

Rainbow Trout invasion in the Lamar River Watershed, Yellowstone National Park: life history and genetics research to preserve native Cutthroat Trout

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Abstract

In Yellowstone National Park, the introduction of Lake Trout, Rainbow Trout, and Brook Trout have had a profound impact on native Yellowstone Cutthroat Trout (YCT), and active fisheries management is needed to prevent further population decline. Of particular concern is the impact of Rainbow Trout (RBT), which not only competes for resources with fluvial YCT, but also hybridize with them and interrupt locally-adapted genetics. The Lamar River, which has long been considered a stronghold for YCT, is now being invaded by RBT and their influence is spreading upstream at an unknown rate. While park managers are attempting to curtail invasion through active management practices, the lack of basic life-history information and spatial extent of hybridization in this drainage preclude the formation of a comprehensive management strategy. The goal of this study is to fill in knowledge gaps and develop a strategy to preserve the integrity of YCT in the Lamar River and its tributaries. From 2015 – 2017 we will use radio telemetry and passive integrated transponder tags to determine the timing and location of YCT, RBT, and hybrid spawning, and the degree of reproductive overlap throughout the watershed. Continued monitoring of individuals will also help to better understand the seasonal migration patterns of YCT and RBT and the fluvial connectivity requirements of YCT. We will also collect genetic samples to supplement ongoing work by the Park Service, and perform genetic analysis to determine the spatial extent of hybridization within the watershed and identify the source of invasion. We will work with park managers in developing a comprehensive strategy for Lamar River fisheries management, continued monitoring of hybridization status, and ongoing evaluation of management action effectiveness.

Problem Statement:

Invasive non-native trout are one of the greatest threats to native trout of the intermountain west, and have contributed to the decline and extirpation of many Yellowstone Cutthroat Trout (*Oncorhynchus clarkii bouvierii*, YCT) and Westslope Cutthroat Trout (*O. c. lewisi*, WCT) populations. Of particular concern is the impact of Rainbow Trout (*O. c. mykiss*, RBT), that influence native cutthroat trout both through competitive interactions and through the consequences of hybridization and genetic introgression. Rainbow trout are the most widely introduced fish species in the world (Halverson 2010), and their success at colonizing new habitats may in part be due to their ability to tolerate a variety of environmental conditions (Bear et al. 2007) and the expression of life-history plasticity (Meka et al. 2003). Relative to native cutthroat trout they prefer slightly warmer temperatures and lower spring-time river discharge (Bear et al. 2007, Muhlfeld et al. 2009b). Across a broad geographic scale, invasion success has been linked to variability in discharge, with low springtime flows facilitating juvenile RBT emergence and successful invasion (Fausch et al. 2001). While these slight physiological and life-history differences may to some extent protect headwater populations of native cutthroat populations in Montana (Yau and Taylor 2013), large and intermediate sized river systems at lower elevations have been widely invaded. Yellowstone Cutthroat Trout and WCT now occupy less than half of their current geographic range and populations have been extirpated (Meyer et al. 2003, Shepard et al. 2005, Gresswell 2011).

Rainbow trout and cutthroat trout can hybridize to produce viable offspring, a situation that can progress to the formation of hybrid swarms and the complete loss of native genomes (Muhlfeld et al. 2014). A hybrid swarm population occurs where few if any of the parent species remain and mating is random among an entirely hybrid population. Such is the case in portions of the Flathead River in Northwest Montana, where millions of RBT were stocked between late 1800s and 1969 and are having a pronounced impact on WCT. More frequently in this watershed hybridization is realized at various levels of introgression (e.g. the % of RBT alleles present in the WCT genome). As predicted by the thermal habitat preferences of RBT, levels of introgression appear to be highest at lower elevations within the watershed and near historical stocking locations (Boyer et al. 2008). However, even slight levels of levels of genetic introgression can reduce the fitness of WCT in this watershed (Muhlfeld et al. 2009a), and the spread of hybridization upstream is a serious concern. Elsewhere hybridization appears to be more limited, for example in the Yellowstone River between Gardner and Springdale. Here RBT are the most abundant trout, levels of hybridization with native YCT appear low, and many tributaries support presumably unhybridized YCT (De Rito 2004). Differences in the progression of hybridization among watersheds have led to a wealth of studies to identify the underlying mechanisms of RBT introgression.

Spatiotemporal differences in spawning behavior are believed to be one of the primary mechanism limiting hybridization of WCT (Muhlfeld et al. 2009a) and YCT (Henderson et al. 2000) with RBT (Figure 1). For example, in the Yellowstone River radio-telemetry was used to determine that temporal reproductive isolation was the predominant

mechanism that limited the degree of hybridization, as RBT spawn 5—9 weeks earlier than YCT (De Rito 2004). Similar differences in spawn timing between WCT and RBT have also been identified in the Flathead River(Muhlfeld et al. 2009b) system.

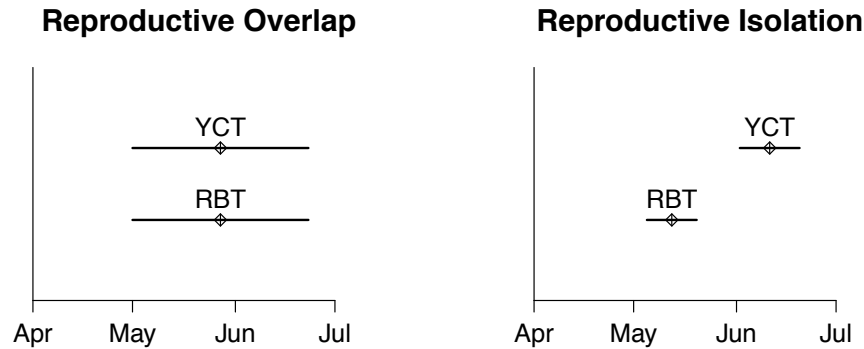


Figure 1. Hypothetical example of temporal mechanisms of reproductive overlap and isolation. Lines represent range of spawning dates of YCT and RBT while stars indicate median spawning date. Isolation and overlap can also be attributed to spatial differences.

However, it appears that reproductive isolation (due to differences in spawning timing and location) between native cutthroat trout and introduced RBT, in many cases, is not a strong enough mechanism to prevent eventual hybridization. Once hybridization occurs, the resulting hybridized individuals may be more likely to backcross with parents, contributing to more rapid spreading of introgression. As an example, in the Yellowstone River and Flathead River, hybrids tend to spawn at an intermediate time periods of RBT and cutthroat trout (De Rito 2004, Muhlfeld et al. 2009b), which may facilitate further introgression through temporal overlap in spawning. Furthermore, hybridized individuals may be more likely to stray long distances from natal spawning areas facilitating a stepping-stone pattern of RBT genetic material into native population genetics (Boyer et al. 2008).

There is also growing concern that the probability of successful hybridization, and the extent of hybridization, is correlated to climate change (Muhlfeld et al. 2014). Many of the anticipated hydrological impacts of climate change (e.g. warmer water temperatures, decreased snow-pack, and earlier spring flooding events) may create habitat conditions more favorable to RBT, accelerating hybridization with native cutthroat. In the Flathead River, the spread of hybridization since 1984 was negatively associated with high flow events, and positively correlated with stream temperature (Al-Chokhachy et al. 2014). This is alarming, and raises concern for the security of native cutthroat trout strongholds, and strengthens the need for implementing management strategies to curtail the spread of RBT.

Management efforts to limit hybridization and non-native salmonid invasion have involved either the chemical or mechanical removal of unwanted species, or the construction of barriers to fish movement to protect genetically pure populations of native

fish. The use of the piscicide Rotenone is a common approach, however this chemical is not selective for a particular species and when applied, kills all aquatic species (including macroinvertebrates and amphibians). Mechanical methods are labor intensive and must be continued for multiple years to be effective (Shepard 2004), which requires considerable funding. Barrier construction can impact the migratory life history of native fish that utilize a suite of spatially separated locations within a migratory circuit (Schlosser and Angermeier 1995) and influence metapopulation dynamics in ways difficult to predict. Isolation can also increase extinction risk due to genetic effects and the stochastic population dynamics of smaller populations (Fausch et al. 2009). Given these concerns, a careful consideration of the trade-offs of a particular approach should be analyzed prior to implementation (Peterson et al. 2008, Fausch et al. 2009). These decisions should consider ecological, socioeconomic, and evolutionary consequences (Fausch et al. 2009). However, in many situations, the ecological consequences are difficult to predict due to lack of basic life-history and genetic information on the population of interest.

Despite it's relatively unchanged and pristine aquatic habitats, YCT in Yellowstone National Park (YNP) are not immune to these anthropogenic impacts. In YNP over nine million Rainbow Trout and nearly 2 million Brook Trout were introduced into rivers and lakes, however in 1936 the park policy on fish stocking dictated, "Non-native fish shall not be stocked into waters containing native fish" (Varley 1981). This curtailed the further intentional spread of non-native species through the park, however the impacts of earlier introductions, and continued illegal stocking events, continue to be primary threats to native fish in YNP.

The Lamar River watershed, long considered one of Yellowstone's most important riverine strongholds for native YCT, is now threatened by invading RBT. In recent years, angler reports and genetic analyses have indicated that RBT are now entering the system and hybridizing with the native YCT. Of primary concern is the population near the Slough Creek confluence with the Lamar River, where densities of RBT and hybridization appear higher than elsewhere within the watershed. The population in the popular Slough Creek fishery was thought to be isolated from rainbow trout invasion due to a falls/cascade complex in the lower river, but the discovery of a number of rainbow trout in Slough Creek in recent years has created substantial concern about the integrity of the YCT population. To date, efforts to identify the source of the invasion have drawn no definitive conclusions. Moreover, in the upper Lamar River near Soda Butte Creek, there is an upstream invasion of nonnative brook trout that now appears to be expanding. Park management has taken action to curtail further spread of hybridization through various methods, but is in need of more detailed information about the mechanism of hybridization in order to most efficiently protect YCT.

The overall goal of this project is to investigate key life history and genetic attributes of YCT, RBT, and hybrid populations/metapopulations in the Lamar River Watershed (including Slough Creek and Soda Butte Creek) in order to guide management efforts to preserve genetically pure YCT. With information revealed through this study, a

comprehensive strategic plan for Lamar River YCT preservation, RBT suppression, and monitoring of population status will be developed.

Objectives:

1. **Determine locations and timing of spawning by YCT, RBT, and hybrids in the Lamar River drainage.** The current understanding of timing and location of spawning in the Lamar River is limited. However, knowledge of the spatiotemporal overlap in spawning behavior of YCT, RBT, and hybrids is critical to understand the process of hybridization and to guide management action. This knowledge will guide efforts for strategic removal of spawning adult RBT. If there is sufficient spatiotemporal separation in spawning behavior of RBT and YCT, chemical treatments during the peak of RBT spawning could be employed with little impact on YCT. If considerable overlap in spawning behavior is detected, mechanical methods to capture and selectively remove RBT could be employed. Furthermore, understanding the migratory patterns of YCT will help determine the seasonal habitat connectivity requirements of YCT, which will be critical to evaluating the potential impacts of barrier construction as a management strategy. Finally, understanding the migratory patterns of RBT and hybrids will help determine the relative threat of introgression to distant, genetically pure, populations within the watershed.
2. **Assess spatial patterns of introgression, and determine genetic variation within and among populations, within the Lamar River system.** A thorough evaluation of the extent of hybridization is critical to developing management priorities. For example, this should help to identify genetically intact YCT populations for conservation, and populations where introgression has progressed too far to warrant preservation and protection. Assessing the spatiotemporal pattern of introgression will help to identify if Lamar River hybridization is following a stepping-stone or continent-island type invasion. Should a predominant *source* population of RBT be identified, this could be targeted for suppression efforts, and be expected to have a significant impact on further hybridization. If *stepping-stone* dynamics and long-distance dispersal are facilitating hybridization, the strategic use of barriers to conserve genetically pure populations would be strongly supported.
3. **Develop a comprehensive plan to conserve YCT in the Lamar River system, YNP, and a strategy to evaluate the effectiveness of management actions.** The results of this work will provide life history and genetic information integral to developing a management strategy for the Lamar River. Specifically, the plan will identify important conservation units of genetically pure YCT and evaluate available options for continued preservation of those units, addressing the trade-offs related to threats of invasion and intentional isolation. This plan will also provide a framework for annual data needs and analysis needed to 1) continue monitoring spatiotemporal patterns of introgression and 2) evaluate the response to management action (e.g. mechanical or chemical removal, barrier construction, harvest regulations).

Methods:

Study area

Fieldwork will be conducted under this permit from April 2015 – October 2017 in the Lamar River watershed. Our fieldwork will be focused primarily in the Lamar River near its confluence with Slough Creek, however sampling and radio tracking will be conducted throughout the entire watershed including upper Slough Creek, Soda Butte Creek, Buffalo Creek, and the upper Lamar.

Objective 1

We will utilize radio-telemetry and passive integrated transponder (PIT) tag technology to address this objective. Radio tags actively transmit a radio signal and allow animals to be tracked over long distances and no prior knowledge of movement patterns is necessary. PIT tags contain no battery (e.g. are passive), but rather have an internal magnetic coil that is energized to transmit a signal once it comes in contact with a resonating antenna. These tags are 1/100th of the cost of radio-tags, permitting much larger sample sizes, however can only be detected at close range with stationary stream-wide antennae or handheld antennae. We will utilize the strengths of each technology to address aspects of the first objective.

Will use radio telemetry to determine the location and timing of spawning in 2016 – 2017. Fish will be captured via angling, backpack and boat/raft mounted electrofishing, and fyke nets in collaboration with NPS staff and their Volunteer Angler Program. Sampling will be conducted at various times and locations, pending weather, availability of staff, and equipment availability. Ideally, most radio tagging will be conducted in August – September. Fish will be anesthetized with AQUI-S20E (10% Eugenol) as an anesthetic in light of recent recommendations by the American Fisheries Society and the FDA (Bowker and Trushenski 2013). A small surgical incision will be made in the abdominal cavity anterior to the pelvic girdle and offset from the midventral line by 1 cm (Ertel 2011). A small hole will be punctured 3 cm posterior to the incision to facilitate threading the trailing whip antenna through the abdominal wall. After the implantation of radio tag, incisions will be closed with several sutures. Only mature fish will be tagged, which generally will be determined by a size greater than 300 mm (Clancy 1988), however it is known that some resident fish in tributaries to the Lamar River mature at smaller sizes (personal communication, Brian Ertel) and tagging these fish may also be conducted. In either scenario, the minimum size chosen will also ensure that tags are less than 4% of the body mass of the fish a threshold recommended by Zale et al. (2005). A fin clip will be taken from each radio tagged fish to determine hybridization status (further description in objective 2).

The anticipated tagging and tracking schedule is as follows, however will be modified as needed based on initial results. In the summer of 2015 we tagged 83 fish with radio transmitters, distributing tags evenly among RBT, YCT, and hybrids. We used transmitter models MCFT2-3FM and MCFT2-3BM, which weigh 10g and 8g

respectively. We worked with the park fisheries personnel and radio department to choose frequencies that would not interfere with ongoing work (information below).

ID: 1505712

Project: Lamar River Project - MT, WY

Bureau: NPS

Request Date: 05/04/15

Start Date: 05/30/2015

End Date: 10/30/2018

Animal: Trout (Yellowstone cutthroat trout, rainbow trout, hybrid trout)

Endangered? F

Dangerous? F

Contact: Brian Ertel, Rich Noble

Contact Phone: 307-344-2282

Contact Email: brian_ertel@nps.gov;richard_noble@contractor.nps.gov

Liaison: Carroll Alexander

Liaison Email: carroll_alexander@nps.gov

Number of Transmitters: 150

Number of Channels: 4

Frequencies:

172.00625 172.01875 172.03125 172.04375

Both of these tag models also include a motion sensor. When inactive (e.g. not moving) for a user-defined period of time, the tag transmits a *mortality* signal. The effective life of this tag would allow individuals tagged in the summer of 2015 to be tracked over the course of two spawning seasons (2016 – 2017), and fish tagged in 2016 to be tracked in 2017 – 2018). We anticipate tagging another ~60 fish in the summer of 2016.

Monitoring techniques included highway vehicle, on-foot tracking and fixed wing aircraft surveys with Tracker Aviation, and a fixed telemetry station at the confluence of the Lamar and Yellowstone River (Figure 2). Tracker Aviation conducts regular services for YNP and complies with all permitting and safety requirements of the Park. Fish will continue to be monitored and re-located monthly through the winter, via highway vehicle skiing trips, and flights.

From mid-April through mid-June (2016 – 2017) (the anticipated spawning period) attempts will be made to locate fish on a weekly basis, and at least twice a week during the peak periods of spawning. When fish have gone missing for more than one week attempts will be made to locate them via aircraft. Without apriori knowledge of fish spawning migration distances, it is difficult to predict the need for frequency of telemetry flights. If fish display short spawning migrations, into areas readily accessible by foot, few flights will be needed. If extensive migrations ensue, more regular telemetry flights will be conducted in order to obtain the necessary data. In the scenario with extensive movements to hard to reach locations, up to two flights may be required per week during the spawning period from mid-April – late June.

Fixed Monitoring Sites for Spring 2016

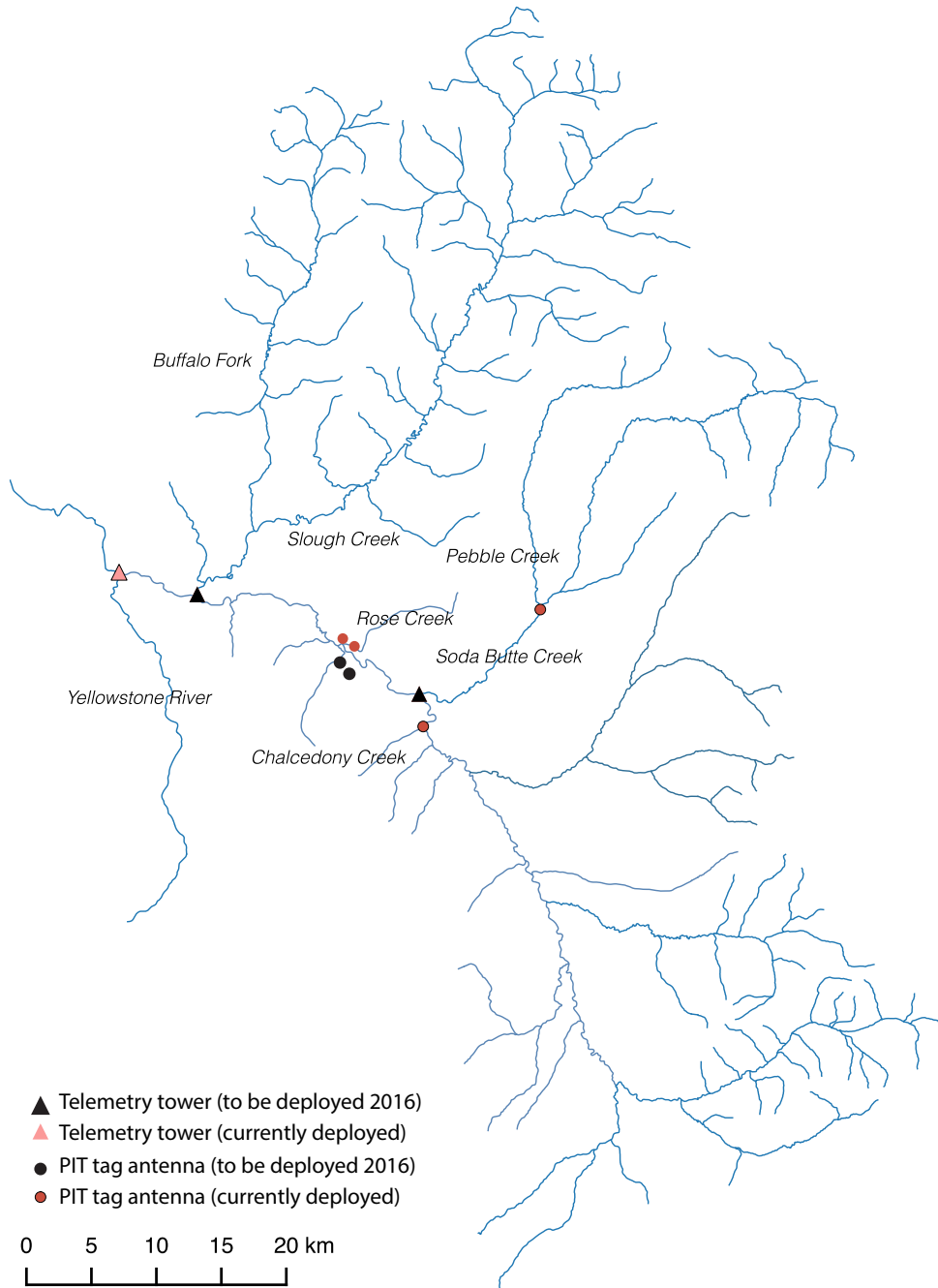


Figure 2. Fixed monitoring locations for radio and PIT tagged fish.

Tagging events in 2016 will largely be guided by our success or failure to identify spawning location and timing in Spring 2016. If the strategy described above (summer tagging) is successful a similar strategy will be employed to tag additional fish in the summer of 2016, in order to monitor their spawning behavior in Spring 2017. If insufficient numbers of tagged fish survive the winter of 2015, do not spawn, or are lost during the elapsed time from 2015 – Spring 2016, a new strategy will be considered. An alternate strategy would be to follow the example of Henderson (*et al.* 2000) and Muhlfeld (*et al.* 2009b) and capture pre-spawn individuals in March for tagging, and monitor their movements to spawning areas in April-June. This method could be advantageous because fish can be examined for pre-spawn condition using an otoscope, and only tagged if fish are in pre-spawn condition. Furthermore, fish tagged in this manner are at-large for a shorter period of time prior to spawning, and there should therefore be a smaller probability of mortality prior to spawning.

Radiotags provide detailed information on movement and location, but are limited in the number of tags that can be used. To increase sample size for monitoring movement and spawning location of YCT, RBT, and hybrids, PIT tags will also be used. Initially in 2015, before the main tributaries used for spawning are identified via radio telemetry, sites for monitoring with stationary PIT tag antennae will be chosen based on professional opinion and early season scouting with backpack electrofishers and test sets with fyke nets. As of December 2015, we have installed PIT tag antenna at Rose Creek East fork, Rose Creek West fork, Pebble Creek, and Chalcedony Creek. Two more sites will be equipped with PIT tag antennae in 2016, top candidate sits include Amythest Creek and Amphitheater Creek.

All fish captured during sampling events (using fyke nets, electrofishing, and angling) will be anesthetized (as described above), measured, weighed, and those > 250 will be tagged with 23-mm half duplex PIT tags and adipose fin clipped. PIT tags will be inserted into the dorsal sinus. This location for tagging was chosen because it will likely result in the highest tag retention rates (Dieterman and Hoxmeier 2009), however does raise concerns about anglers inadvertently consuming PIT tags. Because YCT and Hybrids are protected in the park, the only concern is that anglers will encounter PIT tags in RBT. We will adipose fin clip all PIT tagged fish, as a secondary mark, which will allow anglers to identify PIT tagged fish (flyers will be posted to inform anglers of PIT tag presence in adipose fin clipped fish, see attached for example of 2015 Flyer). This also will allow us to determine rate of tag loss from recaptured fish. Fish will be identified as either a Yellowstone Cutthroat Trout, Rainbow Trout, or Hybrid based on a morphological assessment (Behnke 1992). All PIT tagged fish will also be caudal fin clipped (see section on genetics below) for assessment of hybridization status, and to verify the accuracy of morphological assessment. We anticipate PIT tagging between 1,000 – 3,000 fish over the course of the study (about 400 were tagged in 2015).

Solar powered PIT tag monitoring stations will be set up at selected locations in each tributary stream near its confluence with the Lamar River (Figure 3). These stations will consist of two in-stream antenna approximately 2 meters apart, a rugged enclosure to

house electronics and batteries, and a ~180 W solar panel. When a fish with a PIT tag passes through or over the antenna, the ID, date, time, and antenna number are recorded by a datalogger.

The sequence of detections at antennae allow for the determination of directional movement. Antennae will be placed in a shallow location within the stream in order to maximize probability of detection. Care will be taken to choose locations for antenna stations and associated solar powering systems that are out of sight from the road. If possible, locations near trees will be considered to provide visual cover, and equipment (e.g. field boxes) will be green in color to reduce visual impact. Solar panels will be oriented for maximum solar absorption, but care will be taken to position in order to reduce visual impact and observed “glare” from road locations. For front country locations (e.g. Rose Creek), we will consult Park Ranger to discuss the most suitable antenna station locations. At each station, a water-level and temperature logger (e.g. HOBO U20L-04) will also be deployed in the stream.



Figure 2. Fixed radio telemetry station located at the confluence of the Lamar and Yellowstone River, September 2015.



Figure 3. Example of a small solar powered PIT tag antenna station. This station is located on the East Fork of Rose Creek (note Buffalo Ranch in background), and is entirely concealed from view of observers (when viewing from the North) by local topography.

On subsequent sampling events, after a sufficient number of individuals have been PIT tagged, we will conduct mobile tracking surveys to locate individual PIT tagged fish. Mobile tracking surveys will be conducted at each visit to the site, while nets are fishing. These will consist of hiking along a pre-determined length of stream and scanning its entirety with the wand. GPS locations will be recorded for each detected fish. Visual observations during this time will be recorded during each stream survey, taking note of spawning activity, location, number of fish spawning, and number of redds observed.

Additional PIT tags will be deployed during the summer and fall in collaboration with the volunteer fisherman program, and during sampling events to capture fish for radio telemetry. In 2015 we will be able to determine upstream migration timing for RBT, YCT, and hybrids via capture date, and departure from spawning areas by utilizing the PIT tag records of fish moving downstream out of tributaries. Furthermore we will be able to calculate the total time spent in spawning tributaries by each fish and can use fish detections from mobile wand surveys to determine major areas of use within the tributaries. In subsequent years once more fish are PIT tagged, we will no longer require the use of regular fyke netting to determine upstream migration timing, as fish PIT tagged in previous years should return to streams and be detected moving into and out of spawning tributaries at antenna stations. This method will provide excellent data on

spawning migration timing, but also reduce our presence at streams and disturbance to wildlife and visitors. In 2016 – 2017 we will use radio telemetry results to determine spawning areas of interest to focus future PIT tagging and stream-wide antenna monitoring.

Radio telemetry and PIT tag data will be analyzed via various methods. For each radio tagged individual we will develop a series of summary variables that include “beginning migration date, beginning migration temperature, beginning migration flow, spawning date, spawning temperature, spawning flow, migration period, and migration distance”(Muhlfeld et al. 2009b). The calculation of these variables will be guided by criteria similar to other similar studies (Henderson et al. 2000, De Rito 2004, Muhlfeld et al. 2009b) designed to remove subjectivity from assigned dates. A mixed modeling approach will be used to determine if differences in spawning behavior differ among YCT, RBT, and hybrids (Zuur et al. 2009), while controlling for the effects of year.

Using PIT tag data, we will generate summary information for each individual including migration date (into the spawning tributary), migration date out of the spawning tributary, duration in stream, spawning date, and flow and temperature measurements associated with each event. Similar criteria will be developed to assign spawning variables, in order to remove subjectivity. The PIT tag data set should provide a sufficient sample size at multiple spawning tributaries to address between site differences in spawning timing. A mixed modeling approach will be used in this analysis (Zuur 2013). Furthermore, this dataset will allow us to quantify inter-annual site fidelity and metrics of individual growth based on recaptured fish.

Objective 2

We will collect genetic samples throughout the Lamar River watershed during the summers of 2015 – 2017, in order to better understand the spatial and temporal patterns of RBT introgression. The first step in this process is to thoroughly inventory the availability of samples already collected by the NPS, some of which have been previously analyzed by the University of Montana Conservation Genetics Laboratory. A database will be built that identifies all previously collected samples, their availability, and all sequence data generated from these samples. Based on assessment of available data, we will formulate a collection strategy to collect additional samples to fill in data gaps within the watershed. Furthermore, a thorough assessment of previous sequencing efforts (e.g. which markers have been used for individuals) will allow us to develop a strategy that, if possible, incorporates existing data to reduce laboratory costs and duplication of information. The following describes anticipated methodologies for collection trips, and genetic analysis.

Much of the Lamar River drainage is not road accessible, and therefore collection trips will often involve backcountry travel and overnight camping. All appropriate backcountry permits and campsite reservations will be attended to as far in advance as possible. The majority of trips will be conducted on foot, however it is likely that some trips will be coordinated with the NPS fisheries staff and stock will be used. A list of

streams likely to be sampled for genetics include; Little Buffalo Creek, Buffalo Creek, Upper Slough Creek, Rock Creek, Pebble Creek, Miller Creek, Calfe Creek, Cold Creek, Little Lamar River.

Fish will be collected by electrofishing and angling. For sampling protocols, we will closely follow the recommendations by Rogers (2007). When populations and areas are identified for sampling (e.g., upper Buffalo Fork) we will sample fish from a range of locations within the pre-determined area (250m – 5km) in order to avoid capturing related individuals (Boyer et al. 2008). The UTM coordinates will be recorded at the upper and lower boundaries of sampling reaches. We will aim to capture 30 fish for genetic samples from each reach. Portions of fin tissue will be excised and stored individually in vials of preservative. Genetic sampling will be conducted after the spawning period is over, in order to ensure that the fish collected from designated reaches are indeed individuals that have been produced from reproduction in the stream that they were collected (not migrants to the area) (Boyer et al. 2008). Overall we anticipate collecting and analyzing up to 600 fin clips, collected from 20 sampling areas.

DNA will be extracted from samples using standard methods and genotyped at loci yet to be determined. The use of genomics in conservation has advanced quickly over the last decade, and new techniques allow for the rapid and inexpensive genotyping of individuals at thousands of genetic markers (Allendorf et al. 2010). Among the most commonly used markers for genetic analysis include microsatellite loci and single nucleotide polymorphisms (SNP's). Many of these markers are diagnostic between RBT and YCT, allowing for very precise estimates of hybridization status, and the detection of minute amounts of introgression. The genetic markers be utilized in this research are yet to be determined, but do not preclude the collection of samples, which will be conducted in the same way regardless of future laboratory work.

Objective 3

The culmination of this work will be an analysis of different management strategies, based on information learned through addressing chapters one and two. There has been a great deal of research to investigate the potential impacts of various management strategies to preserve genetic integrity of native salmonids (Peterson et al. 2008, 2013, Fausch et al. 2009). In particular, this research has focused on the effects of intentional isolation, and in general has shown that the outcome of isolation depends largely on the environmental conditions, migratory tendency of the population, patch size, and hydraulic regime (Peterson et al. 2008). With these considerations in mind, we will work with NPS staff to develop a series of potential management strategies, and evaluate their potential impacts on 1) effectively curtailing the further spread of RBT introgression 2) their impacts on YCT population persistence in the face of isolation or connectivity.

Schedule

Spring Semester 2015 (Jan – May)

- ~ April 8th sample weekly with fyke nets in tributaries, PIT tag fish
- Sample more frequently once initial spawning movements are detected
- Set up tributary PIT antennae in April soon after fyke net sampling has begun

Summer 2015 (May – August)

- Continue sampling Lamar tributaries with fyke nets through spawning period and PIT tagging fish
- Regular mobile wand surveys of tributaries during spawning period
- Genetics sampling trips
- PIT tag fish in Lamar with volunteer fly fisherman
- Deploy majority of radio tags in August – September
- Practice/refine telemetry methods, locate each fish at least once/month

Fall Semester 2015 (August 24 – December 11)

- Continue monthly radio tagged fish relocations
- Visit PIT sites to load data/maintain antennae
- Genetics sampling trips

Spring Semester 2016 (Jan – May)

- Set up tributary PIT tag antennas April
- Occasional fyke net sampling at tributary streams
- Intensive radio-tracking effort starting early April to get spawning fish locations at least 2 times per week (may require 2 crews and flights)
- **If not enough fish tagged summer 2015 are still alive, coordinate a March sampling event to put more tags out. If a decent number of fish tagged in 2015 are still around, no March sampling needed

Summer 2016

- Deploy more radio-tags, genetics sampling trips as needed
- Continue PIT tagging in Lamar
- Genetics sampling

Fall Semester 2016

- Finish ALL genetics sampling, get genetics analyzed
- Continue monthly tracking of radio-tagged fish

Spring Semester 2017

- Maintain PIT antennas at tributaries, possibly pit tag more fish caught in fyke nets
- Intensive tracking efforts of spawning fish, this is the main priority April - June now, good data this year will complete radio tracking efforts!

Summer 2017

- Plan fieldwork as necessary to fill in data gaps

Fall 2017 – Spring 2018

- Write dissertation, defend

Budget

Major expenses are listed below

- Telemetry tags—\$30,000 (100 tags)
- Telemetry receivers – \$9,000 (2 readers and antenna)
- PIT tags – \$3,500 (1,500 tags)
- PIT tag antennae readers and solar stations – \$24,000 (6 stations)
- Temperature/Pressure loggers – \$1,800 (6 PT loggers)
- Genetic lab analysis – \$30,000 (600 samples at 50\$ each)
- Fyke nets – \$1000
- Travel, petty cash, assorted gear – \$5,000
- Tuition and salary – \$108,000 (27,000 for 4 years, probably offset by TAs as well though)
- Telemetry flights - TBD

Funding for this project has been procured through the National Park Fisheries and Aquatic Science program.

Products:

Publications and reports

This study will form the basis of Kurt Heim's PhD dissertation research Montana State University in Fish and Wildlife Biology. As such, a full dissertation will be produced upon completion of his doctoral program and kept available through the Montana State University library. Additionally, Kurt Heim and collaborators will publish results in peer-reviewed scientific journals. *Potential* publication titles/topics include:

1. Spawning migrations of Rainbow Trout, Yellowstone Cutthroat Trout, and hybrids in the Lamar River, Yellowstone National park
2. Spatial pattern of Rainbow Trout invasion in the Lamar River, Yellowstone National Park
3. Population structure of Yellowstone Cutthroat Trout: Implications for the use of barriers as a management strategy in Yellowstone National Park
4. Introgressive non-native hybridization influences migratory behavior and homing of a native salmonid
5. Citizens science facilitates fisheries management in Yellowstone National Park: a case study describing active involvement of fly-fisherman in research and invasive species removal

Furthermore, this research will be disseminated through talks at scientific meetings, and interaction with public during sampling and coordinating volunteer events.

Collections

All unused genetic samples collected during this study will be give to the NPS Fisheries and Aquatic Science program for final disposal.

Literature Cited:

- Al-Chokhachy, R., C. C. Muhlfeld, M. C. Boyer, L. A. Jones, A. Steed, and J. L. Kershner. 2014. Quantifying the Effectiveness of Conservation Measures to Control the Spread of Anthropogenic Hybridization in Stream Salmonids: a Climate Adaptation Case Study. *North American Journal of Fisheries Management* 34:642–652.
- Allendorf, F. W., P. A. Hohenlohe, and G. Luikart. 2010. Genomics and the Future of Conservation Genetics. *Nature reviews. Genetics* 11:697–709.
- Bear, E. A., T. E. McMahon, and A. V. Zale. 2007. Comparative Thermal Requirements of Westslope Cutthroat Trout and Rainbow Trout: Implications for Species Interactions and Development of Thermal Protection Standards. *Transactions of the American Fisheries Society* 136:1113–1121.
- Behnke, R. J. 1992. Native trout of western North America AFS Monograph. American Fisheries Society, Bethesda, Maryland.
- Bowker, J., and J. Trushenski. 2013. Fish drug Questions Answered by the FDA. *Fisheries* 38:549–552.
- Boyer, M. C., C. C. Muhlfeld, and F. W. Allendorf. 2008. Rainbow Trout (*Oncorhynchus mykiss*) Invasion and the Spread of Hybridization with Native Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*). *Canadian Journal of Fisheries and Aquatic Sciences* 65:658–669.
- Clancy, C. G. 1988. Effects of Dewatering on Spawning by Yellowstone Cutthroat Trout in Tributaries to the Yellowstone River, Montana. Pages 37-41 in R.E. Gresswell, editor. Status and Management of Interior Stocks of Cutthroat Trout. American Fisheries Society, Symposium 4, Bethesda, Maryland.
- De Rito, J. N. 2004. Assessment of Reproductive Isolation Between Yellowstone Cutthroat Trout and Rainbow Trout in the Yellowstone River. Master's Thesis, Montana State University.

- Dieterman, D. J., and R. J. H. Hoxmeier. 2009. Instream Evaluation of Passive Integrated Transponder Retention in Brook Trout and Brown Trout: Effects of Season, Anatomical Placement, and Fish Length. *North American Journal of Fisheries Management* 29:109–115.
- Ertel, B. D. 2011. Distribution, Movements, and Life-history Characteristics of Yellowstone Cutthroat Trout *Oncorhynchus Clarkii* Bouvieri in the Upper Yellowstone River Drainage. Master's Thesis, Montana State University.
- Fausch, K. D., B. E. Rieman, J. B. Dunham, M. K. Young, and D. P. Peterson. 2009. Invasion Versus Isolation: Trade-offs in Managing Native Salmonids with Barriers to Upstream Movement. *Conservation Biology* 23:859–870.
- Fausch, K. D., Y. Taniguchi, S. Nakano, G. D. Grossman, and C. R. Townsend. 2001. Flood Disturbance Regimes Influence Rainbow Trout Invasion Success Among Five Holartic Regions. *Ecological Applications* 11:1438–1455.
- Gresswell, R. E. 2011. Biology, Status, and Management of the Yellowstone Cutthroat Trout. *North American Journal of Fisheries Management* 31:782–812.
- Halverson, A. 2010. *An Entirely Synthetic Fish: How Rainbow Trout Beguiled America and Overran the World*. Yale University Press, New Haven, CT.
- Henderson, R., J. L. Kershner, and C. A. Toline. 2000. Timing and Location of Spawning by Nonnative Wild Rainbow Trout and Native Cutthroat Trout in the South Fork Snake River, Idaho, with Implications for Hybridization. *North American Journal of Fisheries Management* 20:584–596.
- Meka, J. M., E. E. Knudsen, D. C. Douglas, and R. B. Benter. 2003. Variable Migratory Patterns of Different Adult Rainbow Trout Life History Types in a Southwest Alaska Watershed. *Transactions of the American Fisheries Society* 132:717–732.
- Meyer, K. A., D. J. Schill, F. S. Elle, and W. C. Schrader. 2003. A Long-Term Comparison of Yellowstone Cutthroat Trout Abundance and Size Structure in Their Historical Range in Idaho. *North American Journal of Fisheries Management* 23(January 2015):149–162.
- Muhlfeld, C. C., S. T. Kalinowski, T. E. McMahon, M. L. Taper, S. Painter, R. F. Leary, and F. W. Allendorf. 2009a. Hybridization Rapidly Reduces Fitness of a Native Trout in the Wild. *Biology letters* 5:328–331.
- Muhlfeld, C. C., T. E. McMahon, D. Belcer, and J. L. Kershner. 2009b. Spatial and Temporal Spawning Dynamics of Native Westslope Cutthroat Trout, *Oncorhynchus clarkii lewisi*, Introduced Rainbow Trout, *Oncorhynchus mykiss*, and their Hybrids. *Canadian Journal of Fisheries and Aquatic Sciences* 66:1153–1168.

- Muhlfeld, C. C., R. P. Kovach, L. A. Jones, R. Al-chokhachy, M. C. Boyer, R. F. Leary, W. H. Lowe, G. Luikart, and F. W. Allendorf. 2014. Invasive Hybridization in a Threatened Species is Accelerated by Climate Change. *Nature Climate Change* 4.
- Peterson, D. P., B. E. Reiman, J. B. Dunham, K. Fausch D., and M. K. Young. 2008. Analysis of Trade-Offs Between Threats of Invasion by Nonnative Brook Trout (*Salvelinus fontinalis*) and Intentional Isolation for Native Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*). *Canadian Journal of Fisheries and Aquatic Sciences* 65:557–573.
- Peterson, D. P., B. E. Rieman, D. L. Horan, and M. K. Young. 2013. Patch Size But Not Short-term Isolation Influences Occurrence of Westslope Cutthroat Trout Above Human-made Barriers. *Ecology of Freshwater Fish*:556–571.
- Rogers, K. B. 2007. A Suggested Protocol for Collecting Cutthroat Trout Tissues for Subsequent Genetic Analysis. Colorado Division of Wildlife. Fort Collins, Colorado.
- Schlosser, I. J., P. L. Angermeier. 1995. Spatial Variation in Demographic Processes of Lotic Fishes: Conceptual Models, Empirical Evidence, and Implications for Conservation. In: Nelson JL (ed) *Evolution and the Aquatic Ecosystem: Defining Unique Units in Population Conservation*. American Fisheries Society Symposium 17:392–401
- Shepard, B. B. 2004. Factors that May Be Influencing Nonnative Brook Trout Invasion and Their Displacement of Native Westslope Cutthroat Trout in Three Adjacent Southwestern Montana Streams. *North American Journal of Fisheries Management* 24:1088–1100.
- Shepard, B. B., B. E. May, and W. Urie. 2005. Status and Conservation of Westslope Cutthroat Trout within the Western United States. *North American Journal of Fisheries Management* 25:1426–1440.
- Varley, J. D. 1981. A History of Fish Stocking Activities in Yellowstone National Park Between 1881 and 1980. Information Paper 35. Yellowstone National Park, Wyoming.
- Yau, M. M., and E. B. Taylor. 2013. Environmental and Anthropogenic Correlates of Hybridization Between Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*) and Introduced Rainbow Trout (*O. mykiss*). *Conservation Genetics* 14:885–900.
- Zale, A. V., C. Brooke, and W. C. Fraser. 2005. Effects of Surgically Implanted Transmitter Weights on Growth and Swimming Stamina of Small Adult Westslope Cutthroat Trout. *Transactions of the American Fisheries Society* 134: 653-660.

Zuur, A. F. 2013. A Beginner's Guide to GLM and GLMM Using R; a Frequentist and Bayesian Perspective for Ecologists. Highland Statistics, Newburgh.

Zuur, A., E. N. Ieno, N. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed Effects Models and Extensions in Ecology with R. Springer Science & Business Media.

Qualifications

Principal investigator Thomas McMahon is a professor at Montana State University. He has worked collaboratively with the YNP Fisheries Office on two past graduate student projects (Munro-Identification of timing and source of lake trout introduction of lake trout into Yellowstone Lake; Ertel: Cutthroat spawning and rearing in the Upper Yellowstone River) and is supervising another current study (Stewart: Identification of natal spawning tributaries of Yellowstone cutthroat trout in Yellowstone Lake using otolith microchemistry).

Kurt Heim is a PhD student at MSU that recently completed his M.S. research at the University of Alaska (August 2014). He has experience planning and conducting remote fieldwork and has been working in the field of fisheries since 2009. He independently designed and constructed 10 PIT tag antennae, and has PIT tagged >1000 fish during his M.S. research. He also has proven his analytical and writing skills through authoring/co-authoring several papers during the last year.

Supporting documentation and special concerns

Safety

Potentially hazardous activities include electrofishing, aircraft use, wilderness travel, and river crossings. All appropriate safety precautions will be taken for each activity.

Kurt Heim will, on all sampling occasions, carry either a satellite telephone or a DeLorme inReach explorer (a satellite text messaging and emergency alert system), or SPOT device. During backcountry trips, a detailed itinerary, emergency protocol, and check in schedule will be distributed to collaborators on project, and to the backcountry permitting office, if requested.

Access to study sites

Access to study sites will include highway vehicles, on foot, and horse. Kurt and a technician will live in YNP from May 18th – August 8th during the summer of 2015, and a technician will live in YNP from Aug 8th – November 1st 2015. In 2016 – 2017 Kurt and technician will also live in YNP during the field season (dates TBD). Sampling activities will be conducted daily during the summer as required by the objectives and methodologies described in this document. We anticipate an average of 5–10 overnight backcountry trips per summer. We will conduct surveys by cross-country ski at least 3

times per winter. These trips will involve backcountry winter camping. K. Heim is an experienced winter traveler, and all appropriate safety precautions will be taken.

Use of mechanized and other equipment

We will be utilizing up to 7 PIT tag antenna monitoring stations, as described above. These will be left in tributaries to the Lamar River year round in order to monitor fish movements. In all circumstances, care will be taken to set up stations in a discrete location not easily noticed by park visitors. Pressure transducers will be deployed in streams near antenna stations, these small devices (about the size and shape of a hot dog) will be wired to a 5lb weightlifting weight, and anchored to the substrate using rebar. The rebar will protrude from the waters surface and be marked with flagging. Fyke nets will be deployed for up to 8 hours in tributaries to the Lamar River.

Chemical use

The only chemical to be used in this work is fish anesthetic AQUI-S20E (10% Eugenol).

Ground disturbance

During construction of each PIT tag antenna, four pieces of rebar will be pole-pounded into the stream and riparian area. One pieces of rebar will be pounded into the stream substrate to anchor pressure transducer. This should have minimal impact.

Animal welfare

This study requires an IACUC which was granted by MSU in 2015.

NPS assistance

We anticipate the fisheries staff of NPS to assist with this research as needed, providing personnel assistance, equipment use, and housing during the summer.

Attention Anglers: Rainbow trout and cutthroat trout in the Lamar River and tributaries may contain internal tags

The National Park Service and Montana State University are conducting a study on the spread of hybridization between introduced rainbow trout and native Yellowstone cutthroat trout in the Lamar River drainage. We are using Passive Integrated Transponder tags (PIT) and radio tags to determine the timing and location of spawning of both species and hybrids.

Release alive all tagged and untagged trout with a cutthroat slash, in accordance with park regulations.

If you kill a rainbow trout with a tag, return tag to nearest ranger station or park entrance, or contact:

Kurt Heim, 856-905-9367
kurtheim@gmail.com

Or

Todd Koel, 307-344-2281
todd_koel@nps.gov



Adipose fin will be removed on
PIT tagged rainbow trout



The small PIT tag will be inserted **internally** at the base of the dorsal fin



Radio tagged fish will have a larger radio transmitter (59 mm) **surgically implanted** into the body cavity. These fish will have a **trailing whip antenna** as shown above.