Investigation Use of Passive Integrated Transponder (PIT) Tags for Rio Grande silvery minnow

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SUBMITTED TO

U.S. BUREAU OF RECLAMATION
555 BROADWAY NE., SUITE 100
ALBUQUERQUE, NM 87102-2352
FOR
MIDDLE RIO GRANDE ENDANGERED SPECIES ACT COLLABORATIVE PROGRAM

8 December 2008

EXECUTIVE SUMMARY

We compared passive integrated transponder tag-induced mortality by two implant methods (surgical and injection), and assessed long-term tag retention after surgical implant in Rio Grande silvery minnow *Hybognathus amarus*. Mean survival (±SEM) on day 32 was 99 (±0.013), 87 (±0.060), and 50% (±0.046) for control, surgically implanted, and injected fish, respectively, in Experiment 1. For Experiment 2, we tagged 280 fish by surgical implant and held them in aquaria for 49 days. Survival and tag retention on day 49 was 90 and 91%, respectively. Survival was significantly higher in fish with greater standard length ($P < 0.0001$), but there was no relationship of tag retention to fish size. We conclude PIT tags are a feasible method to tag Rio Grande silvery minnow, if fish are greater than 60 mm standard length. Fish should be held in captivity for a minimum of 6 days after tagging for best survival. We met the minimum criteria set in the original proposal for survival and retention, and have completed Phase I (comparison and selection of tagging methods). Phase II will begin in 2008 and includes field-testing the weir antenna and large-scale tagging and release of up to 10,000 Rio Grande silvery minnow.

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TIMELINE

We obtained Rio Grande silvery minnow brood stock from Dexter National Fish Hatchery on Dec. 13 2008. Fish were held at NMFWCO wet lab facility for the duration the experiment. On Jan 15-16, 2008, Biomark's representative Audrey Hopkins instructed an open workshop on PIT-tagging. Ms. Hopkins instructed the team of investigators on proper pit tagging procedures on small-bodied fish. We discussed several options for PIT-tagging, and designed comparison of tagging methods. We began the experiment January 18 and ended February 19, 2008. When Experiment 1 was finished, we followed up with experiment to examine how standard length relates to survival and PIT-tag retention. We began the second experiment March 5 and ended April 30, 2008. We completed Phase I with acceptable levels of mortality and survival, and Phase II, field testing the antenna and weir, and large-scale tagging, will begin in 2008.

INTRODUCTION

Rio Grande silvery minnow *Hybognathus amarus* is a federally endangered species formerly found throughout the Rio Grande basin of New Mexico and Texas, including the Pecos River, and Mexico. By 1979, Rio Grande silvery minnow was extirpated from the Pecos River, apparently displaced through competition and hybridization with introduced plains minnow *H. placitus* (Cowley 1979). By 1990, Rio Grande silvery minnow occupied about 5% of its original range in New Mexico, and continue to be threatened by habitat and flow modification, and non-native species (Bestgen and Platania 1991). Data from 1996 through 2001 show an annual decline in number of Rio Grande Silvery minnow (Dudley and Platania 1999, 2000, 2001, 2002), and unbiased data on movement, habitat use, and population parameters is needed.

Passive integrated transponder (PIT) tags are commonly used to monitor growth, movement, survival, and population size of fishes (Achord et al. 1996, Armstrong et al. 1999, Hildebrand and Kershner 2000). Passive monitoring stations can be effective for tracking movements of individuals (Brännäs et al. 1994, Armstrong et al. 1996), and portable units are effective for tracking behavior of individuals with minimum disturbance (Roussel et al. 2000, Cucherrousset et al. 2005, Hill et al. 2006). However, knowledge of long-term tag retention, and the effect of PIT-tagging on survival and behavior is required to determine the feasibility of tagging large numbers of Rio Grande silvery minnow, and collecting unbiased data.

Multiple methods of PIT-tag implantation have been examined, including syringe injection, surgical implantation, and surgical implantation followed by suturing, but most research focuses on large-bodied or cold-water fishes. PIT-tagging does not have a significant effect on survival of age-0 salmonids (Gries and Letcher 2002, Dare 2003, Bateman and Gresswell 2006, Acolas 2007), but Rio Grande silvery minnows are warm-water fish, which may have differences in wound healing (Knights and Lasee 1996). PIT-tagging does not significantly lower survival of the warm-water fishes, rudd *Scardinus erythropthalmus*, and roach *Rutilus rutilus* (Skov et al. 2005); however, the smallest fish tested were still considerably larger than Rio Grande silvery minnow, which reach an adult size of about 90 mm total length in New Mexico (Sublette et al. 1990). In general, surgical implantation results in higher survival and tag retention, especially in smaller fishes (Baras et al. 1999, 2000).

To assess feasibility of uniquely marking adult Rio Grande silvery minnows with PIT tags, we determined the effects of injected tags and surgically implanted tags on survival over 32 days, and examined the relationship between standard length and probability of survival and tag retention over 49 days.

OBJECTIVES

- Compare survival among surgically implanted, injected, and control fish
- Determine tag retention over 49 days
- Develop a protocol for tagging and subsequent release of 10,000 fish

METHODS

Fish Maintenance

We obtained Rio Grande silvery minnow brood stock from Dexter National Fish Hatchery and Technology Center, Dexter, New Mexico. We held fish in a 5,100-L recirculating system, consisting of seven individual fiberglass tanks, one containing filtration equipment. Each tank measured 180 x 75 x 60 cm. We filled the system with municipal water passed through a reverse-osmosis filter. Water drained through a standpipe to a sand filter (Jacuzzi© ST-24, 115 L/h flow rate), then was circulated back to each tank. Mean volume of water in each treatment tank was 743 ± 40 L. Return flow to each tank was ~1,000 L/h. Fish were held approximately 1 month before the experiment began.

Experiment 1-Comparison of Implant Methods

We withheld food for 2 days (d) prior to tagging. We anesthetized four to six fish at one time in MS-222 (0.1 g/L), selected a single fish greater than 50 mm, then randomly assigned one of three treatments (control, surgical implant, or injected implant), and one of four tanks in a balanced randomized block design. We chose fish larger than 50 mm standard length based on prior observations of minimum size for successful implantation (Prentice et al. 1990b). Each tank contained 60 fish, 20 each of three treatment groups. Control fish were immediately placed in assigned tank to recover from anesthesia. We used a 12-gauge hypodermic needle to insert a PIT tag (Biomark model TX1411SST, 134.2 kHz, 12.50 x 2.07 mm, 0.102 g) into the abdominal cavity (Prentice et al. 1990b). For surgically implanted fish, we used a scalpel to make a 3 to 5 mm incision in the abdominal cavity in the same insertion point as injected fish (Prentice et al. 1990a, Baras et al. 1999, Baras et al. 2000). We inserted a PIT tag into the incision, and gently massaged the area until the tag was fully inserted in the abdominal cavity. We immediately placed surgically implanted and injected fish in assigned tanks to recover. We recorded treatment and tank assignment, standard length (mm), tag identification number (for tagged fish), and person performing implant procedure. We sterilized all surgical equipment and PIT tags in 70% ethanol between each procedure. After completing all tagging, we stopped filtration and treated each tank with 15 ppm nitrofurazone and a 1.0% salt bath as a prophylactic. After 8 hours (h), we turned the filtration system on and replaced ~50% of the water in the system with fresh water. We performed routine water changes on days 10 and 20. We fed fish ~ 2 grams (g) of spirulina flake food (veggie grazer flake, Jehmco, Inc) per tank, twice per day, for 32 d.

Experiment 2-Effect of Standard Length on Survival and Tag Retention

We prepared, anesthetized, and surgically implanted 280 fish (mean standard length 64.4 mm, Figure 1). We tagged all fish that appeared healthy, without respect to standard length. Three individuals tagged fish at one time. We recorded person tagging and standard length of each fish, and total time elapsed for the tagging session. Each of four tanks contained 70 fish. We fed fish ~3 g of spirulina flake food per tank, twice per day, for 49 d.

Data Collection and Analyses

Experiment 1-We checked tanks twice daily for dead fish. We scanned dead fish for tags, and recorded time and date. We assumed fish that died with no tag and showed no abdominal scars were control fish. We considered fish that died less than 8 h after tagging as day 0 mortalities. On day 32, we scanned all remaining live fish to determine tag retention rates, and measured standard length.

We used a 3x4 randomized block design, each tank representing a blocking unit. We used the mean of each treatment as a response variable; each treatment-tank combination contained only one observation and the treatment by tank interaction was used as the error term. We used Tukey-HSD post-hoc analysis ($\alpha = 0.05$) on the initial mean standard length of fish in each treatment group and tank combination to determine if any initial size bias existed among treatment groups or tanks. Survival data did not meet assumptions of normality and homoscedasticity; therefore, to test effects of PIT tagging on survival we used the Friedman test (Zar 1999, Dalgaard 2002). We used a 2x2 contingency table to compare the proportion of tags retained between injected and surgically implanted fish.

Experiment 2- We monitored tanks for dead fish, and swept each tank with a magnet daily to collect shed tags. Fish that died less than 8 h after tagging were considered day 0 mortalities. On day 49, we scanned all remaining live fish to determine final tag retention. We used only fish still alive on day 49 to calculate retention. We used logistic regression to assess effect of standard length on survival and tag retention to day 49. The logistic response form is

$$Y_i = e^{(\beta_0+\beta_1 X_i)} / [1 + e^{(\beta_0+\beta_1 X_i)}]$$

Where Y_i = probability of survival (or tag retention) of fish i on day 49, β_0 = regression intercept, β_1 = regression slope, and X_i = standard length of fish i. We used a full/reduced model drop-in-deviance test (Dalgaard 2002) to assess the fit of the logistic response function.

We performed all statistical analyses in program R, version 2.6.2 (The R Project for Statistical Computing, http://www.r-project.org/).

RESULTS

Experiment 1

Initial mean standard length was 64.7 ± 1.1 mm (control), 67.0 ± 1.3 mm (injection), and 65.5 ± 0.4 mm (surgical), but differences among treatments were not

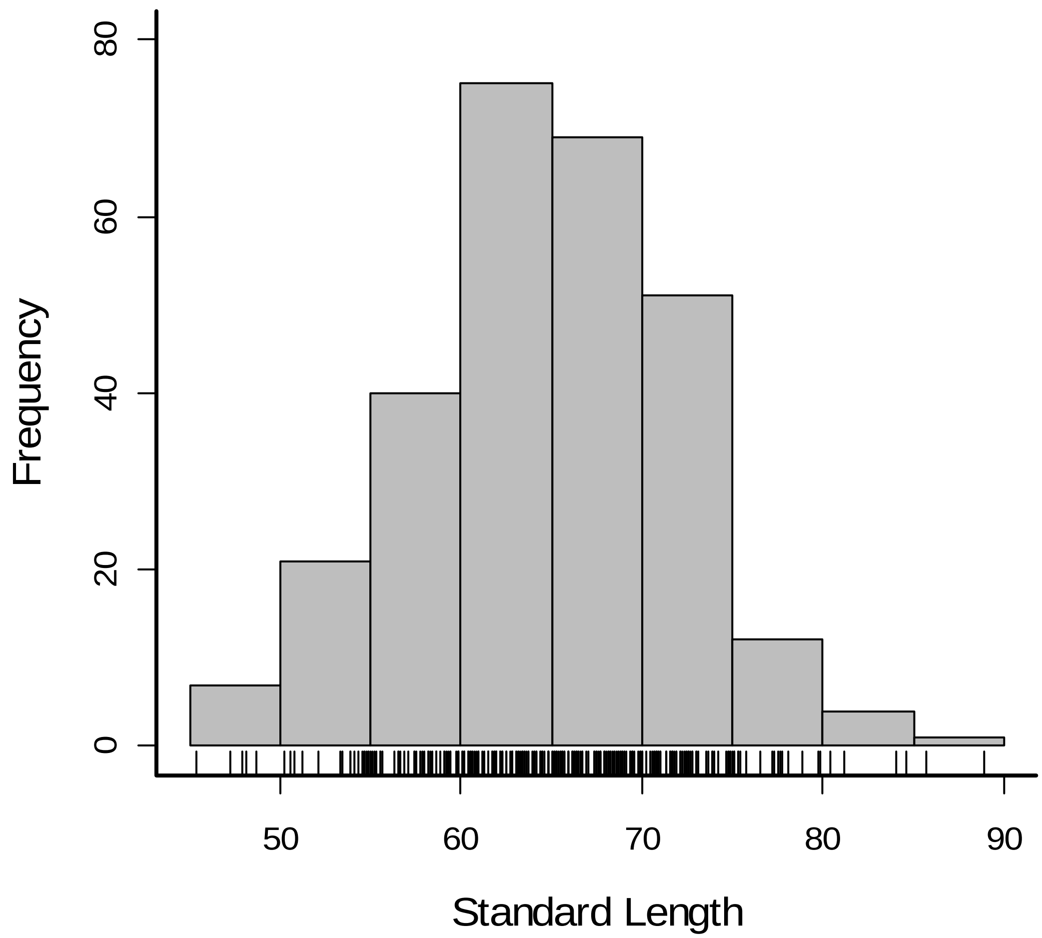

Figure 1-Initial standard length (mm) of Rio Grande silver minnow PIT tagged via surgical implant, held in aquaria 49 d, Albuquerque, New Mexico.

significant (Tukey-HSD, α = 0.05). Mean survival of Rio Grande silvery minnows on day 0 differed between treatments, and was 100% (± 0), 94% (± 0.032), and 66% (± 0.024), for control, surgically implanted, and injected fish, respectively. Mean survival to day 32 significantly differed among treatments (χ^2_2 = 7.6, *P* = 0.0224), and was 99% (±

0.013), 87% (± 0.060), and 50% (± 0.046), for control, surgically implanted and injected fish, respectively (Figure 2). However, 90% of total mortality (46 of 51) in all groups

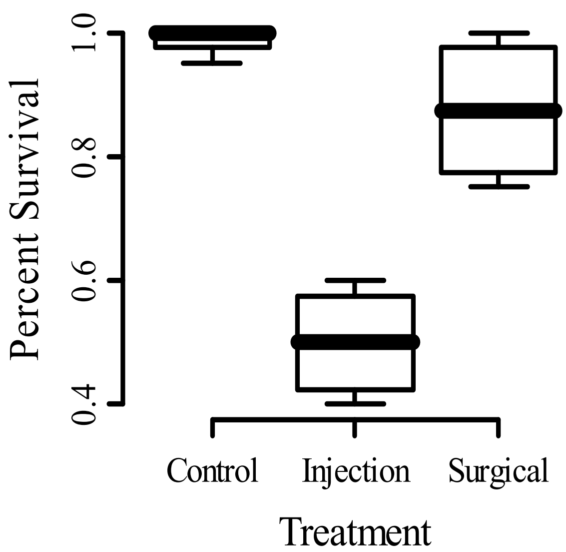

Figure 2-Survival of Rio Grande silvery minnow PIT tagged via injection or surgical implant, held in aquaria for 32 d, Albuquerque, New Mexico.

combined occurred during the first five days. Tag retention did not differ between methods for fish still alive on day 32 ($\chi^2_1 = 0.11$, $P = 0.74$), and was 90% for injected fish and surgically implanted fish (n = 40, n = 70, respectively). Of 160 tags, only one tag was not accounted for in live fish, dead fish, or shed tags recovered in tanks.

Experiment 2

We inserted surgically implanted PIT tags into 280 fish in approximately 3 hours. On day 0, survival was 97% (n = 271) and tag retention was 99% (n = 278). A single tagger was responsible for 78% of the mortality on day 0.

Survival on day 49 was 90%. Tag retention of fish still alive on day 49 was 91%. Most of the mortality occurred within 5 d of tagging, while most tag loss occurred between days 9 and 16 (Figure 3). Probability of survival increased with increasing standard length (Figure 4). The predicted standard length for 90% survival was 63 mm, while 60 mm fish had an estimated survival of 86%. Standard length was not related to tag retention (Figure 5). Survival and tag retention stabilized after 40 days. Fish that died after day 30 were emaciated, suggesting PIT tags interfered with digestion.

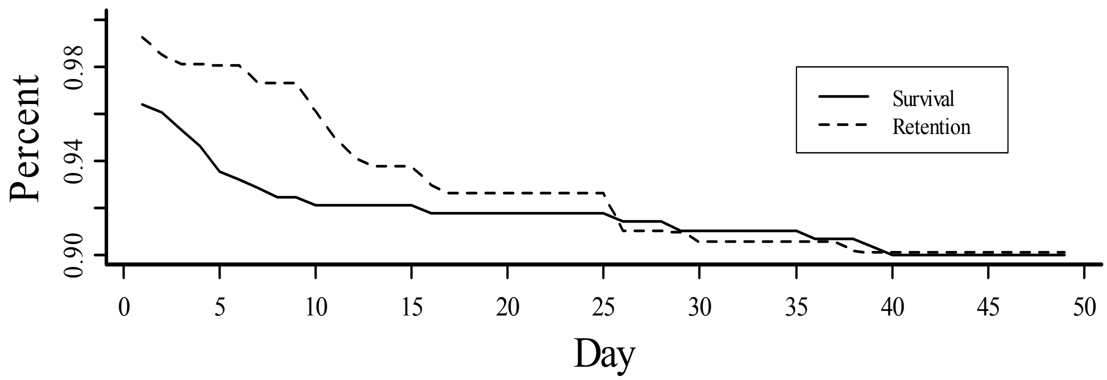

Figure 3-Daily survival and PIT tag retention of Rio Grande silvery minnows tagged via surgical implant, held in aquaria, New Mexico Fish and Wildlife Conservation Office, Albuquerque, New Mexico.

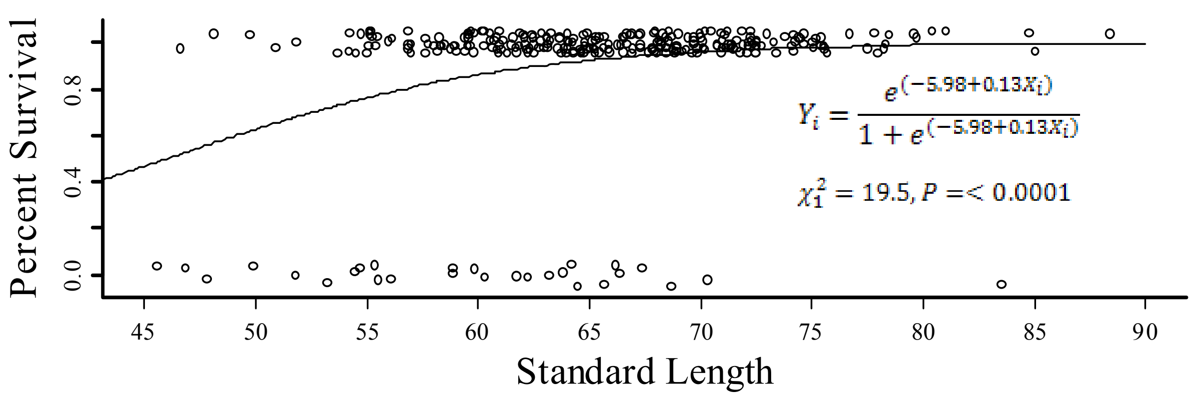

Figure 4-Logistic regression of survival predicted by standard length of Rio Grande silvery minnow PIT tagged by surgical implant, held in aquaria over 49-d, Albuquerque, New Mexico. A small amount of jitter was added to each point to avoid overlap.

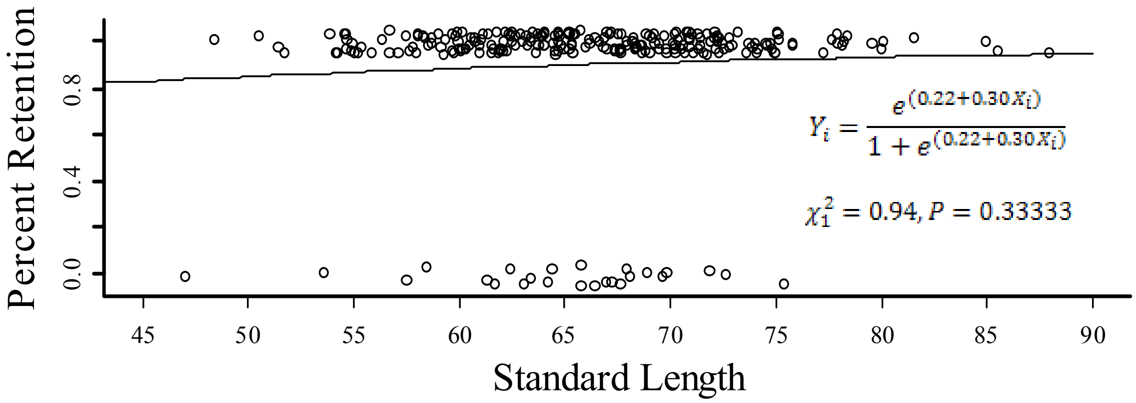

Figure 5-Logistic regression of PIT tag retention predicted by standard length of Rio Grande silvery minnow PIT tagged by surgical implant, held in aquaria over 49-d, Albuquerque, New Mexico. A small amount of jitter was added to each point to avoid overlap.

DISCUSSION

Our results clearly demonstrate surgical implant method for PIT-tagging Rio Grande silvery minnow results in the highest survival. Injection with a hypodermic needle was difficult because of scale size and body type (Page and Burr 1990). Rio Grande silvery minnows have a long, coiled intestine, and, even when food is withheld for 24 h prior to tagging, the abdomen was soft and penetration with a needle was difficult. Immediate mortality of injected fish was high, and post-tagging mortality was greater for injected fish than for surgically implanted fish. Scalpel incisions required less handling time than injection with a hypodermic needle, and penetration depth was more controlled. Scalpel incisions often could be made in less than 30 seconds, where as injections took over 1 minute. Handling during incision procedures was much gentler, due to the relative ease of penetrating the body wall. Although anecdotal, we noted most of the fish that died on day 0 had ruptured gall bladders from needle injections.

Our data are in agreement with several other studies examining surgically implanting PIT tags in small fish (Baras et al 1999, Baras et al. 2000), suggesting that fish greater than 60mm standard length, and possibly smaller, can be tagged with greater than 90% survival and 90% tag retention. PIT-tagging juvenile Nile tilapia *Oreochromis niloticus* reduced survival, and, survival over 49-d, and was 0% for injected fish compared to 83% for surgically implanted fish (Baras et al. 1999). Similarly, surgically implanted Eurasian perch *P. fluviatilis* had higher survival than injected fish (Baras et al. 2000). Our data also suggest experienced taggers can tag fish as small as 52 mm standard length with nearly 90% survival; however, 96% of the fish we tagged were greater than 52 mm standard length.

Additionally, we examined the effects of PIT-tagging in a relatively controlled, sterile, laboratory setting. Immediate release into a stream would likely increase stress and mortality and tag loss. We recommend treatment with antibiotics and holding fish in captivity for a minimum of 6 days to allow wound healing prior to release in the wild, and to accurately record the number of live, marked fish released.

Uniquely tagged animals provide an opportunity to study changes in population demographics as well as movements. Our study does not examine the effects of PIT tagging on physiology or behavior. These effects should be examined on any species before large-scale population analysis is conducted. Fish less than 52 mm standard length may be tagged successfully, but behavior and physiological capabilities may be altered significantly more than for larger fish. Rio Grande silvery minnow may require a year in captivity after hatching to reach 60 mm standard length, an important consideration when planning a long-term, large-scale study of hatchery-reared fish.

Adult Rio Grande silvery minnow can feasibly be PIT-tagged in large numbers via surgical methods, with acceptable levels of mortality and tag retention. Although labor-intensive, surgical implant methods may be applicable to other small-bodied, warm-water fishes.

CONCLUSIONS

This completes Phase I of the original proposal. Minimum criteria set in the original proposal were control fish must have greater than 90% survival, the best treatment survival must be greater than 80%, and tag retention of the best treatment must be greater than 90%. Phase I objectives were met with approximately 90% survival and retention in surgically implanted fishes. We will proceed with Phase II objectives in 2008, which include field-testing the weir antenna, and large-scale tagging and release of up to 10,000 Rio Grande silvery minnow.

ACKNOWLEDGMENTS

We thank the Middle Rio Grande Endangered Species Act Collaborative Program and the Bureau of Reclamation for funding this study, and Manuel Ulibarri and William Knight (Dexter National Fish Hatchery and Technology Center) for providing fish. We also thank Audrey Hopkins and Scott McCutcheon (Biomark) for providing tagging demonstrations and advice. Eric Gonzales and Matt McMillan (SWCA), Evan Anderson and Leeanna Torres (NMFWCO) provided assistance during tagging operations. Ryan Wilson (Utah State University) and William Matter (University of Arizona) provided valuable editorial comments that improved the report.

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