Do Piscicide Treatments Used to Restore Trout Populations Have Unintended Negative Consequences on a Non-Game Fish Species?

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Introduction

Invasive fishes in Montana represent one of the major threats to native fish species. A common management approach is to install a barrier and treat the water with a piscicide to remove non-native species. Desired species are then reintroduced to the system. This approach is often done with game species in mind, without consideration of non-game species. Recently it was found that Rocky Mountain Sculpin (*Cottus bondi*) have survived piscicide treatment in severatreatment streams, but apparently occur at low abundance following treatment. We tested for a population bottleneck by quantifying genetic variation following treatment. This work will allow us to assess population viability following piscicide treatments.

Methods

Field

Backpack single-pass electrofishing from above and below barrier at both Greenhorn Creek (2020,2021) and Sixteenmile Creek (2021)

	GHN_20	GHN-21	STM_21
Samples above	20	20	31
Samples below	19	23	32

Table 1: Sample size from each site

Lab

Salt-precipitation DNA extraction

2 batches of Microsattlite PCR (PCR 1: *Cott100, Cott687, Cott582,Cba42, Cgo33, LCE29;* PCR 2: *Cott255, CottES19, Cott113, Cgo1114, Cott310, Cott127*)

Microsatellites were size fractioned on a capillary sequencing machine at the UM Genomics Core

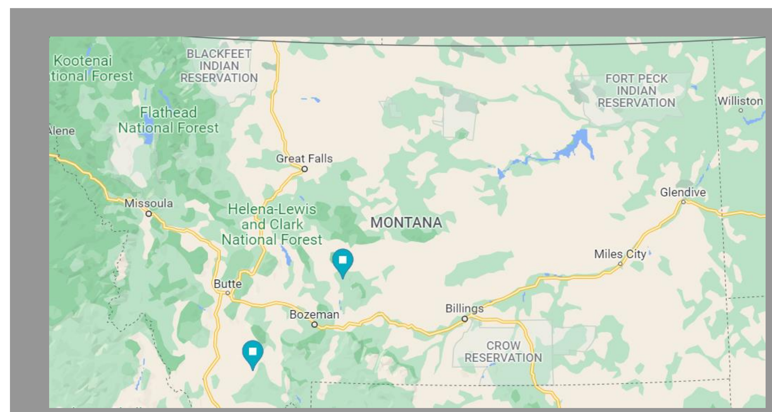
Alleles determined with the software Geneious

Analysis

Heterozygosity was estimated with R package *strataG*

N_e was estimated using an equation from Crow and Kimura (1970): $N_e = 1/(2*(1-e^a))$, where $a = \ln(H_t/H_0)/t$, *t is* in generations, and H_t/H_0 is the ratio of heterozygosity at time *t* (after the bottleneck; estimated from the above-barrier sample) and initial heterozygosity (H_0; estimated from below-barrier sample). This approach assumes that the below-barrier fish are representative of the above-barrier population before piscicide treatment. Sculpin generation interval could be from three to six years, so calculations were performed with both values to determine a likely range of N_e values.

Study area



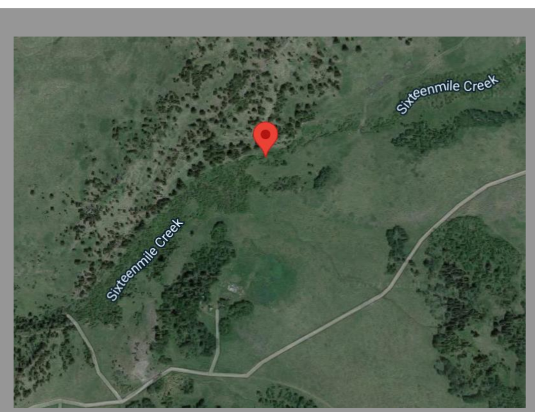


Fig 1: Map of the two study sites in the state of Montana; the right pin is Sixteenmile Creek; the left pin is Greenhorn Creek
Fig 2: Satellite image of Sixteenmile Creek; 46.236733, -110.586132
Fig 3: Satellite image of Greenhorn Creek; 45.107596, -112.057133

Results

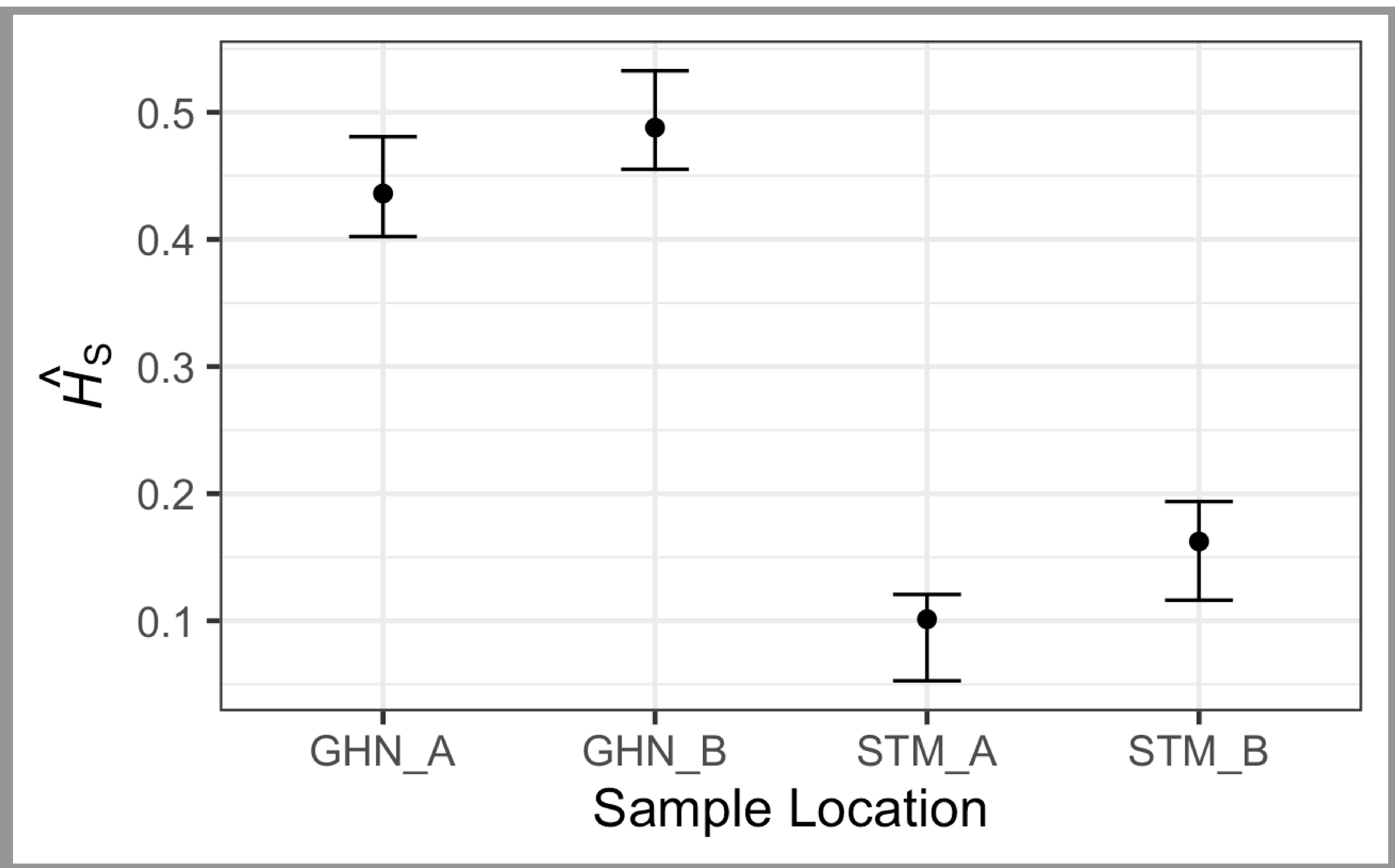


Fig 4: H_s is the mean expected heterozygosity for each population. GHN- Greenhorn Creek, STM- Sixteenmile Creek, _A is for above barrier or treated population, _B is below barrier or the original population.

	H_t	H_0	H_t/H_0	Difference in Genetic diversity	*t*	Generation interval	N_e
Greenhorn Creek	0.436	0.488	0.893	-10.60%	1	6	4.7
	0.436	0.488	0.893	-10.60%	2	3	9.1
Sixteenmile Creek	0.101	0.162	0.623	-37.60%	1	6	1.3
	0.101	0.162	0.623	-37.60%	2	3	2.4

Table 2: H_t is the mean expected heterozygosity in the affected population (above-barrier) and H_0 is the mean expected heterozygosity for the original population (below-barrier), *t* is the number of generations, and the generation interval is defined as the mean age of reproducing adults in a population. N_e is the estimate of effective population size necessary to explain the loss in heterozygosity over the given time in a generations. Due to the uncertainty in generation interval for this species, we calculated based on low (3 years) and high (6 years) possible values.

Discussion

We observed a loss in heterozygosity in the above-barrier populations. To explain the 10.6% loss in heterozygosity in Greenhorn Creek, the range of possible N_e values was between 4.7 (assumed a 3-year generation interval) and 9.1 (assumed a 6-year generation interval). In Sixteenmile Creek, to explain the 37.6% loss in heterozygosity, N_e would only be between 1.3 (assumed a 3-year generation interval) and 2.4 (assumed a 6-year generation interval) individuals. Thus, to explain heterozygosity results and effective population size, an N_e value below 10 is necessary for both Greenhorn and Sixteenmile Creeks.

Such low N_e values are consistent with extreme genetic drift and could lead to inbreeding and inbreeding depression in these now isolated above-barrier populations. Inbreeding depression could lead to low population viability (Kardos et al. 2021). Further, low genetic variation might lead to a low adaptive potential for changing environmental conditions (Kardos et al. 2021). Population extirpation could occur without management intervention, such as through the implementation of genetic rescue (Whiteley et al. 2015).

The Rocky Mountain Sculpin is a recently reclassified species that is morphologically and genetically distinct and is threatened by anthropogenic activities (Ruppert et al., 2017). As a species affected by human activity, conservation and awareness should be extended to populations affected by piscicide treatments.

Management Implications

- Knowing the loss in heterozygosity in Rocky Mountain Sculpin populations following the treatment of piscicide, efforts should be made to genetically restore and monitor surviving sculpin populations after treatment.

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