely to have large demographic effects. The relationship between migration rate and rescue effects is influenced by life-history, the magnitude of the genetic load, habitat constraints, and the extent of local adaptation in the recipient population (Figure 4-IB). Experimental tests of genetic rescue across various scenarios will help to identify these intermediate optima for diverse taxa and maximize genetic rescue effects.



Box 4-3; Figure 4-I. The influence of population divergence and migration rates on hybrid relative fitness in F2 and later generations. Intermediate amounts of population divergence (A) and migration rates (B) typically maximize genetic rescue effects (e.g., the fitness of hybrids relative to residents). However, relationships between these factors vary considerably due to taxonomic, evolutionary, and environmental differences. For example, divergent crosses or high immigration rates may be less risky for a generalist species (Species A; blue line) than a species with fine-scale local adaptation (Species B; orange line). Equal fitness between resident and hybrid individuals is represented by the dashed line.

CHAPTER 5: Experimental test of genetic rescue using inbred source populations of imperiled cutthroat trout

Abstract

Translocations aimed at increasing persistence by alleviating genetic problems (i.e., genetic rescue) and boosting genetic variation are a promising conservation strategy to combat human-driven habitat loss and fragmentation. However, our understanding of genetic rescue is hampered by few empirical studies that are confounded by multiple ongoing conservation efforts. We conducted an experimental test of genetic rescue in wild westslope cutthroat trout (WCT) populations in the Missouri River basin of Montana, USA, where remaining populations are isolated and have substantially depressed genetic variation. We translocated 6-8 mature fish into four isolated recipient populations that spanned a gradient of inbreeding risk and carefully monitored the genetic and demographic outcomes. The two smallest populations had substantially increased heterozygosity (39% and 215%) and increased survival (71% and 379% for hybrids compared to residents), suggesting that genetic rescue occurred. The mid-sized population had a smaller increase in genetic variation and minimal effects of gene flow on fitness, and the largest population had very low transplant reproductive output, suggesting limited effects of gene flow or potentially reduced fitness (i.e., outbreeding depression). We did not find clear evidence for an increase in population growth rate (i.e., the strongest evidence for genetic rescue) owing to gene flow in any population. The increase in vital rates without an increase in population growth could be due to the unique ecology of freshwater fishes compared to previous study organisms, and suggests that the population effects of genetic rescue may be more limited in fishes than some other taxa. Nevertheless, massive increases in genetic variation following translocations will likely translate into increased adaptive potential and promote the persistence of isolated fish populations. These results highlight that effective, broadscale implementation of genetic rescue will require examination of diverse translocation scenarios and taxa.

Introduction

Human-driven habit loss and fragmentation have confined enumerable populations to small habitat fragments with little or no connectivity to other populations. Small, isolated populations face heightened extinction risk due to demographic and genetic factors (Soulé and Mills 1998, Tallmon et al. 2004). Ideally, habitat should be restored and reconnected, but these actions are often impractical or impossible for at-risk populations. A promising conservation strategy is to institute a small amount of gene flow into isolated populations to alleviate genetic problems, increase vital rates, and ultimately, increase persistence probability, termed genetic rescue (Whiteley et al. 2015b, Bell et al. 2019). Despite numerous documented successes (Johnson et al. 2010, Weeks et al. 2017), genetic rescue is rarely attempted in populations of conservation concern (Frankham et al. 2017).

Recent calls have been made for a paradigm shift towards more widespread genetic rescue attempts (Ralls et al. 2018). However, concerns remain that augmenting gene flow will have limited or negative effects in some populations. For example, evolutionary theory predicts that gene flow should often reduce local adaptations (e.g., outbreeding depression). Further, detailed studies of genetic rescue in wild populations remain rare, and controlled and replicated studies are almost non-existent, necessitating further research to address uncertainties before genetic rescue can be confidently implemented on a broad scale (Bell et al. 2019).

Another important uncertainty surrounding genetic rescue is the degree to which inbred source populations can induce genetic rescue in other inbred populations. Genetic rescue using inbred source populations remains very rare (but see Heber et al. 2012). Outbred source populations are generally thought to result in greater fitness benefits (Frankham 2015), but large populations can carry a greater number of harmful mutations, potentially making the use of larger populations as the source of translocated individuals a greater risk in some cases (Kardos et al. 2021, Kyriazis et al. 2021, Pérez-Pereira et al. 2022). Importantly, many threatened species may no longer have any large, outbred populations left, leaving inbred source populations as the only option for genetic rescue attempts. Determining the efficacy of using isolated source populations will be crucial for the conservation of many taxa.

Freshwater ecosystems are among the most threatened on earth (Tickner et al. 2020) and have undergone extensive habitat fragmentation (Brauer and Beheregaray 2020). Genetic rescue has received limited attention in freshwater ecosystems (Frankham et al. 2017), yet many

freshwater taxa could likely benefit from gene flow. One such taxon is the westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) in the Missouri River basin. Westslope cutthroat trout (WCT) are now limited to a small fraction of their historical range due to habitat degradation and invasive species (Shepard et al. 2005, Bell et al. 2021a). In particular, hybridization with invasive rainbow trout is widespread and can lead to genomic extinction of populations (Muhlfeld et al. 2014, Kovach et al. 2016b), and was the main impetus for a petition to list WCT as threatened under the Endangered Species Act (Allendorf et al. 2004). All remaining non-hybridized populations are fragmented from one another, and are often confined to short stream reaches (Kovach et al. 2021). These populations have significantly reduced genetic variation (Fig. 5-1) and could be at increased risk of extinction (Drinan et al. 2011, Kovach et al. 2021). Given the severe threats from invasive species, restoring connectivity is unadvisable and pragmatically impossible, leaving augmented gene flow as one of the only viable conservation strategies for these populations.

We conducted an experimental test of genetic rescue using inbred populations as the sources for translocations into other small, isolated WCT populations that spanned a gradient of inbreeding risk. We translocated 6-8 fish into each of four small, completely isolated populations (Figure 5-1a) and also removed the same number of fish of the same sex and similar length as we introduced to isolate the genetic contribution of the immigrants. The recipient populations had only 10% to 56% of the mean heterozygosity found in >200 WCT populations in Rocky Mountains of Montana and southern British Columbia (Figure 5-1b). Three of the recipient populations were previously identified as candidates for genetic rescue (Kovach et al. 2021) and have effective population sizes that indicate inbreeding could be an immediate threat (Jamieson and Allendorf 2012; Chapter 3), while the largest recipient population was likely at less immediate risk. We intensively monitored the four recipient populations and two additional control populations for 5 years to document the influence of gene flow on genetic metrics, vital rates and population growth rate and to examine how these effects differ across the gradient of inbreeding risk.

Results

Effect of gene flow on genetic composition

At least one translocated fish reproduced in all study populations but the number of transplants that reproduced and total number of transplant offspring varied considerably among populations. In the largest population (Little Belt), three of eight transplants reproduced, but only had a total of four detected offspring. By the final year of the study, we found no evidence for any remaining ancestry from the migrants in Little Belt (Figure 5-2a). In the second largest population (Gold Run, N_e = 45, Chapter 3), 7 of 8 transplants produced 56 detected offspring, which lead to a migrant ancestry of 4.2% in 2021. In Hall Creek, which had the lowest effective size (N_e = 9, Chapter 3), 3 of 6 transplant produced 32 detected offspring, resulting in a migrant ancestry of 5.7% by 2021 (4 years after transplantation). In the population with the lowest genetic variation (Staubach, H_o = 0.027), 4 of 6 reproductively-successful migrants produced 235 F1 hybrids and 28 F2 hybrids that were detected, resulting in a migrant ancestry of 17.4% by 2021.

Individual heterozygosity was considerably greater for F1 hybrids relative to residents in all populations. Despite large differences in resident heterozygosity, F1 hybrids had similar heterozygosity in all populations (Figure 5-2b). This created a gradient in the difference in heterozygosity between hybrids and residents across populations of 84% in Little Belt, 70% in Gold Run, 285% in Hall, and 1025% in Staubach (Figure 5-2b). This translated to population increases in heterozygosity in all populations except for Little Belt (Figure 5-2c). For Gold Run and Hall, respectively, heterozygosity was 4% and 39% greater in 2021 than before gene flow in 2017. In Staubach Creek, the 4 reproductively successful migrants increased the observed population heterozygosity by 215% (H_o = 0.027 to 0.085).

Effect of gene flow on vital rates and population growth rate

To determine if gene flow influenced fitness, we first examined family size (an indicator of survival to age-1) and stage-specific survival of residents versus F1 hybrids and then combined vital rates to obtain a proxy of survival from fertilization to maturity (composite fitness). We omitted Little Belt from these analyses due to the very low gene flow. Hybrids in Gold Run had slightly (nonsignificant) depressed early life survival compared to residents, but higher age-2+

survival, which resulted in hybrids having a 20% lower survival to age-4 (*pd* = 0.67; Figure 5-3a-e), the age at which most fish have reached maturity. Hybrids in Hall had higher fitness than residents for all fitness components, but family size was the only significant difference. This resulted in hybrids having a 379% greater probability of surviving to maturity in Hall than residents (*pd* = 0.98), although with considerable uncertainty in parameter estimates. In Staubach, hybrids had 108% larger family size than residents (*pd* = 1), but non-significantly lower survival for all life-stages, which led to a 71% higher survival to maturity for F1 hybrids than residents (*pd* = 0.99). Overall, our examination of composite fitness revealed that the higher relative fitness of hybrids was statistically and biologically significant in Hall and Staubach.

An increase in abundance is often considered the best evidence for genetic rescue (Tallmon et al. 2004, Bell et al. 2019, but see Hedrick 2005, Hogg et al. 2006), and we calculated abundance and geometric population growth rate (λ_G) in both the recipient and control streams (Figure 5-4). λ_G of age-2+ fish from 2017 (pre-translocation) to 2021 ranged considerably from 0.93 in Hall to 1.26 in Staubach. Notably, the large increase in the abundance of Staubach occurred the year before F1 hybrids reached age-2 (Figure 5-4). Generally, the extent to which gene flow influences population growth is challenging to separate from environment influences. λ_G was slightly positive for the control populations (1.02 and 1.03), falling in the middle of the estimates for the recipient populations. However, the correlation in abundance between the recipient and control streams was variable and generally low (-0.88 to 0.86), suggesting low correlation in the population dynamics, limiting the usefulness of these comparisons.

Comparison of genetic rescue across populations

The recipient populations spanned a gradient of heterozygosity and effective size (Chapter 3), which is expected to influence genetic rescue outcomes. The number of detected offspring per transplant had a strong negative correlation with both the pre-translocation heterozygosity (r = -0.52) and the effective population size (r = -0.80) of the recipient population (Figure 5-5). Notably, migrants had an average of 39 detected offspring in Staubach (N_e = 18, H_o = 0.03) and only 0.4 offspring in Little Belt (N_e = 108, H_o = 0.16), which suggests 100 times greater reproductive success in Staubach. As stated above, composite fitness was the highest in hybrids in the two smallest populations.

Discussion

The influence of gene flow from inbred source populations on fitness differed among the four isolated recipient populations, consistent with the gradient of heterozygosity and effective population size. We found evidence for genetic rescue in the two smallest populations (N_e of 9 and 17): both Hall and Staubach had a large increase in early life survival, the life-stage in which lethal and highly deleterious mutations cause mortality (e.g., Isle Royale Wolves; Robinson et al. 2019), which translated into increased survival from conception to age-4. Gene flow appeared to have a minimal effect in Gold Run despite the population having an N_e of 45 that can often pose short-term risks from inbreeding (Jamieson and Allendorf 2012). Conversely, the largest population (N_e = 108) had no signs of any remaining migrant ancestry by the final year of the study, suggesting that transplants had no fitness advantage or possibly lower relative fitness (i.e., outbreeding depression).

Understanding how the use of inbred sources influences the outcome of translocations for genetic rescue is critical as it will be the only option in many cases. The effect size of genetic rescue in our two smallest populations was apparently smaller than many previous studies documenting genetic rescue (Johnson et al. 2010, Weeks et al. 2017), especially given that we did not see obvious increases in population growth rate. These smaller effect sizes could be due to our use of inbred source populations. Notably, our effect sizes were smaller than a similar study in a different headwater trout species that examined genetic rescue using gene flow from a large outbred source population (Robinson et al. 2017). Inbred sources populations are typically expected to carry fewer highly deleterious mutations due to purging, thus posing a lower risk (Kyriazis et al. 2021, Pérez-Pereira et al. 2022), but are also expected to result in lower heterosis (Frankham 2015). Studies examining the genetic architecture of inbreeding depression and genetics rescue (e.g., the number of loci and their effect size) will further improve our understanding of using inbred source populations (Fitzpatrick and Funk 2019).

The smaller genetic rescue effects could also be in part due to salmonids being more resilient to isolation than many previously studied taxa. Several factors may limit the impact of inbreeding depression on the population dynamics of trout populations. Salmonids have residual tetraploidy, which may allow for the retention of variation in some critical genomic regions (Frankham et al. 2017). Additionally, in high fecundity species with stages of strong competition, natural selection may have minimal influence on population growth both because

selection can determine which but not how many individuals make it through a density regulated life stage (i.e., soft selection) or because mortality due to selection could be compensatory (Wallace 1975, Bell et al. 2021b). Staubach had a pattern consistent with compensatory mortality. Hybrid fish in Staubach appear to have considerably elevated survival to age-1, as evidenced by increased family sizes, then reduced survival at later life-stages. A possible explanation is that high densities created by these large families reduced survival in the later life-stages. This suggests early life inbreeding depression could result in compensatory mortality because density determines the number of juveniles a stream section can support. It remains possible that gene flow has increased r_{max}, which would allow for more rapid recovery from environmental catastrophes.

A major concern with genetic rescue attempts is that gene flow may sometimes depress fitness (i.e., outbreeding depression). Outbreeding depression offers a potential explanation for the reduction in some vital rates of hybrids and for the complete failure to the translocation into Little Belt, although alternative explanations cannot be ruled out (i.e., the stochastic nature of translocations). Salmonids are thought to have fine-scale local adaptation (Taylor 1991, Eliason et al. 2011, Fraser et al. 2011, Mobley et al. 2019) which could increase their susceptibility to reductions in these local adaptations from gene flow. Gold Run and Little Belt have higher N_e, and therefore could be more likely to be locally adapted and adaptively differentiated, which increases the risk of disrupting local adaptations via translocations.

Verifying that genetic rescue occurred is a considerable challenge. Almost all of our understanding of genetic rescue has come from laboratory experiments and opportunistic monitoring of translocations that are part of a suite of conservation efforts to protect threaten species (Bell et al. 2019), making it challenging to separate genetic from environmental effects. Further, genetic rescue studies may face inherent power limitations because inbreeding is the most problematic in the smallest populations which by definition will have low samples sizes even with intensive monitoring efforts (Robinson et al. 2021). Hall Creek provides a good example. Sample size could have been increased by translocating more fish, but this risks genetic swamping and with an average of only 4 detected age-1 families a year, the habitat could be placing a very low upper limit on sample size. This conundrum with small sample sizes is not unique to our study. Our samples sizes, even in the smallest populations, were consistent with or larger than many previous high-profile studies (Madsen et al. 1999, Johnson et al. 2010, Weeks

et al. 2017, Hasselgren et al. 2018). Unless the effect size is large, genetic rescue outcomes will be challenging to determine in many small populations (Robinson et al. 2021).

Finally, despite population growth providing the best support for genetic rescue in most cases (Tallmon et al. 2004, Whiteley et al. 2015b), trends in abundance may be difficult to document in some taxa, which is likely the case for salmonids. High fecundity of salmonids likely allows for populations to track carrying capacity despite inbreeding depression, and depressed vital rates may not result in depressed population growth. Further, salmonids can have highly variable population dynamics, making the detection of trends in population growth a considerable challenge.

In addition to increasing fitness (i.e., heterosis), translocations can boost genetic variation, which correlates with heightened evolutionary potential (Kardos et al. 2021). We show that translocating a small number of individuals can cause massive increases in heterozygosity in small, isolated salmonid populations. Although differences in marker types limit comparisons, the increase in heterozygosity in Staubach appears to be among the largest documented in any genetic rescue study (e.g., Weeks et al. 2017). This is consistent with a recent study on WCT that documented low variation and high levels of divergence between nearby WCT populations, and also demonstrated that gene flow could potentially results in large increases in genetic variation (Kovach et al. 2021). We also found that hybrid fish in all populations had similar levels of heterozygosity, suggesting much of the basin level variation that could have been present pre-isolation can be restored via translocations. Together, this suggests that translocations will likely be beneficial for small, isolated fish populations even if fitness benefits are smaller than in other taxa.

Our findings of potentially-weaker genetic rescue effects in cutthroat trout using inbred sources highlights that need for further research using a greater diversity of implementation strategies and taxa. Mammals have been the focus of much genetic rescue, and may other taxa remain underrepresented or nearly absent from the genetic rescue literature (Frankham et al. 2017). Nevertheless, fragmentation now threats countless populations and species spanning diverse taxa and ecosystems, many of which could benefit from restored gene flow into small recently-isolated populations. For example, freshwater ecosystems are at greater risk than most terrestrial ecosystems (Tickner et al. 2020) and are highly fragmented (Brauer and Beheregaray 2020), yet have been the focus of little genetic rescue research. On limited conservation budgets,

understanding the influences of gene flow across diverse taxa and scenarios will help to determine when, how, and if genetic rescue should be attempted for the species of concern.

Methods

Study Populations and Translocations

We selected seven genetically depauperate, isolated westslope cutthroat trout (WCT) populations in the Missouri Basin for this study (Figure 5-1a). Four of the populations were designated as recipients of gene flow (Little Belt, Gold Run, Hall, and Staubach), two streams were left unaltered as controls (Crawford and McClellan), and the remaining stream was used as a source of gene flow (Quartz). Criteria for population selection included complete isolation above a barrier in a small first order stream (i.e., less than 5 km of perennial stream habitat) and low heterozygosity compared to the mean of >200 WCT populations in Montana (Figure 5-1b). All populations tested negative for non-native genetic ancestry and whirling disease. The selected populations span a range of H_e (0.03 to 0.17) and N_e (9 to 108). Three of the four recipient populations (Hall, Gold Run, and Staubach) have recently been identified as top candidates for genetic rescue attempts (Kovach et al. 2021), while one stream (Little Belt) was not. Little Belt thus offers a useful comparison to the other populations, as genetic rescue effects are not expected to be as large.

We translocated 6 to 8 mature adults into each recipient population from nearby isolated populations in 2017. We removed the same number of fish as we introduced and of the same sex and similar size to minimize demographic influences of translocations (Figure S5-1). In all study sites, fish were translocated to recipient populations within the same subbasin (HUC8) as the source population to reduce the risk of outbreeding depression. We reciprocally translocated four males and four females between Little Belt and Gold Run (Table S5-1; F_{ST} = 0.43), and translocated three males and three females from Quartz Creek into Hall Creek (F_{ST} = 0.70) and Staubach Creek (F_{ST} = 0.76). Translocations took place between June 6 and 8, which is just prior to spawning to increase the probability of transplant reproduction.

Sampling Procedures

We sampled all populations using backpack electrofishing. Gold Run Creek, Hall Creek and Staubach Creek were sampled in their entirely or near entirety. We sampled roughly half of the occupied stream length of Little Belt Creek, and we sampled 400-500 m of the control streams. For all captured fish, we measured length and clipped a small piece of the upper caudal fin to provide tissue for genetic analyses. We additionally inserted Passive Integrated Transponder (PIT) tags in to the body cavity of all fish over 70 mm at the study sites on the first capture of the fish. PIT tags have been found to have minimal effects on growth and survival for trout (O'Donnell and Letcher 2017). Starting in 2018, we scanned all fish for PIT tags.

We returned to the streams to perform a recapture sample one to two weeks after the first sampling occasion in 2017 and 2018 to allow for estimation of individual detection probability. Fish were scanned for a PIT tag and visually examined for a fin clip to determine if they were marked. Fish that had not been previously captured underwent the same sampling protocol as fish captured in the initial stream visit. Recaptures were limited to a subset of the initially sampled stream sections, and we randomly selected eight to 12 forty-meter stream sections to resample.

Bioinformatics, Filtering, and Genotyping

We genotyped all captured fish using GTseq (373 markers, including a sex marker), and additionally genotyped fish from Hall Creek, Staubach Creek, and Gold Run Creek using Rad-capture (see supplementary text S1 for detailed laboratory and bioinformatic methods). To increase read depth in individuals with low DNA concentrations, we included some individuals on multiple sequencing runs and then combined reads. Genotype error rates were 0.02% for GTseq (83 duplicated individuals) and 0.09% for Rad-capture (53 duplicated individuals).

We tested for conformity to Hardy-Weinberg (HW) and Linkage Disequilibrium (LD) expectations for each population. We limited tests to fish sampled in 2017 as this was prior to the pulse of age-1 hybrids, which would cause large deviations from both HW and LD. We tested for HW expectations using chi-squared tests in the R program *pegas* (Paradis 2010), and we examined LD using chi-squared tests in the *genetics* package in R (Warnes et al. 2021). Markers in which the chi-squared test was significant (p = 0.001) in 2 populations were removed in several analyses. For GTseq, we removed 14 markers that deviated from HW proportions in two or more populations (P <= 0.01), both of which had the same direction of F_{IS}.

We removed loci that had greater than 40% missing genotypes across individuals for both Rad-capture and GTseq. Further, individuals with > 75% missingness on GTseq and > 50% on Rapture were not given genotypes for that panel. We were more stringent with Rad-capture as the error rates appeared to be higher for individuals with low read depth using that method. For both GTseq and Rapture, we filtered based on a minor allele count of 5.

To avoid close physical linkage and multiple SNPs on the same bait, we thinned markers so that only one SNP was selected every 10,000 base pairs. To avoid additional linkage, we removed markers that had a mean *r* of > 0.5 across at least 2 populations. Finally, we found a block of rainbow trout ancestry in Hall Creek that covered roughly half of chromosome 6. We only selected one marker on this block. When deciding which markers to keep, we favored markers on the GTseq panel as genotypes were available for all populations, and we chose the loci with the highest average allele frequency across populations.

Genetic metrics and analyses

We used genotype data to identify resampled individuals that were too small to PIT tag initially (e.g., > 70 mm), or that shed their PIT tags. PIT shed rates are very low, but do occur in larger females during spawning. We used the dupGenotype function from the R package StrataG (Archer et al. 2017) to identify duplicate genetic samples, which uses pairwise comparisons of all individuals in a population to calculate the proportion of identical genotypes. We used a 99% percent match as a threshold to call the same individual, which typically provided adequate power.

We identified hybrids (i.e., outcrosses) and determined hybrid class (i.e., F1, F2, or resident backcross) using NewHybrids (Anderson and Thompson 2002) run using the R program *parallelnewhybrid* (Wringe et al. 2017). We used 2017 captures and transplants as individuals of known population origin to help determine population specific allele frequencies. We used a burn-in of 5,000 and ran 10,000 sweeps. This showed perfect consistency with an alternative method in which we identified loci with fixed differences in recipient and source populations, and used the number of heterozygous ‘diagnostic’ loci to determine hybrids. Finally, parentage analysis identified a similar set of hybrid individuals.

Gene flow causes a deviation from Hardy Weinberg expectations, which is required as an assumption in many parentage analyses. We thus used a combination of exclusion-based

parentage, which does not require HW proportions, and maximum likelihood based sibship and parentage, which has higher power but assumes populations are close to HW proportions. Exclusion based methods also lack an implicit expectation of LD, allowing for more markers to be used in the analysis.

Parentage analyses require the specification of potential parents and offspring. Potential parents for a cohort were allowed from all sampling years, but potential parents were omitted based on being an unreasonable length to have produced offspring in the cohort of interest. Length cutoffs for parents were informed by growth modeling (described below and in Chapter 2) and previous estimates of size at maturity (Downs et al. 1997). Offspring for a cohort were determined based on being age-1 at time t+1.

Exclusions were based on both offspring and the parent have opposite homozygote genotypes and on both parents being homozygous for the same allele while the offspring was heterozygous (Cockburn et al. 2021). Additionally, we used full-likelihood joint sibship and parentage estimation in Colony2 allowing for polygamy in both males and females and for inbreeding, which relaxes HW assumptions (Wang and Santure 2009, Jones and Wang 2010, Wang 2012). We ran Colony separately for each population and each cohort.

To combine results, we used Colony results to determine resident by resident crosses, and to cluster families. When Colony determined two potential parents as having similar probabilities of being the true parent, we checked if exclusion had identified either of these parents as the top parent. Family size was calculated as the number of detected age-1 fish assigning to the same parents.

Component and composite fitness

We did not capture juveniles until they reached age-1, but early life survival is perhaps the most important stage for inbreeding depression (Keller and Waller 2002). Estimating the difference in the number of age-1 juveniles in resident versus hybrid families while controlling for maternal length provides a proxy for survival from embryo to age-1. This method first requires an estimate of maternal length at the time of reproduction, which often did not align with years in which the fish was captured. We thus predicted maternal lengths in all years by modeling individual growth using a GLM with a normal distribution and a log link. The log link prevented negative growth. Growth was modelled as a function length (quadratic) in the previous year, and this relationship

was fit separately for each population. We used parameter estimates from this GLM to estimate length in all years in which the fish of interest was not captured. As growth is equal to length at time t+1 minus length at time t, these values can be rearranged to estimate lengths in both future and previous time steps.

Family size was then modelled using length estimates and pedigree results. Family size was the dependent variable in a GLM with a log link and a zero-truncated Poisson distribution with maternal length and cross type (i.e., resident versus hybrid) as covariates. Colony infers unobserved moms for families, for which we did not have lengths estimates. To include these families in the analysis, maternal length was specified as a normal distribution, and allowing for full uncertainty of missing lengths of missing moms to be included into the analysis.

We estimated differences in survival by life-stage using a multistate CJS (Kery and Royle 2020). The models allowed for uncertainty in the length-class an individual fell in during years in which it was not captured and corrected for individual detection probability, allowing for unbiased estimates of stage-specific survival. Specifically, survival was dependent on age and length-based size categories: age-1 (juvenile), age-2+ < 150 mm (sub-adult), and >= 150 mm (adult). Stage-based survival and transitions among states were modeled as a function of cross type (i.e., resident versus hybrid).

We combined estimates of family size and age/class specific survival to obtain a measure of composite fitness. We first converted family size to survival from conception to age-1 by dividing family size (given mean maternal length) by an estimate for mean number of fertilized offspring (50). A precise estimate of the number is not necessary, as inferring relative fitness differences between residents and hybrids is the primary goal of our study. We added this estimate of survival to age-1 to the projection matrix of survival of age-1+. We then simulated survival from age-0 to age-4, which is the age at which many WCT reach maturity.

Number of mature adults (N_c) and population growth rate

Individual detection probability was needed to estimate abundance, since detection probabilities of less than 1 lead to biased estimation of demographic parameters (Kery and Royle 2020). We used the within year recaptures from 2017 and 2018 to estimate detection probability as the proportion of fish captured on the return visits to the streams that were captured earlier in the same year. This was done using generalized linear models (GLM) with a logit link and a

Bernoulli distribution. We estimated detection probability separately for every stream and for different size classes, including fish < 120 mm (juveniles), ≥ 120 and < 150 mm (sub-adults), ≥ 150 and < 180 (smaller adults), and ≥ 180 (large adults), which was necessary since smaller fish have lower detection probabilities. We estimated abundance as the number of detected fish in a given year divided by the detection probability. This was done separately for each size class and population. The summation of age-2+ size-class provides an estimate of the total age-2+ abundance. Annual population growth rate (λ_t) was calculated as N_{t+1}/N_t. We then took the geometric mean of λ_t (λ_G) to obtain an average population growth rate spanning from pre-translocation to 4 years post-translocations. Age-2 F1 hybrids were first detected in 2019, which resulted in one pre-translocation estimate of λ_t and three post-translocation estimates.

Bayesian model analysis

All demographic models described above were analyzed using Bayesian inference in the program JAGS (Plummer 2003) in the R program jagsui (Kellner 2019). Models were run with a burn in of 50,000 iterations, 50,000 addition iterations, five chains, and were thinned by 10. This resulted in successful convergence of all models based on $\hat{R}$ values less than 1.1 and visual inspection of MCMC chains. We report 95% CRIs and probabilities of direction (*pd*; the proportion of estimates the more common direction), and test for statistical significance for 95% CRIs the do not overlap 0 or *pd* greater than 0.975.

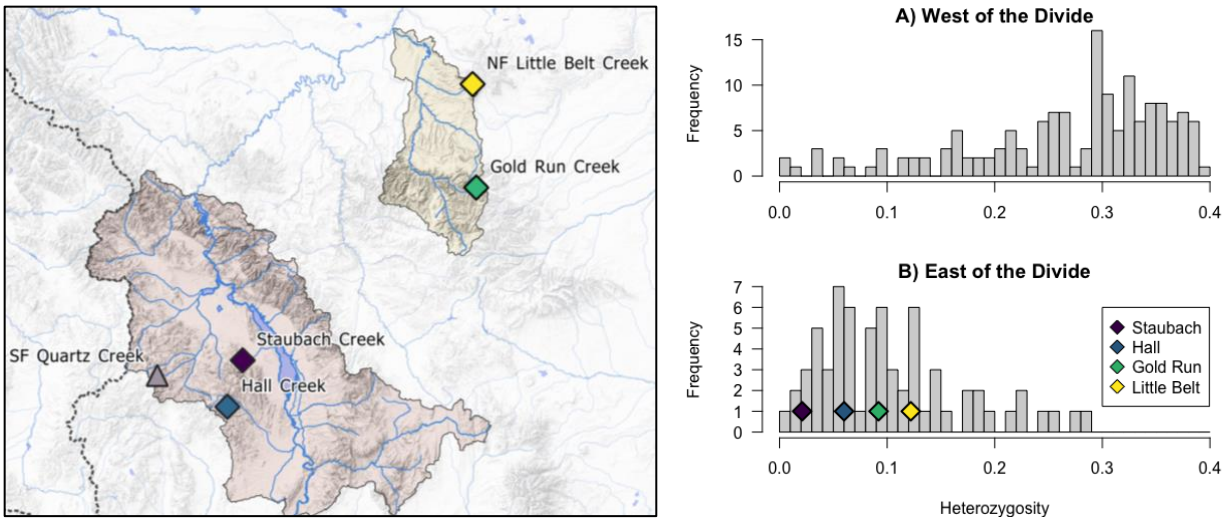


Figure 5-1. Summary of study populations and translocations. The dashed line on the map is the Continental Divide. The histograms show the mean observed heterozygosity for >200 populations of westslope cutthroat trout in Montana and southern Canada found east and west of the Continental Divide (adapted from Kovach et al. 2021). Diamonds show heterozygosity in the four recipient populations.

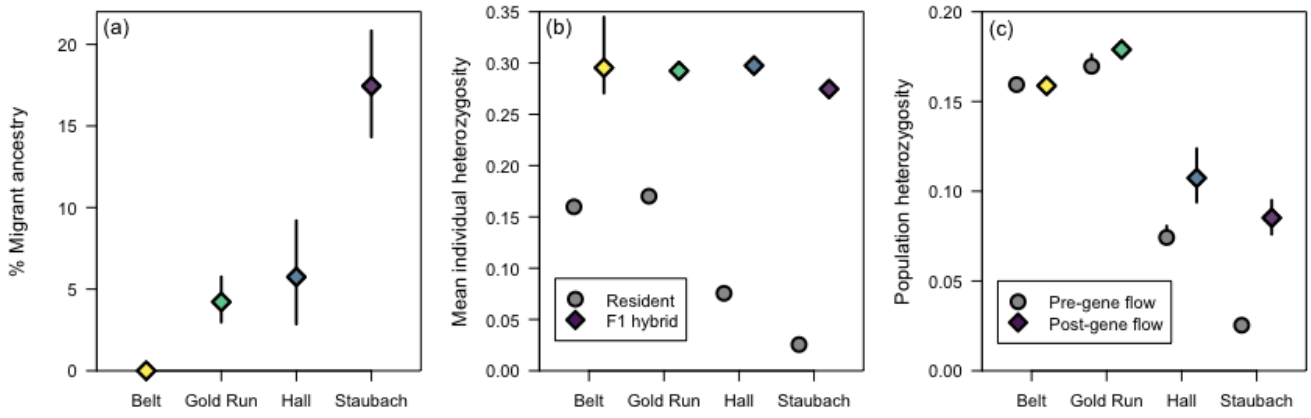


Figure 5-2. Genetic summary statistics in the recipient populations, including (a) % migrant ancestry in 2021, (b) heterozygosity of resident versus F1 hybrids, and (c) population observed heterozygosity (H_o) before (2017) and after (2021) gene flow. Bars represent 95% bootstrap CIs.

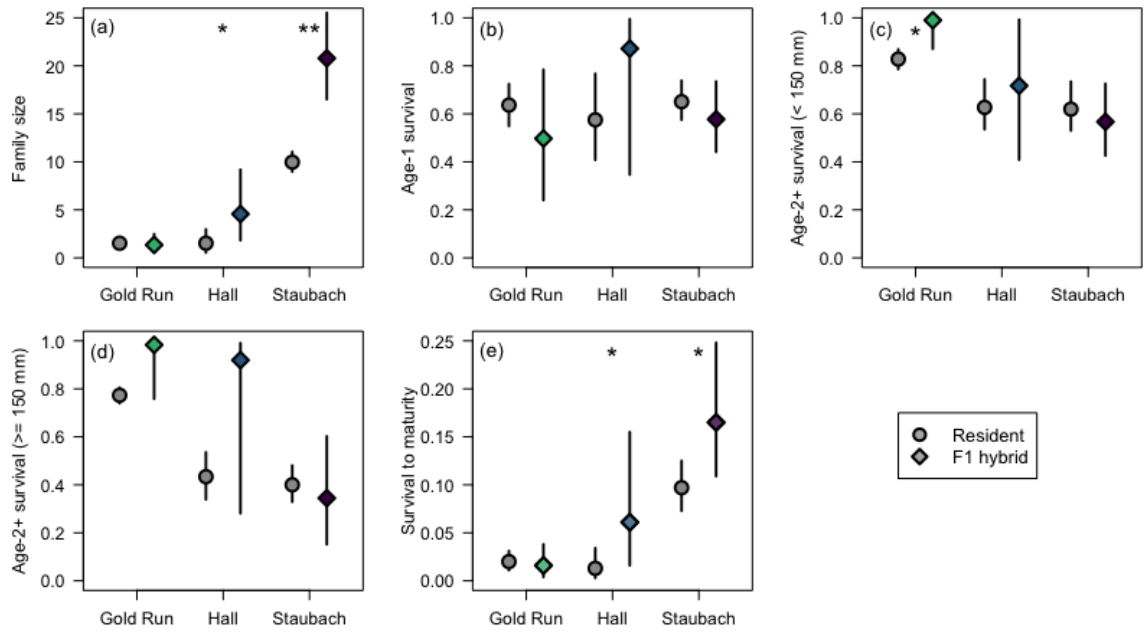


Figure 5-3. Fitness of residents versus F1 hybrids, including family size (a), annual survival probabilities (b-d) and survival from age-0 to maturity, a measure of composite fitness (e). Asterisks represent probabilities of direction (*pd*) for the difference between resident and hybrid fitness > 0.975 (*) or 0.99 (**). Vertical lines on point estimates 95% credible intervals.

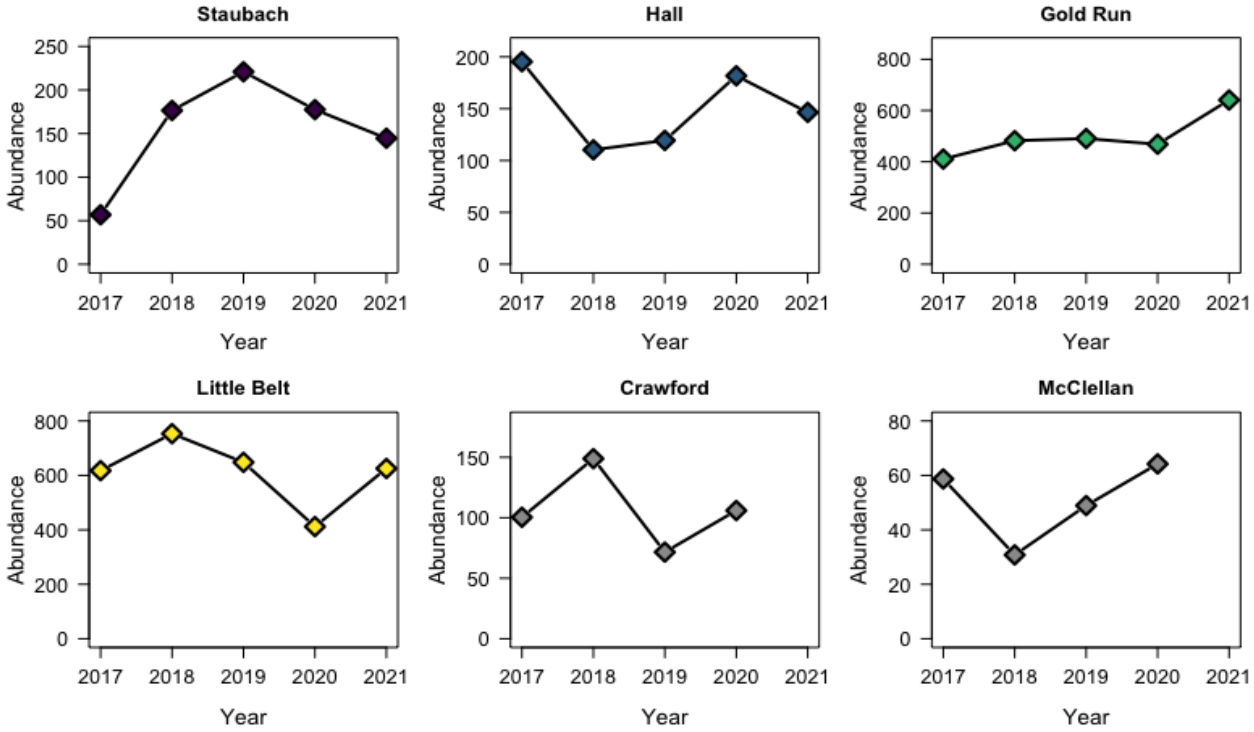


Figure 5-4. Abundance of fish > 120 mm (sub-adults and adults) in the four recipient and two control populations (Crawford and McClellan). 2019 was the first year with sub-adult F1 hybrids.

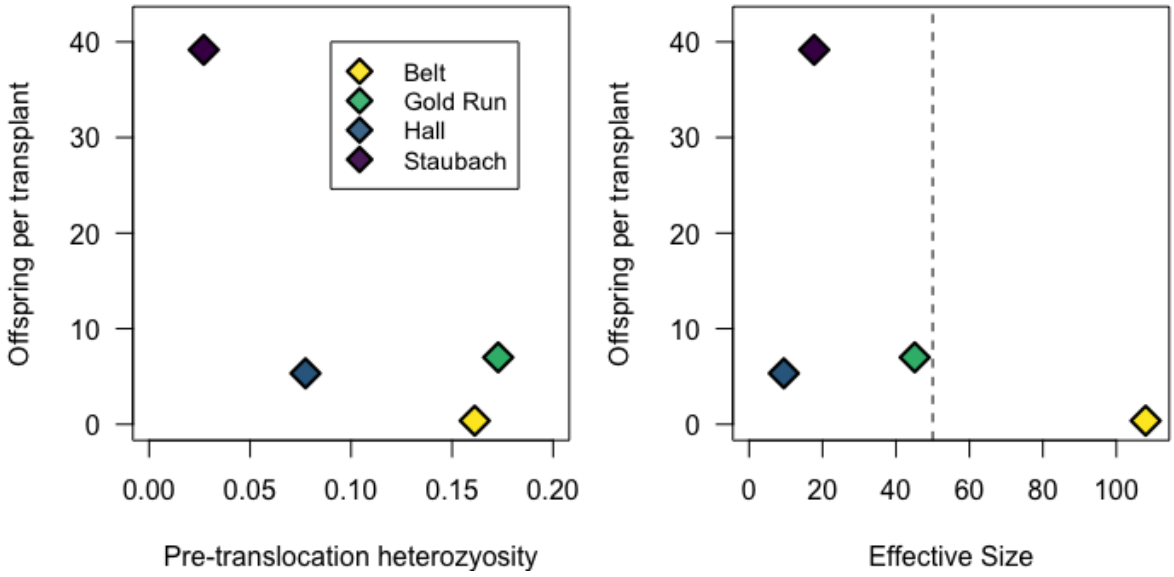


Figure 5-5. Relationship between transplant reproductive success (detected offspring per transplant) and genetic metrics (H_o and N_e) of recipient populations. The vertical dashed line shows an N_e of 50, a commonly used guideline for an effective size of concern.

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APPENDIX A. Chapter 2 Supplementary Materials

Model validation

Model validation was based on observed versus predicted species detections, which is a product of occupancy probability and detection probability. Notably, distribution models that do not explicitly account for detection probably are estimating the product of occupancy and detection probability (30, 31, 61), and our model validation is thus directly comparable to models that ignore detection probability. We predicted detection histories using parameter estimates from 200 MCMC iterations to incorporate model uncertainty. Observed and predicted detection histories were then used to calculate the area under the receiver-operating characteristic curve (AUC), predictive accuracy, and goodness-of-fit. We calculated AUC and predictive accuracy for both sites within the study species possible range (i.e., sites that were predicted based on estimated parameters), and throughout the entire study region (i.e., including sites that were forced to 0 due to being outside of the species possible range). The latter was calculated to determine our overall representation of the species distribution within the study region, which was especially important for invasive species since their presence were used as covariates in models for native species.

Predictive accuracy was determined by rounding the predicted probability that a species was both present and detected to 0 or 1. We then calculated the proportion of samples that were true positives or true negatives separately for each species. For our goodness-of-fit test, we summed the total number of observed and predicted detections by year for each species. We then used chi-squared tests to determine if the observed and predicted detections significantly differed, which would suggest that our model did not fit the data well.

AUC values were fair for brook trout (0.74), good for bull trout, cutthroat trout, and rainbow trout (0.83-0.87), and excellent for brown trout (0.92; Table S5). Our AUC values and predictive accuracy slightly exceed previous studies on trout covering the same region (17, 36). Mean chi-squared p-values for the goodness-of-fit test ranged from 0.12 to 0.96, indicating the model fit the detection history data well.

Climate change projections and emissions scenarios

Our dynamic occupancy model and projections of future distributions used the Western U.S. Stream Flow Metrics and Norwest Stream Temperature Database, both of which accurately characterize stream conditions (33, 34). The Western U.S. Stream Flow Metrics were derived from a variable infiltration capacity (VIC) hydrological model, and the NorWeST stream temperatures were based on spatial-stream-network models. NorWeST stream temperatures were modeled as a function of several covariates, including summer stream flow from the VIC model and downscaled estimates of August air temperature (15 km^2 gridded). These stream temperature and stream flow metrics are widely used for research and management in the Pacific Northwest, USA, and are among the highest quality stream metrics available for any region globally.

Future stream flow and stream temperature projections were based on a composite of 10 global climate models (GCMs) used to simulate the A1B scenario. These 10 GCM scenarios were the best at capturing key features of the climate in the Pacific Northwest, USA, and included hadcm, cnrm_cm, ccsm3, echam5, echo_g, cgcm3.1_t47, pcm1, miroc_3.2, ipsl_cm4, and hadgem1 (69). Although CMIP3 emissions scenarios have now been replaced by CMIP5, our future projections were limited to the A1B emissions scenario because this was the only scenario available for the U.S. Stream Flow Metrics and NorWeST Stream Temperatures database. However, the middle-of-the-road scenarios for CMIP3 (e.g., A1B) and CMIP5 (e.g., RCP 6.0) are similar, and CMIP3 and CMIP5 projections have produced similar ecological outcomes (70).

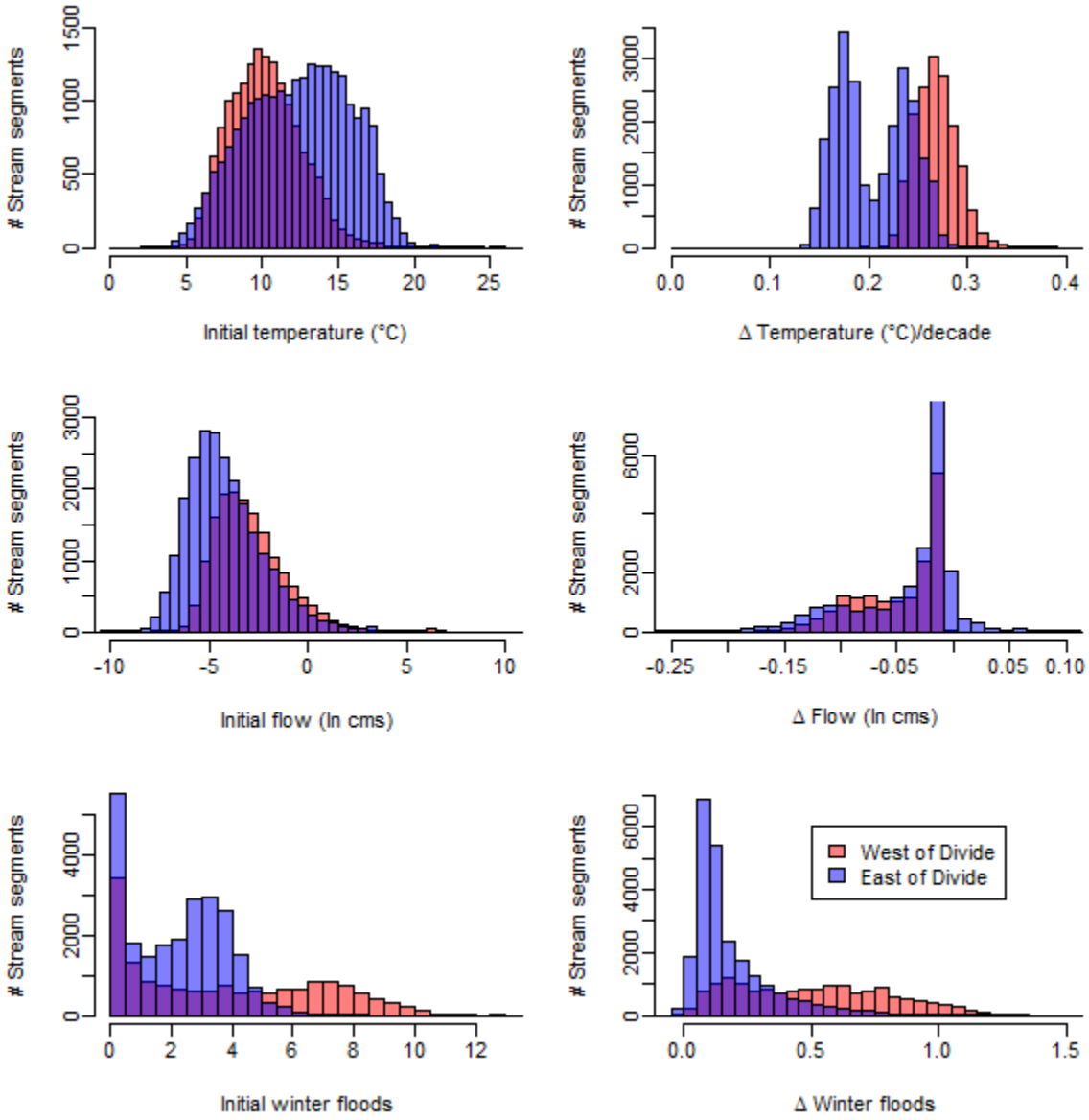

Fig. S2-1. Summary of the abiotic variables, including summer stream temperature, summer stream flow, and winter flood frequency, by stream segment, including the initial 1993 value and the predicted decadal rate of change. Winter floods are the number of winter days in the top 5% of annual flow. The natural logarithm of stream flow is shown in cubic meters per second. Red represents stream segments on the west of the Continental Divide, blue represents stream segments east of the Divide, and purple shows the overlap in these distributions.

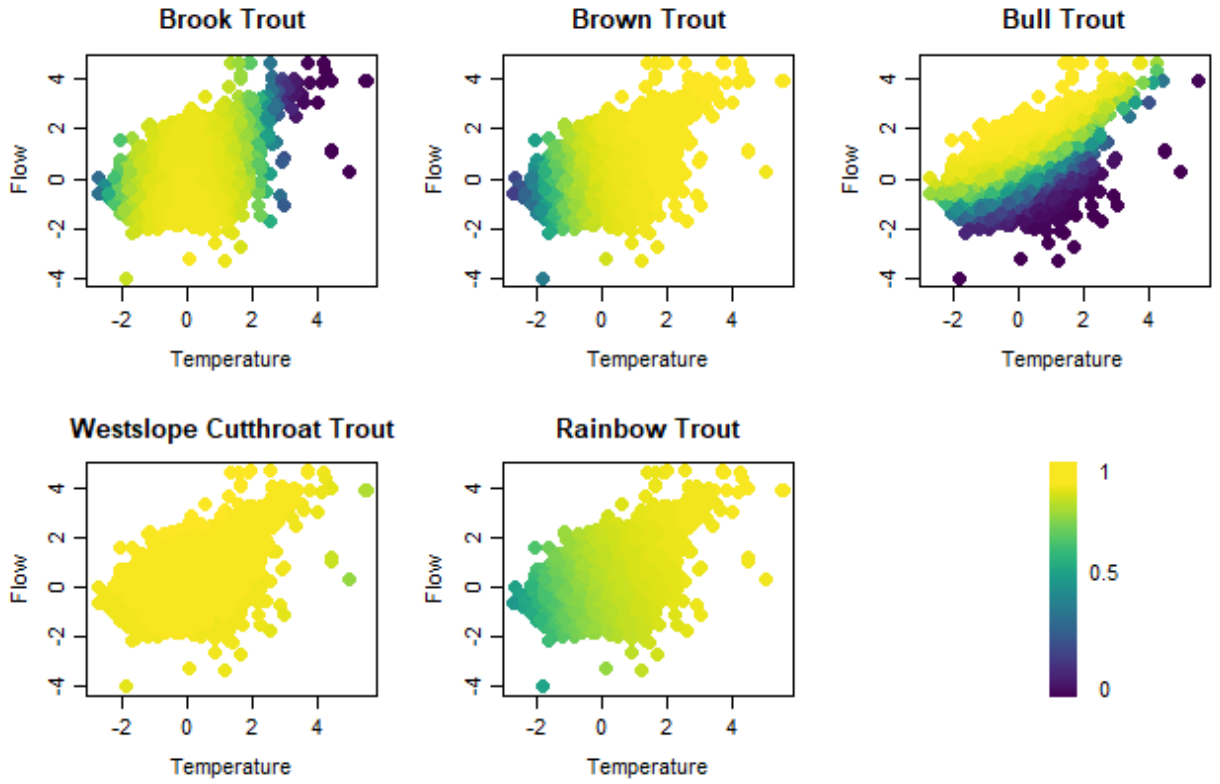

Fig. S2-2. The influence of stream flow and stream temperature on persistence probability, where purple depicts stream segments where the focal species is not predicted to persist. The relationships are shown with winter flooding held at its mean value and without species interactions. Temperature and flow are standardized.

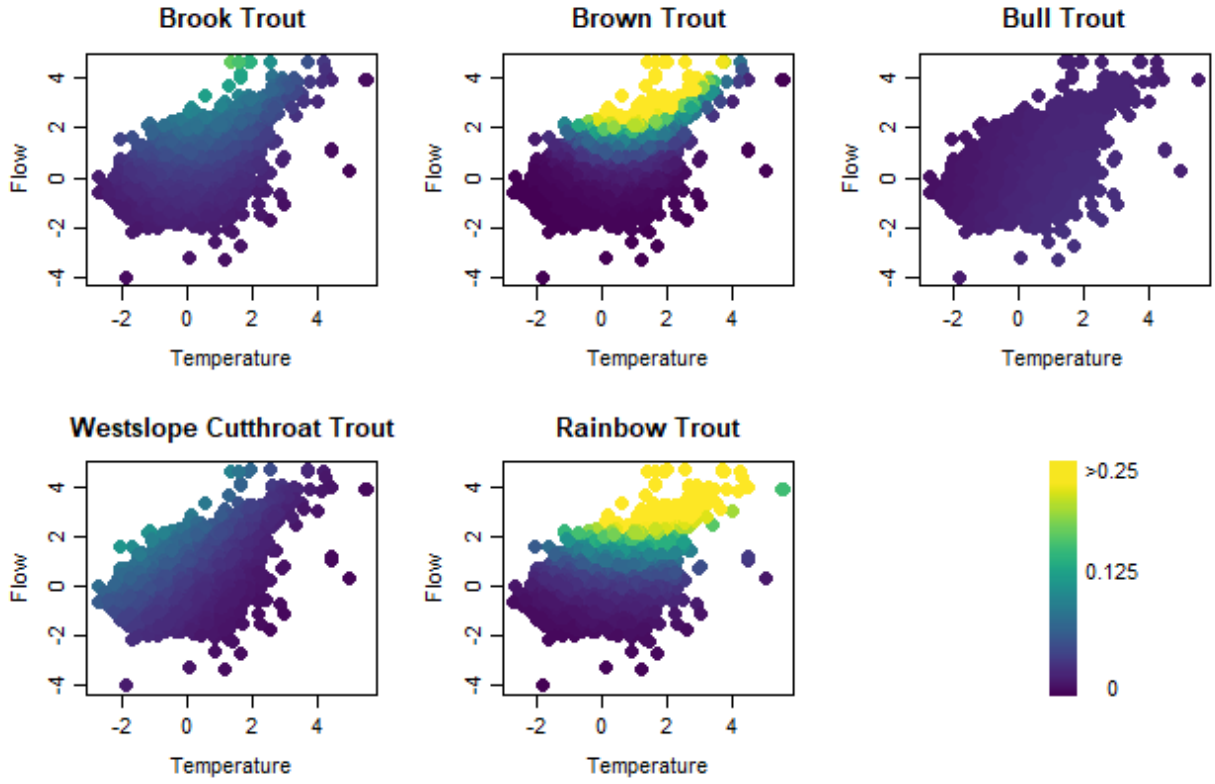

Fig. S2-3. The influence of stream flow and stream temperature on colonization probability, where purple depicts stream segments the focal species is not predicted to colonize. The relationships are shown with winter flooding held at its mean value and without species interactions. Temperature and flow are standardized.

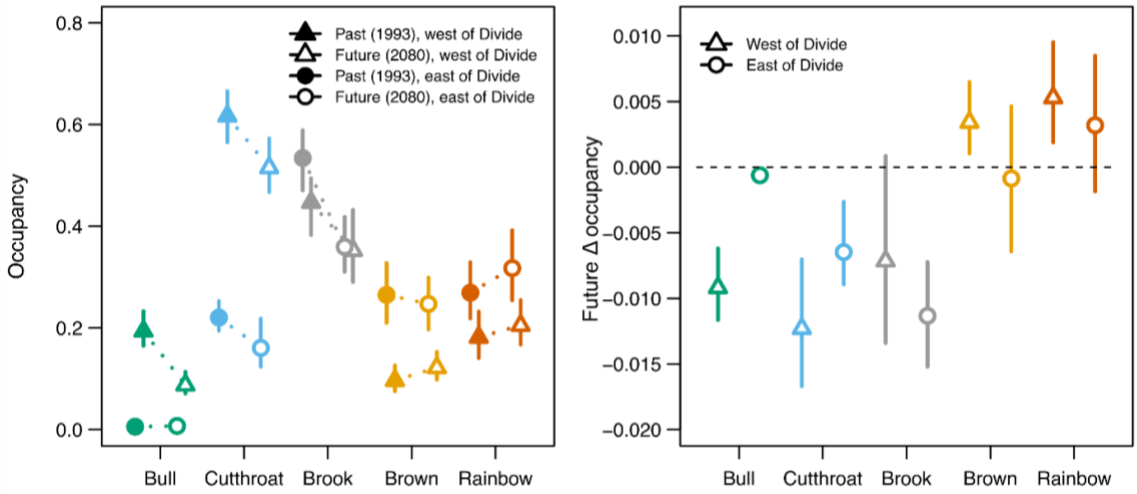

Fig. S2-4. Past (1993) and future (2080) occupancy estimates and future change in occupancy per decade (2019-2080) on the east and west of the Continental Divide, with bars representing 95% credible intervals.

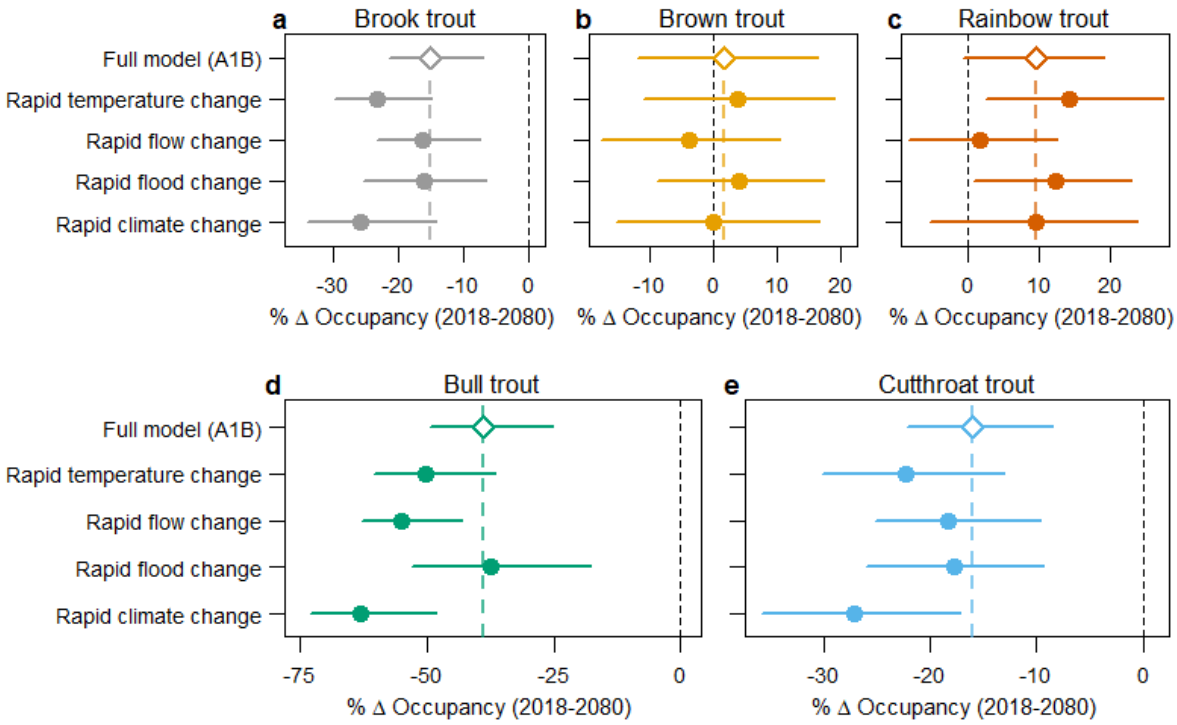

Fig. S2-5. Sensitivity analysis of how more extreme climate change influences predicted occupancy in 2080. (a-e) The % change in predicted occupancy (2080) when a climate variable changed 50% more than under the A1B emissions scenario compared to the 2018 occupancy estimate for the full model. This rate of climate change is consistent with a high-emissions scenario such as the SRES A2 or the RCP 8.5. The diamonds and colored dashed lines represent the % change in occupancy for the full model under the A1B emissions scenario. Horizontal, colored bars are 95% credible intervals.

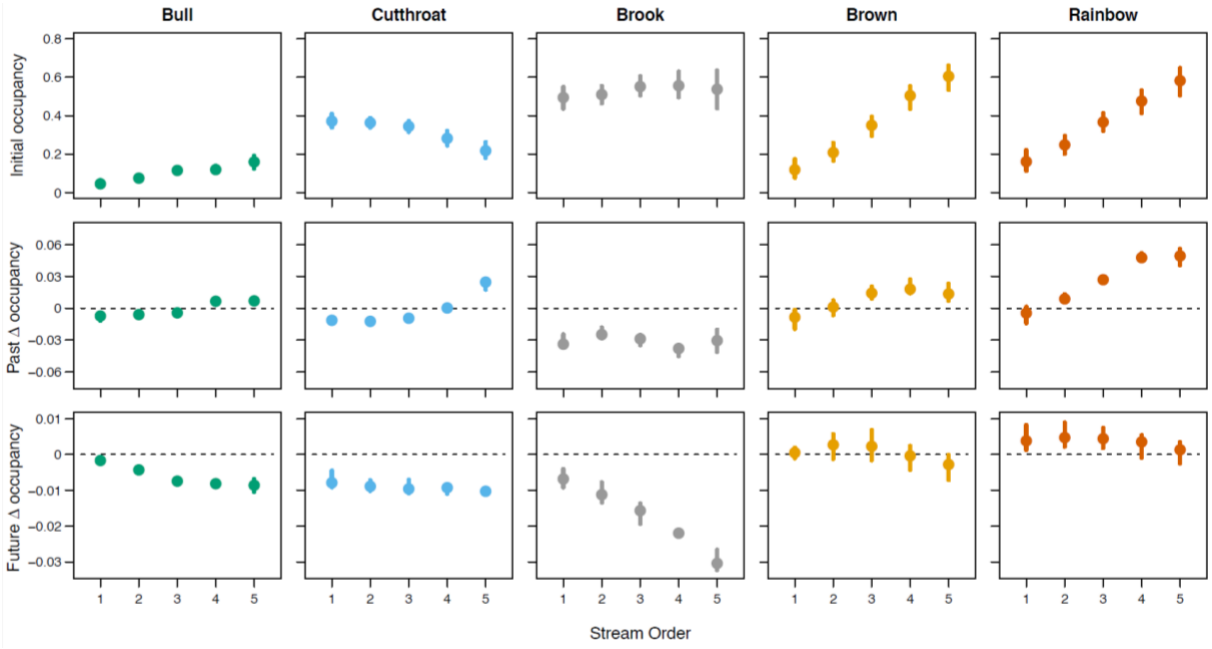

Fig. S2-6. Initial occupancy and decadal changes in past and future occupancy by stream order. Bands are 95% credible intervals.

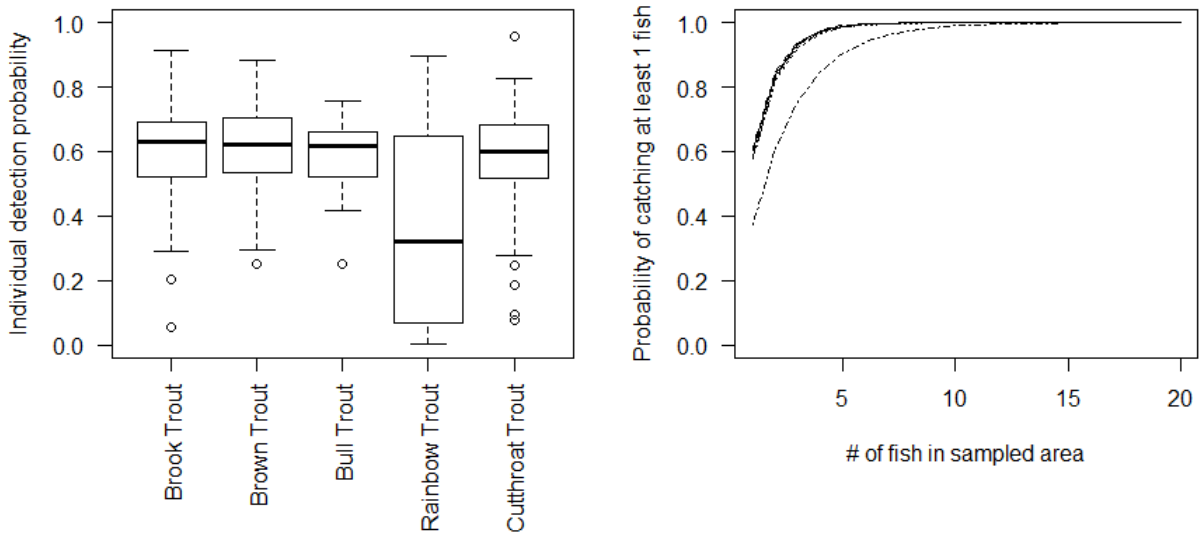

Fig. S2-7. Individual detection probabilities by species based on multi-pass depletion estimates within the study region (left). Probability of catching at least 1 fish of the focal species (i.e., species-level detection probability) versus the number of fish in the stream section being sampled, based on the median individual detection probabilities for each species (right). Lines strongly overlap for all species besides rainbow trout, which had a lower individual detection probability. Note that occupancy models use species level detection probabilities, not individual detection probabilities.

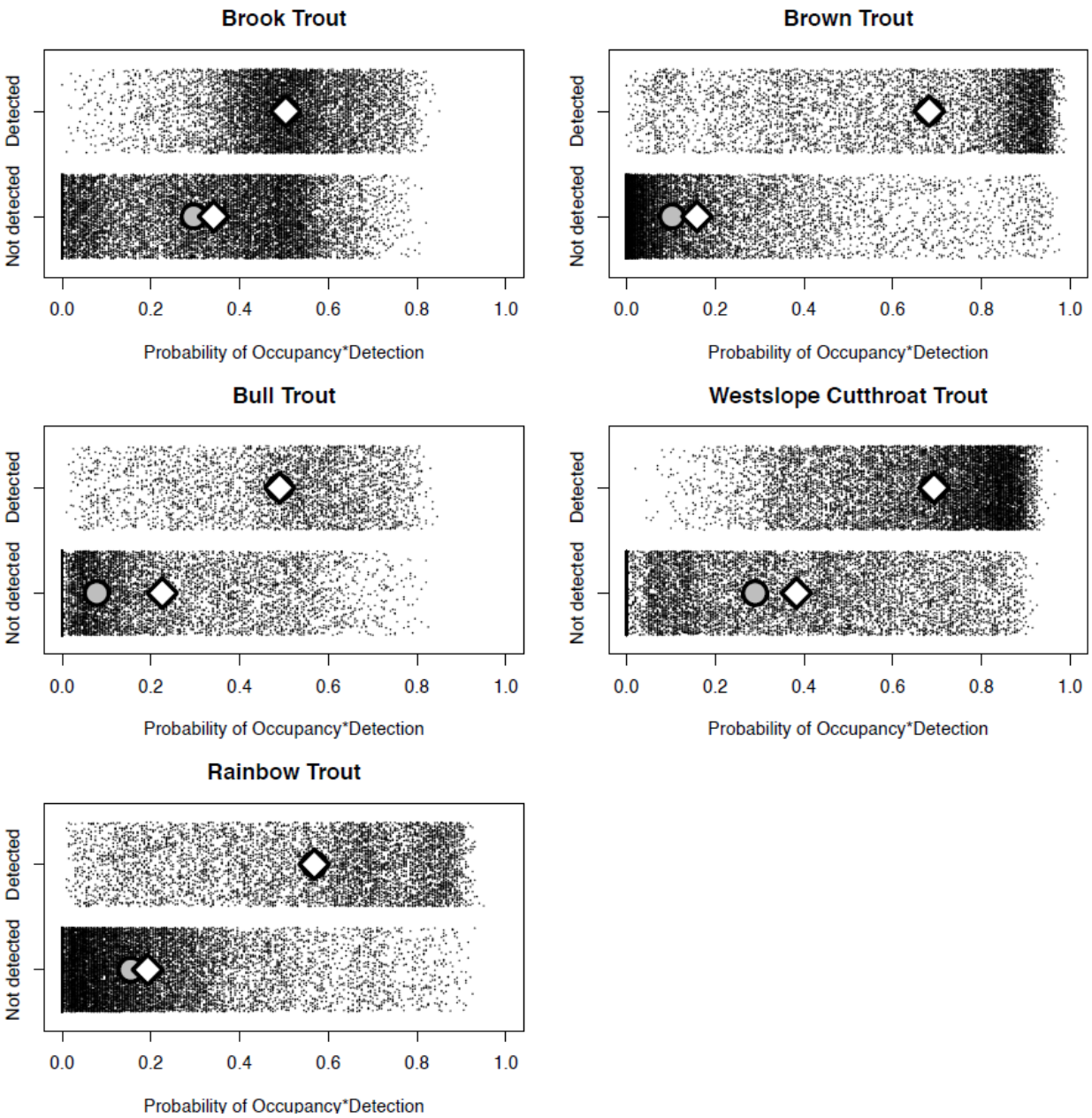

Fig. S2-8. Predicted probability of species occupancy x detection for surveys that did and did not detect the focal species. White diamonds indicate the mean probability of occupancy x detection for samples within the species possible range and grey circles for all stream segments within the study region.

Table S2-1. Parameter estimates for all biological models with 95% credible intervals shown in parentheses.

Covariate	Brook trout	Brown trout	Bull trout	Cutthroat trout	Rainbow trout
Occupancy					
Intercept	0.93(0.39,1.48)	-1.88(-2.53,-1.26)	-2.23(-3.42,-0.98)	3.03(1.82,4.38)	-1.41(-2.33,-0.7)
Temperature	0.21(-0.05,0.46)	1.07(0.67,1.5)	-1.55(-2.06,-1.04)	-1.37(-1.81,-0.96)	0.52(0.21,0.82)
Temperature^2	-0.4(-0.55,-0.24)	-0.1(-0.28,0)	-0.36(-0.63,-0.11)	-0.6(-0.87,-0.36)	-0.04(-0.13,0)
Flow	-0.06(-0.31,0.19)	1.03(0.69,1.38)	1.62(1.05,2.29)	0.65(0.27,1.05)	0.68(0.37,1.01)
Floods	0(-0.3,0.3)	0.28(-0.16,0.72)	-0.68(-1.2,-0.19)	0.26(-0.27,0.85)	0.03(-0.34,0.42)
Stocking	0.04(-0.13,0.2)	1.01(0.72,1.34)	NA	NA	0.08(-0.14,0.33)
Stream Length	0.06(0,0.16)	0.07(0,0.21)	0.08(0,0.25)	0.02(0,0.08)	0.07(0,0.21)
Brook Pres.	NA	NA	-0.42(-1.11,0.24)	-1.77(-2.34,-1.26)	NA
Brown Pres.	NA	NA	-1.16(-2.19,-0.25)	-3.37(-4.65,-2.21)	NA
Rainbow Pres	NA	NA	NA	-0.14(-0.88,0.64)	NA
HUC8 (RE)	1.28(0.83,1.86)	1.06(0.54,1.77)	1.39(0.73,2.57)	2.41(1.63,3.48)	1.93(1.24,2.94)
Colonization					
Intercept	-3.09(-3.6,-2.65)	-3.96(-4.59,-3.37)	-3.7(-4.62,-2.97)	-3.18(-3.76,-2.62)	-3.18(-3.64,-2.76)
Temperature	0(-0.29,0.33)	0.35(0,0.74)	0.21(-0.16,0.59)	-0.42(-0.69,-0.16)	0.17(-0.1,0.46)
Temperature^2	-0.11(-0.21,-0.01)	-0.31(-0.57,-0.15)	-0.04(-0.14,0)	-0.04(-0.12,0)	-0.09(-0.2,-0.01)
Flow	0.4(0.15,0.62)	1.25(0.9,1.64)	-0.07(-0.4,0.29)	0.38(0.14,0.62)	0.85(0.6,1.13)
Floods	0.21(-0.1,0.51)	0.23(-0.11,0.56)	-0.12(-0.47,0.26)	0.54(0.24,0.84)	0.5(0.2,0.81)
Brook Pres.	NA	NA	-0.14(-0.66,0.39)	0.28(-0.16,0.71)	NA
Brown Pres.	NA	NA	1.12(0.43,1.8)	-0.03(-0.49,0.43)	NA
Rainbow Pres	NA	NA	NA	0.39(-0.08,0.84)	NA
HUC8 (RE)	0.97(0.62,1.42)	1.06(0.59,1.73)	1.22(0.62,2.16)	0.72(0.42,1.1)	0.86(0.54,1.27)
Persistence					
Intercept	3.78(3.37,4.18)	3.06(2.4,3.82)	1.09(-0.16,2.23)	4.72(4.23,4.99)	2.13(1.66,2.61)
Temperature	0.07(-0.18,0.3)	1.12(0.7,1.56)	-1.37(-1.8,-0.97)	-0.06(-0.32,0.22)	0.59(0.34,0.86)
Temperature^2	-0.56(-0.67,-0.45)	-0.18(-0.31,-0.04)	-0.23(-0.38,-0.07)	-0.12(-0.22,-0.02)	-0.04(-0.11,0)
Flow	-0.16(-0.37,0.05)	0.18(-0.16,0.48)	2.31(1.69,2.93)	0.29(0,0.56)	0.15(-0.08,0.37)
Floods	-0.28(-0.54,-0.01)	-0.05(-0.44,0.35)	0.17(-0.31,0.62)	-0.69(-1.02,-0.38)	-0.37(-0.67,-0.07)
Brook Pres.	NA	NA	-0.05(-0.62,0.53)	-0.7(-1.14,-0.28)	NA
Brown Pres.	NA	NA	-1.25(-2,-0.5)	-0.24(-0.79,0.34)	NA
Rainbow Pres	NA	NA	NA	-3.05(-3.55,-2.53)	NA
HUC8 (RE)	0.85(0.57,1.27)	1.28(0.68,2.15)	1.9(1,3.41)	1.24(0.87,1.79)	1.07(0.73,1.55)

* RE indicates that the covariate was included as a random effect

Table S2-2. Summary of past and future occupancy shifts. Occupancy shifts are summarized for the entire study area, as well as east and west of the Continental Divide. Raw changes and percent changes in occupancy are shown. 95% credible intervals are shown in parentheses. Note that bull trout have a very limited range east of the Continental Divide in Montana—in the Saskatchewan River Drainage—and were only captured in a small number of surveys east of the Divide.

	Brook trout	Brown trout	Bull trout	Cutthroat trout	Rainbow trout
Change in occupancy per decade					
Past total	-0.032(-0.057,-0.008)	-0.004(-0.019,0.015)	-0.005(-0.011,0)	-0.008(-0.022,0.009)	0.006(-0.023,0.029)
Past west	-0.016(-0.041,0.016)	0.002(-0.007,0.011)	-0.016(-0.028,-0.005)	-0.005(-0.025,0.018)	-0.001(-0.02,0.016)
Past east	-0.035(-0.061,-0.007)	-0.006(-0.025,0.015)	0.002(0,0.003)	-0.006(-0.02,0.012)	0.009(-0.022,0.033)
Future total	-0.01(-0.014,-0.005)	0.001(-0.004,0.005)	-0.003(-0.004,-0.002)	-0.008(-0.011,-0.005)	0.004(0,0.008)
Future west	-0.007(-0.013,0.001)	0.003(0.001,0.007)	-0.009(-0.012,-0.006)	-0.012(-0.017,-0.007)	0.005(0.002,0.01)
Future east	-0.011(-0.015,-0.007)	-0.001(-0.006,0.005)	-0.001(-0.001,0)	-0.006(-0.009,-0.003)	0.003(-0.002,0.009)
Percent change in occupancy					
Past total	-16.3(-27.5,-4.2)	-4.6(-20.2,20.7)	-18.2(-36.1, 1.5)	-6(-16,6.9)	6(-18.6,39.7)
Past west	-10.4(-24.2,11.6)	3.6(-24.3,41.2)	-24.7(-41.4,-5.9)	-3.8(-14.1,7.9)	-5(-32.1,31)
Past east	-18.9(-31.3,-4.3)	-6.1(-23,19.8)	99.7(15.7,460.8)	-8.8(-26.3,14.7)	10(-17.5,44.7)
Future total	-14.8(-21.4,-7.2)	1.7(-11.7,15.3)	-38.7(-49.1,-25.2)	-15.7(-21.6,-8.3)	9.9(-1.1,19.1)
Future west	-11(-21.3,1.2)	21.2(6.8,39)	-39.5(-49.7,-27.5)	-12.9(-17.6,-7.4)	19.3(7,32.8)
Future east	-16.6(-22.6,-10.2)	-2.1(-15.5,10.9)	-34.4(-52.2,-13.1)	-20.5(-30,-7.3)	6.7(-4.1,15.7)

Table S2-3. Correlations between covariates used in our analyses. All transformations used in our analysis were performed before testing for correlations among covariates. Correlations that did not include invasive presence were performed on site-level data. Correlations involving invasive presence included data for sites in all years in which sampling occurred. Invasive presence was determined if the focal species was detected within a site at a given year.

	Stream temperature	Stream flow	Winter floods	Stream length	Brook stocking	Brown stocking	Brook Presence	Brown Presence
Stream flow	0.47	-	-	-	-	-	-	-
Winter floods	0.33	0.38	-	-	-	-	-	-
Stream length	0.16	-0.32	-0.09	-	-	-	-	-
Brook Stocking	0.34	0.22	0.22	0.06	-	-	-	-
Brown Stocking	0.33	0.16	0.21	-0.06	0.52	-	-	-
Rainbow Stocking	0.3	0.2	0.24	0.08	0.55	0.51	-	-
Brook Presence	-0.05	-0.12	0.1	-0.07	-	-	-	-
Brown Presence	0.6	0.53	-0.06	0.42	-	-	-0.12	-
Rainbow Presence	0.53	0.51	-0.14	0.38	-	-	-0.1	0.55

Table S2-4. Parameter estimates for the detection probability models with 95% credible intervals shown in parentheses.

Covariate	Brook trout	Brown trout	Bull trout	Cutthroat trout	Rainbow trout
Intercept					
Order 1-2	1.41(1.26,1.56)	0.18(-0.16,0.53)	0.67(0.4,0.95)	1.82(1.68,1.97)	0.53(0.28,0.78)
Order 3-4	1.13(0.95,1.31)	1.28(1.07,1.5)	1.43(1.21,1.65)	1.57(1.39,1.75)	1(0.79,1.2)
Order 5-6	-0.1(-0.46,0.26)	2.35(2.05,2.64)	0.98(0.6,1.39)	1.35(1,1.71)	2.57(2.27,2.89)
Order 7-8	-1.17(-1.92,-0.24)	3.71(3.04,4.45)	-1.07(-1.91,0.06)	2.79(1.44,4.33)	1(0.69,1.34)
Year					
Order 1-2	0(-0.01,0.01)	0.03(0.01,0.06)	0.02(0,0.03)	0.02(0.01,0.04)	0(-0.02,0.02)
Order 3-4	0.02(0,0.03)	0.01(0,0.03)	0.01(-0.01,0.02)	0.02(0,0.03)	0.01(0,0.03)
Order 5-6	0.01(-0.02,0.03)	0.03(0,0.05)	-0.03(-0.06,-0.01)	-0.01(-0.04,0.01)	-0.03(-0.05,-0.01)
Order 7-8	0.03(-0.03,0.09)	-0.08(-0.12,-0.04)	0.06(-0.01,0.11)	-0.07(-0.16,0)	0.01(-0.01,0.03)

Table S2-5. Model validation statistics including AUC, predictive accuracy, and chi-squared p-values for the goodness-of-fit test.

	AUC – excluding sites outside of possible range	AUC - including sites outside of possible range	Predictive Accuracy - excluding sites outside of possible range	Predictive Accuracy - including sites outside of possible range	Goodness-of-fit chi-squared p-value
Brook Trout	0.74	0.78	0.66	0.69	0.12
Brown Trout	0.92	0.95	0.85	0.89	0.96
Bull Trout	0.83	0.94	0.76	0.89	0.62
Cutthroat Trout	0.83	0.87	0.76	0.79	0.17
Rainbow Trout	0.87	0.9	0.83	0.85	0.36

APPENDIX B. Chapter 3 Supplementary Materials

Table S3-1. Life table for Crawford Creek.

Age (x)	$s_{x(\text{male})}$	$l_{x(\text{male})}$	$b_{x(\text{male})}$	$s_{x(\text{female})}$	$l_{x(\text{female})}$	$b_{x(\text{female})}$
1	0.66	1.00	0.0	0.51	1.00	0.0
2	0.36	0.66	0.1	0.42	0.51	0.0
3	0.49	0.24	0.9	0.49	0.21	0.4
4	0.47	0.12	3.5	0.59	0.10	2.5
5	0.44	0.05	7.7	0.56	0.06	6.3
6	0	0.02	11.9	0.53	0.03	9.5
7	0	0.00	0.0	0	0.02	11.7

Table S3-2. Life table for Hall Creek.

Age (x)	$s_{x(\text{male})}$	$l_{x(\text{male})}$	$b_{x(\text{male})}$	$s_{x(\text{female})}$	$l_{x(\text{female})}$	$b_{x(\text{female})}$
1	0.72	1.00	0.0	0.52	1.00	0.0
2	0.63	0.72	0.0	0.63	0.52	0.0
3	0.64	0.45	0.1	0.57	0.33	0.0
4	0.54	0.29	0.7	0.50	0.19	0.2
5	0.50	0.16	3.5	0.45	0.09	0.9
6	0.47	0.08	8.7	0.42	0.04	2.2
7	0.46	0.04	13.3	0.00	0.02	4.0
8	0.00	0.02	15.8	0.00	0.00	0.0

Table S3-3. Life table for Little Belt Creek.

Age (x)	$s_{x(\text{male})}$	$l_{x(\text{male})}$	$b_{x(\text{male})}$	$s_{x(\text{female})}$	$l_{x(\text{female})}$	$b_{x(\text{female})}$
1	0.38	1.00	0.0	0.47	1.00	0.0
2	0.27	0.38	0.3	0.36	0.47	0.0
3	0.27	0.10	1.2	0.27	0.17	0.7
4	0.00	0.03	2.7	0.32	0.05	2.1
5	0.00	0.00	4.8	0.00	0.01	4.2

Table S3-4. Summary of stream characteristics.

Stream	NHD Above Barrier Flowline Length (km)	Start Elevation (ft)	Above Barrier Drainage Area (km^2)	Sampled Mainstem (m)
Little Belt	3.4	4,788	7.98	1,160
Gold Run	2.5	5,880	3.38	1,520
Hall	3.8	5,755	5.44	1,360
Staubach	4.9	4,955	4.76	1,480
Crawford	3.4	5,856	5.56	400

APPENDIX C. Chapter 5 Supplementary Materials

Sampling details

We began sampling at the barrier and worked upstream all populations. At Staubach Creek, we began sampling approximately 1 km above the barrier in 2017 and 0.5 km above the barrier in 2018. We sampled Gold Run Creek in its entirety in all years, and Hall Creek in its near entirety in all years. Hall Creek does not have a defined and the core population of both Hall Creek and Staubach Creek. We sampled through the core of the population to a partial barrier in all other study streams. The presence of fish above the partial barriers was confirmed in all of these streams. Densities were very low above the sampling reaches in Staubach Creek and Hall Creek, but remained high in NF Little Belt Creek and the control streams. Study reaches ranged from 1,400 m to 1,760 m, and we sampled the entirety of these reaches. We additionally sampled two tributaries in NF Little Belt Creek. Study reaches in all control streams began at the barrier and ended 500 m upstream. Study reaches were broken into sections to allow for spatial analyses.

Table S5-1. Summary of translocations.

Subbasin (HUC8)	Creek	Creek Abbr.	Experimental Type	Source Creek	# of Fish Introduced/ Removed	Translocation Date (2017)
Belt	NF Little Belt	LB	Study	Gold Run	8/8	6/6
Belt	Gold Run	GR	Study	NF Little Belt	8/8	6/6
Upper Missouri	Hall	HA	Study	Quartz	6/6	6/8
Upper Missouri	Staubach	ST	Study	Quartz	6/6	6/8
Belt	Crawford	CR	Control	*NA*	0/0	*NA*
Upper Missouri	McClellan	MC	Control	*NA*	0/0	*NA*

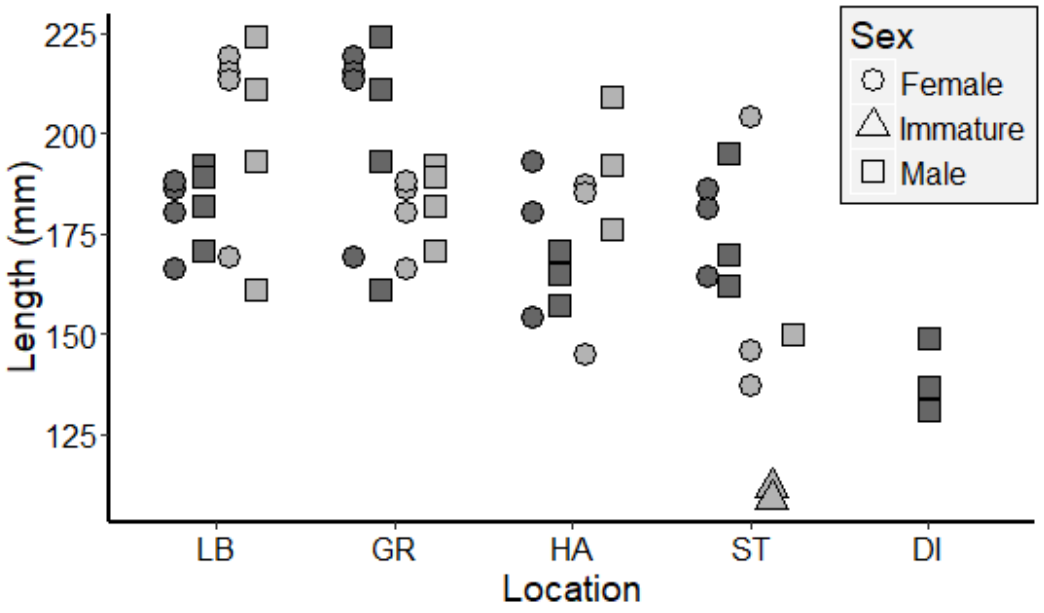

Figure S5-1. Length of fish that were introduced (dark grey) and removed (light grey) from each study population during 2017 translocations.