

# Fossil Creek Native Fish Restoration Project

Final Project Report, November, 2005

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The implementation of this project did not come without cost. **Mark Whitney**, Coconino National Forest Fisheries biologist, was driving to Fossil Creek for a meeting when his vehicle inexplicably went off the road and he was killed. Mark had been instrumental in much of the early work with APS leading up to and following their decision to de-commission the Childs-Irving power plants. He was very dedicated to the management of Fossil Creek and took students from Sinagua High School in Flagstaff there for field trips. In April of 2004, we all lost a dedicated biologist and many lost a good and dear friend. The American Fisheries Society, Arizona-New Mexico Chapter recognized Mark's life-long achievements by posthumously recognizing him as Professional of the Year in February 2005. He is well remembered and will be missed.

List of Figures	vi
List of Tables	vi
Project Background	1
Project Introduction:	1
Fossil Creek Water Quality	4
Fish Salvage, Transport and Holding Operation	5
Introduction	5
Methods	5
Results	7
Fossil Creek Renovation	9
Introduction	9
Methods	10
General	10
Reaches 1-2	11
Reaches 3-4	16
Results	18
Fossil Creek Post-Renovation Monitoring	21
Introduction	21
Methods	22
Results	22
Discussion	23
Fossil Creek Watershed Stock Tank Renovations	23
Introduction	23
Methods	25
Coordination	25
Plan Development and Consultation	25
Results	26
Renovation	26
Monitoring	27
Discussion and Recommendations.	27
References	29
Appendix 1. Fossil Creek Renovation First Bioassay	30
Appendix 2. Fossil Creek Renovation Second Bioassay, Controlled Experiment	35
Appendix 3. Fossil Creek Renovation Third Bioassay	41
Appendix 4. Fossil Creek Renovation Fourth Bioassay	44
Appendix 5. Fossil Creek pool volume calculations for reaches 1-4	46
Appendix 6. A Modified bucket system for dispensing chemicals into streams	49
Appendix 7. Priority areas for post-renovation monitoring	51

# **Table of Contents**

# LIST OF FIGURES

Figure 1. Fossil Creek Native Fish Restoration Project Area and location of salvage and	
treatment reaches 1-4. Gila and Yavapai counties, Arizona	3
Figure 2. Fossil Creek fish salvage efforts.	5
Figure 3. Fossil Creek native fish holding facility design. The facility was constructed at Irving	
prior to project implementation	6
Figure 4. Fossil Creek holding facility as constructed at Irving, Az.	7
Figure 5. Drip bucket in operation showing typical stream channel placement	10
Figure 6. Drip station placement and pool locations for renovation of Fossil Creek Gila and	
Yavapai counties, AZ, Reach 1, October 2004	12
Figure 7. Drip station placement and pool locations for renovation of Fossil Creek, Gila and	
Yavapai counties, Az. Reach 2, October 2004.	13
Figure 8. Irving Power plant during drawdown for renovation with Fintrol, October 22, 2004	15
Figure 9. Fossil Creek Native Fish Restoration crews assisting with assembly of drip buckets for	)r
placement along the stream.	Γ7
Figure 10. Fossil Creek Native Fish renovation, neutralization station at downstream end of	
targeted reach, Fossil Creek Fish Barrier	18
Figure 11. Drip station placement and pool locations for renovation of Fossil Creek, Gila and	
Yavapai counties, AZ, Reach 3, November 2004	19
Figure 12. Drip station placement and pool locations for renovation of Fossil Creek, Gila and	
Yavapai counties, AZ, Reach 4, November 2004	20
Figure 13. Fossil Creek Fish Barrier as constructed.	21
Figure 14. Fossil Creek watershed stock tank names and locations treated with rotenone during	
2005	24

# LIST OF TABLES

Table 1. Fossil Creek Water Quality Samples collected August 10, 2004	.4
Table 2. Number of salvaged native fish from Fossil Creek.	.8
Table 3. Fossil Creek Native Fish Restoration holding facility tank dimensions, capacities and	
treatment parameters.	.8
Table 4. Amount of Fintrol used to charge drip buckets for renovation of Fossil Creek reaches 1	-
2, October 2004	14
Table 5. Priority areas within Fossil Creek identified in the Short-Term Monitoring Plan, and the	le
amount of effort completed using netting, snorkeling, and electrofishing sampling methods	5.
, 	22
Table 6. Amount of Prentox® used to treat stock tanks and Boulder Canyon in the Fossil Creek	
drainage	26

#### PROJECT BACKGROUND

Arizona Public Service (APS) owns and operates the Childs and Irving hydroelectric facilities on Fossil Creek through a Special Use Permit from the U.S. Forest Service (USFS). Built in the early 1900s, these facilities utilize stream flow diverted from Fossil Creek to generate hydroelectric power. An important element of the hydroelectric system is the Fossil Springs diversion dam, which captures and directs nearly all of the stream's 43 cubic feet per second (cfs) base flow through a series of flumes, tunnels, pipes and a small impoundment to supply the Childs and Irving power plants.

In December 1992, APS filed an application with the Federal Energy Regulatory Commission (FERC) to relicense the Childs-Irving Hydroelectric Project for 30 years. On August 14, 1997, FERC issued a draft EA on the relicensing proposal and invited public comment. After a period of negotiation with a coalition of groups including American Rivers, The Nature Conservancy, the Yavapai-Apache Tribe, the Northern Arizona Audubon Society, the Sierra Club, and the Center for Biological Diversity, APS signed an Agreement in Principle in 1999 to decommission the facilities and return full flows to Fossil Creek. FERC analyzed the effects related to decommissioning and facility removal in the stream corridor and watershed under the NEPA process and issued a record of decision to decommission the project and return full flows to the stream.

Decommissioning of the Childs-Irving Hydroelectric Project was the driving force behind the schedule for implementing native fish restoration actions in Fossil Creek. If decommissioning occurred according to the terms of the Agreement in Principle, APS would have returned base flows of approximately 43 cfs to Fossil Creek no later than December 31, 2004. Native fish restoration work would need to be completed before full flows were returned to the stream. Once full flows are returned, renovation and any in-stream work would be logistically and economically difficult to accomplish.

In May 2004, a final Environmental Assessment (USBR 2004) was completed by the Bureau of Reclamation (USBR) and Tonto and Coconino National Forests and provided for public review. The NEPA process was completed when the USFS issued its Decision Notice and Finding of no Significant Impact on June 8, 2004. The decision reached was to construct a concrete fish barrier in the Mazatzal Wilderness area and to renovate15 km of stream using Fintrol ®, a commercially produced piscicide. Cooperators working on the logistics, planning and implementation of the Native Fish Restoration Project began meeting in March 2004 in preparation for the completion of the NEPA process that would allow the project to move forward. These cooperating agencies included the Coconino and Tonto National Forests, USFS Southwestern Region, USBR, Arizona Game and Fish Department (Department), US Fish and Wildlife Service (USFWS), Northern Arizona University (NAU) and APS. A mandatory appeal period lasting 45 days, during which no appeals were filed, was followed by a five-day waiting period. Implementation of the Fossil Creek Native Fish Restoration Project was allowed to commence on August 2, 2004.

# PROJECT INTRODUCTION:

The project area is located in Fossil Creek, in the Mazatzal Mountains of central Arizona. Fossil Creek forms the boundary between Yavapai and Gila Counties, as well as the Tonto and Coconino National Forests over most of its course (Figure 1). Fossil Creek is one of Arizona's

rare warm water perennial streams, flowing from a complex of springs, known as Fossil Springs, 23 km through rugged and isolated terrain before entering the Verde River. Fossil Springs produces a constant flow of about 43 cfs (slightly more than 320 gallons per second) at a temperature approximately 21 C, most of which is captured by APS at the 7.5 m tall Fossil Springs diversion dam located 0.5 km downstream of the springs. Base flows below the diversion dam vary between 0.4 to 5 cfs, although episodic flows of much higher magnitude are possible from rainfall, snowmelt or temporary closure of the diversion at the Fossil Springs dam.

Fossil Creek supports existing populations of six species of native fish; Sonoran sucker (*Catostomus insignis*), desert sucker (*Catostomus clarki*), roundtail chub (*Gila robusta*), headwater chub (*Gila nigra*), speckled dace (*Rhinichthys osculus*) and longfin dace (*Agosia chrysogaster*). Attempts to reintroduce other native fish were made, including Gila topminnow (*Poeciliopsis occidentalis*) and razorback sucker (*Xyrauchen texanus*). Neither species is known to occur in the stream at present, although razorback sucker survived at Fossil Springs for several years after stocking, and were still present in Stehr Lake (the regulating impoundment between Irving and Child's) prior to renovation.

Nonnative green sunfish (*Lepomis cyanellus*) invaded Fossil Creek or were illegally released into the stream. They were thought to be present from the confluence of the Verde River upstream to the base of the Fossil Springs Diversion Dam. However, surveys conducted prior to renovation, and a review of historic collection data failed to confirm their presence above a small boulder waterfall (hereafter called the "sunfish barrier") located 0.8 km below the diversion dam. Nonnative smallmouth bass (*Micropterus dolomieu*) invaded Fossil Creek and were present from the confluence upstream to a large waterfall acting as a fish barrier located about 5 km below the Fossil Springs Diversion Dam. There are records of yellow bullhead (*Ameiurus natalis*) downstream of Sally May Wash (AGFD Data), plus several other nonnative fishes that enter lower Fossil Creek from Verde River.

The project area was divided into four reaches (Figure 1). Reach 1 began at a short boulder waterfall ("sunfish barrier") below the diversion dam and went downstream to a large waterfall. Reach 2 went from that waterfall downstream to a large waterfall at Irving. Reach 3 went form that waterfall down to the Sally Mae Wash confluence. Reach 4 went from the Sally Mae confluence downstream to the newly constructed fish barrier. Salvage and renovation was implemented in two phases. Phase 1 involved salvage and renovation of Reaches 1 and 2 and the Irving power plant, tailrace and fore bay canal and was conducted October 11-22, 2004. Phase two involved salvage and renovation of Reaches 3-4 and was conducted November 3-13, 2004.

This project consisted of multiple activities: Fish salvage, fish holding and repatriation, stream renovation, fish barrier construction and watershed stock tank renovations. Fish salvage and restocking operations were done in coordination and in conjunction with USFWS and a larger research project being conducted by NAU through separate funding. Brief information on methods and results from those efforts are provided. The Department, utilizing resources provided directly by USBR and via a grant from the FWS, implemented holding facility construction and operation. The Department coordinated the stream renovation with planning and implementation assistance provided by all the aforementioned partners. The USBR conducted all activities related to the fish barrier design and construction, details of which will

not be included in this report. Four bioassays were conducted prior to full-scale renovation to adjust and perfect the application rate of Antimycin, the active ingredient found in Fintrol®. Three were done instream and one was conducted in tanks with captive fish (Appendices 1-4).

Figure 1. Fossil Creek Native Fish Restoration Project Area and location of salvage and treatment reaches 1-4. Gila and Yavapai counties, Arizona.



#### FOSSIL CREEK WATER QUALITY

Fossil Creek is unique among southwestern streams. Fossil Creek is a travertine system – the spring water travels through limestone deposits and when it reaches the surface, it is highly charged with calcium carbonate and dissolved CO2. As the water progresses downstream, the  $CO_2$  degasses and calcium carbonate precipitates out. The result is a cacading, step pool habitat that provides extensive surface area for macroinvertebrate colonization and rearing areas for small fish.

Diversion of most of the water at the Fossil Springs Dam has resulted in the loss of travertine formation in the upper parts of the creek and shifted that formation to two areas downstream (Overby and Neary 1996, Malusa 1997). The first is at the outflow of the Irving power plant, where about 5-7 cfs of flow is returned to the creek. The second is at the Childs power plant outflow to the Verde River, where the remaining 35-38 cfs of flow is ultimately released.

Several Fossil Creek water quality parameters change drastically along the project reach as a result of flow manipulations (Table 1.) Water quality parameters of special note are iron concentration, total suspended solids and turbidity. The elevated iron concentration discovered in the area just upstream from the Irving power plant appeared to affect the toxicity of antimycin to green sunfish, evidenced by the results of the third bioassay, Appendix 3. The iron concentration became diluted at Irving where some diverted Fossil Spring flow is returned to the stream. Turbidity in this area was also found to be higher than anywhere else in the system, likely due to influences of the spring inflow and run-off and sedimentation from the road and Irving Trailhead parking lot located in the vicinity.

Parameter	analysis date	upper diversion dam 1535 hrs.	above sunfish barrier 930 hrs.	above Irving plant 1148 hrs.	below Irving 1217 hrs.	Sally Mae confluence
Temp. C	In field	21.7	19.7	23.23	22.55	24.13
pH	In field	7.02	7.63	8.08	7.89	8.23
Cond. (umhos)	In field	740	617.7	563.5	712.2	523
D.O. (mg/l)	In field	7.52	5.9	7.39	7.97	7.93
TDS (mg/l)	In field	474.1	395.4	320.6	453.8	334.8
Redox (mv)	In field	326	306	322	175	292
% DO sat.	In field	90.3	68.7	91.3	97.2	99.5
Turbidity (ntu)	In field	13.5	1.4	45.6	1.1	15.1
Iron (ug/l)	8/23/2004	<100	98	519	<100	222
alkalinity-total	8/19/2004	383	310	296	367	246
Total sus. solids (mg/l)	8/12/2004	<3.0	<3.0	12.6	<3.0	20.8
Total diss. solids (mg/l)	8/12/2004	414	358	342	402	282
Kjeldahl (mg/l)	8/19/2004	<0.3				
Total phosphorus (mg/l)	8/30/2004	0.02	<.02	0.03	<.02	0.025
BOD	8/15/2004			<2.0		
TOC (mg/l)	8/19/2004	N/A	<0.5	2.1	N/A	<0.5

Table 1. Fossil Creek Water Quality Samples collected August 10, 2004.

# FISH SALVAGE, TRANSPORT AND HOLDING OPERATION

#### INTRODUCTION

On September 18, 2004 an effort was organized using volunteer anglers from the Red Rock Flycasters and Northern Arizona Flycasters to help capture specimens of roundtail chub for holding during the renovation. More than a dozen anglers, Department and USFWS personnel spent the day capturing and transporting chubs to the holding facility constructed at Irving. On September 19, a severe thunderstorm hit the Fossil Creek area, causing a landslide that collapsed a short section of the flume carrying water to Irving. Fossil Creek flooded as a result of the flume collapse and additional run-off from the storm, forcing the postponement of additional salvage operations and the renovation scheduled to begin the following week. Collapse of the flume resulted in the loss of water supply to the holding facility. The captive roundtail chub were returned to the creek so they wouldn't perish in captivity with loss of flow to the raceways. Salvage and renovation of Reaches 1-2 was rescheduled for October 11-22, 2004 following expected repair of the damaged flume.

#### METHODS

The holding facility consisted of twelve circular raceways, independently plumbed and provided with available auxiliary oxygen supply, if needed (Figure 2). A continuous flow of water was provided via a 2-inch high pressure (~240 psi) line fed directly from the penstock of the Irving hydroelectric turbine. Water pressure was lowered via a pressure reduction valve to about 80 psi and plumbed through 1.5 inch PVC pipes to each raceway. Outflow from the raceways was directed via 2-inch lines to a dirt holding basin or to the creek.





The fish salvage was coordinated and implemented by staff from the USFWS Flagstaff Ecological Services and Fisheries Resource offices in conjunction with Department biologists, researchers, students from Northern Arizona University and other volunteers. Fish were captured using backpack electrofishers. baited hoop nets, trammel nets, seines and minnow traps during October 11-15. Fish intended for salvage were held in live cars placed along the stream course

and were transported to the holding facility on October 15 by helicopter long line in 55 gallon drums. Nonnative fishes captured during salvage efforts were returned to the stream for later post-renovation enumeration by NAU researchers conducting separate, but related, research.

Both Smith-Root Model 12 and Model LR-24 backpack electrofishing units were used in shallow riffle and run habitats to capture fish. The primary goal of the salvage efforts was to remove as many native fishes as possible, settings and net sizes and netting efforts varied widely

during collection by multiple crews and were not recorded. Minnow traps were collapsible mesh design and their deployment was in habitats targeted for presence of small-bodied native fishes.



Figure 3. Fossil Creek native fish holding facility design. The facility was constructed at Irving prior to project implementation.



Figure 4. Fossil Creek holding facility as constructed at Irving, Az during Fall 2004.

Transportation of captured fishes from the stream to the holding facility and back following renovation was accomplished in a variety of ways. For remote areas inaccessible by vehicle, a helicopter was employed to carry a 208 L drum on a long line. The drum contained a battery-powered aerator for providing supplemental air while fish were in transit. Fish carried inside the drum were segregated by size into two separate mesh bags. Upon setting of the drum on the ground the bags were removed from the drums and placed in a tank mounted in the bed of a pickup and driven several hundred yards to the holding raceways. In accessible areas, biologists carried fish to hatchery trucks in 19 L buckets and drove them to the holding facility.

#### RESULTS

Salvage operations were conducted in two phases. First phase was done October 11-15, 2004 in Reaches 1-2 prior to renovation. The second salvage operation occurred November 3-8, 2004 in Reaches 3-4 prior to renovation. NAU researchers counted fish captured during salvage efforts (Table 2) as part of a separately funded research study. The data provided are courtesy of Allen Haden at NAU. Care was taken in handling of fish from Reaches 1 and 2 to segregate them from each other. Segregation was done with the intent of preserving any genetic differences that may be present in fish residing above the waterfall that formed the barrier between the two reaches.

Taxa	Reach 1-2	Reach 3-4	Total
Roundtail/headwater chub	174	103	277
Desert sucker	344	49	393
Sonoran sucker	44	204	248
Speckled dace	986	0	986
Longfin dace	13	0	13

Table 2. Number of salvaged native fish from Fossil Creek.

Fish salvaged from Reach 1-2 were transported to the holding facility on October 15, 2004 and held until re-stocking occurred on October 29. Weather events forced re-stocking to be delayed for several days, as flooding in the creek prohibited the helicopter from flying and crews from accessing the more remote areas of the creek. Fish salvaged from Reach 3-4 were transported by truck from holding pens in the stream to the facility periodically throughout the salvage period. Additionally, fish were flown by helicopter from the wilderness area on November 8 to the facility. They were held in captivity until November 17, when they were returned to two different pools located in Reach 3. These pools were easily accessible by road and fish were transported by vehicle. A film crew from National Geographic attended both re-stocking events and is producing a documentary chronicling the decommissioning of the hydroelectric plants.

While held in captivity, fish were monitored for health and well-being. Department hatchery staff periodically treated captive fishes with saline or formalin bath as needed to address parasite load and stress, according to treatment parameters identified in Table 3. Mortality in captivity for most species was minimal, and limited to only a few individuals. Mortality on speckled dace was slightly higher, when approximately 80 perished, apparently as a result of stress. Actual numbers of fish repatriated to the stream were not recorded, but are accurately estimated to be at least 90% of salvage numbers that are reported here.

Tank Id.	Standpipe Ht. (in.)	Volume (ft <sup>3</sup> )	capacity (gal)	carrying capacity (lbs)	1% NaCl bath 15 min (lbs.)	100 ppm 60 min Formalin bath (ml)
A1	19.5	45.9	344	99	29	313
A2	21.0	49.5	370	107	31	141
A3	24.5	57.7	432	124	36	163
B1	16.0	37.7	282	81	24	107
B2	21.5	50.6	378	109	32	144
B3	23.0	54.2	405	117	34	154
C1	17.5	41.2	308	89	26	117
C2	20.5	48.3	361	104	30	137
C3	22.5	53.0	396	114	33	150
D1	19.5	45.9	343	99	29	130
D2	22.5	53.0	396	114	33	150
D3	23.0	54.2	405	117	34	154

Table 3. Fossil Creek Native Fish Restoration holding facility tank dimensions, capacities and treatment parameters.

#### FOSSIL CREEK RENOVATION

#### INTRODUCTION

The cooperating agencies and groups renovated Fossil Creek, below Fossil Springs diversion dam, to restore aquatic habitat for rare native fishes, and to provide future suitable habitat for introduction of threatened and endangered species. Green sunfish and smallmouth bass invasion into upstream areas has been negatively impacting the ability of natives to maintain suitable population levels. Few age 0-1 fish are present in areas occupied by smallmouth bass, and are only present in areas occupied by green sunfish as a result of downstream migration from unimpacted upstream areas.

Our initial plan was to begin the chemical renovation at the base of the Fossil Springs diversion dam and treat downstream to the constructed fish barrier. However, during pre-treatment fish surveys and review of previous data, we determined that non-native fish were not present above the sunfish barrier located about 0.8 km below the diversion dam. Healthy populations of native fishes, including young of year and small-bodied individuals were present in that 0.8 km distance. Thus, the area identified for renovation was altered to begin at the sunfish barrier.

Green sunfish were documented from the confluence of the Verde River, 22.5 km downstream, upstream to the sunfish barrier. Smallmouth bass had only been documented from the confluence upstream to a high waterfall located near the Irving power plant. They had not been detected upstream of this point until one small young of year smallmouth bass was captured during salvage operations in Reach 1. Additionally, we suspected that yellow bullhead were also present from the Verde confluence upstream to that same waterfall. Renovation planning and discussion involved much concern about our ability to affect yellow bullhead with the chosen piscicide, Fintrol.

Fintrol was selected as the piscicide for several of its qualities. First, it works at a much lower concentration 10-25 parts per billion (ppb) than the alternative, rotenone, which is generally applied at 1 part per million (ppm). Second, rotenone is ineffective on eggs, whereas antimycin is toxic to eggs and all life stages of fish and the toxic action is irreversible (Berger et al. 1969). Finally, rotenone has been shown to cause avoidance reactions in fish, whereas antimycin did not (Dawson et al. 1998), an undesirable trait when dealing with complex stream habitats. The complexity of habitats found within the target reach of Fossil Creek would likely provide numerous opportunities for fish to avoid rotenone. Two formulations of antimycin were available for use in the project: Fintrol-concentrate, and Fintrol-15. Fintrol-concentrate is a liquid that was applied to the stream from controlled-release dripstations. Fintrol-15 is a sand formulation that slowly releases the active ingredient as it sinks through the water column.

Concern regarding the applicability of Fintrol to remove yellow bullheads, efficacy of Fintrol in the alkaline and hard waters typical of Fossil Creek, and recommendations from reviews of previous treatments (Dawson and Kolar 2003) led to implementation of several bioassays. Results from the first bioassay (Appendix 1) were not positive, so controlled experiments were conducted in captive tanks, which also addressed yellow bullhead (Appendix 2). These results were more positive and provided some additional insights and guidance, so a third bioassay was conducted in the stream. Results from this bioassay again were not positive (Appendix 3).

Through conversation with the product manufacturer (Nick Romeo pers. comm.) we discovered that high dissolved iron concentrations present within the bioassay area might have negatively affected the toxicity of Fintrol. A fourth and final successful bioassay was conducted (Appendix 4) to confirm application parameters under predicted natural conditions and expected application protocol. Based on the results of this activity, the renovation team decided that an application rate of 50 ppb Fintrol (increased to 100 ppb in the geographic area encompassing the spring inflow) would provide the best chance for success while minimizing total amount of Fintrol needed.

## METHODS

#### <u>General</u>

In July and September, Reaches 1-2 and 3-4 (respectively) were measured and mapped by walking each reach with a hip chain in a downstream direction and flagging points every 150 m to determine drip station placement. Each drip station was shifted upstream or downstream to the nearest suitable single channel with good flow, or if it fell within a large pool. Distance measurement began again from that point. UTM's of bucket locations were recorded with a Garmin 12 GPS, with point localities averaged to get about a +/- 15-foot accuracy. Bucket points and heads of each pool measured were then mapped using Arcview 3.2 (Figures 3-6). Drip station placement is recommended at approximately 150 m intervals to re-charge stream



concentration as dilution, biodegradation and oxidation of Antimycin occurs during travel downstream.

Figure 5. Drip bucket in operation showing typical stream channel placement.

All major pools ( $\geq 1$  M depth) in Fossil Creek treatment reaches were measured to determine application amount of Fintrol-15 to instantaneously establish the target concentration of Antimycin. Fintrol-15 is an Antimycin coated sand grain designed to treat standing

bodies of water or stream pools of great depth and volume. The sand grain is coated with antimycin and then covered with gelatin. The coating and active ingredient are dissolved into the water column as the sand grain sinks. Antimycin would be dissolved in the water column as it sinks, up to a depth of 3m. Length and three width measurements of each pool were taken to the nearest foot using a laser range finder, with the 3 width measurements averaged. Depth down the middle of the pool (following thalweg as close as possible) was measured at a minimum of 3 points using a remote transducer and handheld depth receiver (Humminbird Smartcast® with accuracy +/- 0.3m. Maximum depth for each pool was also found and recorded using this instrument. The head of each pool was located and marked using a Garmin 12 GPS. Each pool

was marked in the field by flagging placed on a nearby branch. Flagging was labeled with pool number. Pool location, measurements, volume calculations and application rate for Fintrol 15 for all pools in reaches 1-2 and reaches 3-4 were entered into a spreadsheet (Appendix 5) and printed for use by field crews during application.

Application of Fintrol-concentrate to the stream was accomplished using 19 L buckets modified to flow at a constant rate. A 19 L bucket design modified slightly from Stefferud and Propst (1996) was used. The modifications are described in Appendix 6. Expected discharge rates were used to calculate amount of Fintrol needed to charge each bucket for a 4-hour exposure period. All field crews were sent into the field with pre-measured individual containers of Fintrol for charging each of their buckets based on flow measurements made the previous evening. Backpack sprayers were used during this renovation project to apply Fintrol-concentrate to margins, backwaters and other slow-moving stream habitats. Backpack sprayers were also recharged with prepackaged containers of Fintrol-concentrate.

Fintrol-15 sand was applied directly to deep stream pools, stream margins, densely vegetated areas, and backwaters by hand. Previous measurements of pool volume were used to determine how much sand formulation was needed for any particular pool.

Detoxification of Fintrol was accomplished by applying a 2.5% solution of potassium permanganate in Reaches 1-2 and 20% solution of liquid sodium permanganate in reach 4. Application occurred at 3 ppm to allow for 1ppm natural environmental oxidation, 1ppm for neutralization of the Fintrol and 1ppm for residual travel downstream from the end of the targeted reach. Application of the permanganate was accomplished using a 113L closed head drum for dispensing. The drum had been modified similar to treatment buckets (Appendix 6) but with larger size brass fittings to allow for increased dispensing rate.

Liquid sodium permanganate was used as the oxidizing agent in Reach 4 instead of powdered potassium permanganate because of several preferred qualities. First, it was available as a liquid that could easily be transported by helicopter to the barrier site and eliminated the need to measure powder and get it into a solution. It was easily dispensed in its concentrated form from the previously described 113L closed-head drum. Secondly, it was available as a 20% permanganate solution that is 8 times more concentrated than the recommended application of a 2.5 % solution of powdered potassium permanganate. Thus a filled permanganate drum would last about 6 hours and could be re-filled by a single person adding fresh permanganate from the 5 gallon containers in which it was shipped. According to the manufacturer and Arizona Game and Fish Department chemist (Mel Underwood pers. comm.) there would be no difference in environmental effects between sodium and potassium permanganate. Furthermore, sodium as the spectator ion in the chemical oxidation process is more common in natural stream settings than is potassium.

# Reaches 1-2

Renovation of Fossil Creek reaches 1-2 commenced on October 18, 2004 with the arrival of the 30+ person field crew. A base camp was established in a large clearing, where communal kitchen, cooks and facilities were available to the fish salvage crew the previous week and renovation crews for this week. A safety briefing outlining the effects of Fintrol and its possible

# Fossil Creek Native Fish Restoration Project November 2005

exposure routes and an explanation of required personal protective equipment was held that evening. Equipment was handed out to participants and a general geographic briefing was provided. Crews received their assignments and were prepared to begin application. Fintrol toxicant kits were opened, mixed and pre-packaged in nalgene bottles for distribution to field crews the following day. In addition, Fintrol 15 sand was emptied from its storage cans and placed in double ziplock bags for ease of transport in backpacks the following day.



Figure 6. Drip station placement and pool locations for renovation of Fossil Creek Gila and Yavapai counties, AZ, Reach 1, October 2004.

Stream discharge was measured that afternoon using a meter tape and Marsh-McBirney flow meter mounted on a stadia rod. A single channel with laminar flow was located and width measured. Ten depths and velocities at 0.6 of each depth were measured and recorded equidistant across the channel. Flow discharge within each of the ten cells was calculated and the results summed to provide a discharge measurement of 0.5 cfs.

Six crews were assigned anywhere from 5-7 buckets to charge and monitor drip rates. The buckets were charged beginning at the upstream end of the reach continuing downstream. Discharge measurement was used to calculate charging amount for each bucket, which was 102 ml for a 50ppb target concentration. Buckets were charged according to Table 4, with some

buckets located in side-channel habitats receiving partial charges. At the recommendation of the Fintrol manufacturer, additional surfactant, Non-oxynal 9, was added at a ratio of 65ml/480 ml Fintrol. Thus each crew was given a 125 ml nalgene container with 102 ml of Fintrol and 14 ml surfactant for a fully charged bucket, less for partial charging.



Figure 7. Drip station placement and pool locations for renovation of Fossil Creek, Gila and Yavapai counties, AZ, Reach 2, October 2004.

The first backpack spraying crew began application of antimycin to isolated water bodies, connected backwaters and vegetated stream margins concurrently with the sand and bucket charging crews. Sprayer crews consisted of two people carrying sprayers and an additional person carrying supplies for the day (water, pre-packaged Fintrol containers, lunches, first aid kit, etc.). Backpack sprayers each worked one side of the creek as they traveled in a downstream direction while the third person scouted around for off-channel or hidden aquatic habitats. While maintaining verbal communication with each other, the crew was able to efficiently and effectively treat habitats that might have otherwise been missed by main channel applications. A second sprayer crew repeated the same procedure, beginning two hours after the first crew began. Backpack spraying crews were provided with nalgene bottles containing 100 ml of Fintrol and 14 ml of additional surfactant. Each filling of the 15 L backpack sprayer with water and pre-

measured Fintrol would treat 7000 cubic feet of water to 50 ppb. Crews were instructed to visually estimate water volumes encountered and make application to the stream accordingly.

Reach 1-2 Fintrol Application Table for 0.5 cfs					
Bucket #	Dosage (ppb)	Fintrol (ml)	Additional Surfactant (ml)		
1 to 15	50	102	14		
16 (side channel)	10	20	3		
17 to 19	50	102	14		
20 (side channel)	10	20	3		
21 to 31	50	102	14		
32 to 37 (area of high Iron)	100	203	28		

Table 4. Amount of Fintrol used to charge drip buckets for renovation of Fossil Creek reaches 1-2, October 2004.

The first application of the fish toxicant was completed on the afternoon of October 19. A total of 8.77 units (1 unit=480 ml) or 4210 ml of Fintrol was mixed and applied to the stream by drip bucket over a four-hour exposure period. Application crews reported bucket drip-rate performance ranging from excellent to poor. When the Fintrol was prepared the previous evening, the Fintrol concentrate contained particles that failed to dissolve completely when mixed with the diluent and additional Non-oxynal 9. The drip-rate variability seemed to result from the undissolved soy lipids blocking the valve opening. Each of the 2 spraying crews was provided with 25-100 ml bottles of Fintrol/surfactant for application. Backpack sprayers applied 10.4 units of Fintrol.

On October 20, the stream application process was repeated. On the second day of application, 10.4 units (4992 ml) of Fintrol were mixed and applied to the stream by drip bucket. When the Fintrol was mixed for day 2 applications, the formulation underwent severe agitation, followed by decanting of the liquid for application. Most solids were retained and disposed. This accounted for the additional units of Fintrol used on the second day of application. This was done in recognition of the problems experienced the previous day with clogging of the drip buckets. The procedure seemed effective, because bucket crews reported better drip-rate performance on this day. Each of the 2 spraying crews was again provided with twenty-five 100 ml bottles of Fintrol for application. Another 10.4 units (4992 ml) of Fintrol was applied by backpack sprayer.

Concurrently with the first bucket crew, the Fintrol 15 sand application crew, comprised of two applicators and an assistant, began applying sand to pools, beginning upstream and moving downstream. As the sand application crews arrived at the first bucket for each consecutive crew downstream, that bucket crew began charging their set of buckets. Sand was applied to each pool according to measurements and calculations provided in Appendix 5. Additional amounts were added to smaller pools previously identified but not measured. A total of 24 kg of Fintrol 15 antimycin coated sand was applied to pools in Reaches 1-2 on each of the two treatment days.

During late afternoon of October 20, live fish of various species were still seen in two different areas of the treated reach. Fish were observed freely swimming just below the waterfall boundary between Reach 1 and 2 in Pool 22 and also lower down in Reach 2 in Pool 30.



Figure 8. Irving Power plant during drawdown for renovation with Fintrol, October 22, 2004.

On October 21, both of these areas were treated a third time with sand and liquid antimycin. Drip buckets were established above large pools and the pools were treated according to the prescribed amount with sand. A total of 2.7 kg of additional sand and one unit (480 ml) of Fintrol was applied in this effort.

On October 22, APS shut off the water from the flume to the Irving power plant in order to allow us to effectively treat the canal diversion and forebay of the power plant. The water was returned to the creek via a sand trap located on the hill above the power plant. The Irving diversion dam was blocked using sandbags and plastic sheeting to keep the returned stream flow from re-entering the forebay. The Irving

diversion canal and forebay were treated with one unit of Fintrol applied by backpack sprayers. In addition, as flows into the flume below Irving were descending during the shutdown, Fintrol was applied to treat the water held there and remove any fishes that may be able to re-enter the forebay upon re-watering. Fintrol use was calculated for an average of 20 cfs over a one-hour period and was applied at a decreasing rate of application over the hour. Two units of Fintrol (960 ml) were used for this activity.

For this phase of the renovation project, a neutralization station was established just below the Irving power plant at the top of a large waterfall that formed the boundary between Reach 2 and Reach 3. Powdered potassium permanganate was pre-measured into ziplock bags at 454 g per bag and stored near the neutralization station. Application of the permanganate was accomplished by dissolving 454 g of permanganate in a 19 L bucket (a 2.5% solution) using a cordless drill and mixing attachment to improve dissolving the powder. The solution was then transferred to the 113 L closed head drum for dispensing. The process was then repeated until the drum contained 95 L of solution. The 2.5% solution was dispensed at a rate of 105 ml/min

according to the formula X=Y(70F) where: X= desired flow in ml/min, F= flow in cfs and Y= desired concentration in ppm. The neutralization station was begun when the sand application crew reached the bottom of the target reach at 1300 hours on October 19 and was maintained until 12 hours after the final application of piscicide to the stream, October 22 noon.

Green sunfish were collected from the target reach during salvage efforts the prior week and held in live cars until the treatment began. These fish were then transported to live cars placed 200 meters below the neutralization station to monitor and confirm effective neutralization of the toxicant. In addition to operation of the neutralization station, a return of about 6 cfs of water by the Irving power plant to Fossil Creek 100 meters below the waterfall further served to dilute the instream concentration of antimycin. Survival of sentinel fish below the target reach confirmed that toxicant effects did not extend beyond the area intended.

#### Reaches 3-4

Renovation of Reach 3 and 4 occurred from November 8-12, 2004. A base camp similar to phase one was established the week prior for salvage operations. Drip buckets were assembled, delivered to the stream and pre-filled with stream water the day prior to arrival of main crew. One section of the APS flume carrying water to the Childs Plant was found to have a low section that allowed overflow to run down the hill and into the stream. This section was sand-bagged to reduce the water return to the stream. Renovation crews arrived for safety briefing and orientation on November 8. The application of Fintrol and Fintrol-15 sand was accomplished in a manner similar to that described for renovation of Reach 1-2. There were 36 drip buckets in Reach 3 (Figure 5) and 36 drip buckets in Reach 4 (Figure 6). During application in Reach 3, six 2-man crews were each assigned 6 buckets to charge and maintain drip rates. Due to access complexities in Reach 4, one crew was sent downstream to do the initial charging of all buckets, followed by a crew of 2 people each hour to check and adjust (if necessary) dispensing rates on the buckets.

Fossil Creek discharge was measured at the Sally May confluence as previously described for Reach 1-2 on the evening of November 8. Discharged was calculated to be 12 cfs, nearly double the discharge that was expected based on historic flow conditions. The increased discharge was the result of recent rainfall on the watershed and resulted in a need to double the amount of Fintrol used to charge drip buckets. The planning team believed that the increased run-off would result in dilution of the dissolved iron in the water and thus alleviate concerns over affects of iron concentration. Each drip bucket was charged with 2440 ml of Fintrol for the first day of application. In addition, at the manufacturer's recommendation (Nick Romeo pers. comm.) additional surfactant (65 ml/480 ml Fintrol) and acetone (130 ml acetone/480 ml Fintrol) was added to the formulation during the mixing process. This resulted in each full drip bucket being charged with 3430 ml of formulation.

On the morning of November 9, discharge was measured again and found to be nearly 17 cfs discharge. The formulation was prepared and placed in 4 L nalgene bottles on the evening of November 8 for transportation and distribution by the field crews at an expected discharge of 12 cfs. It was too late to adjust the quantities in the nalgene bottles to account for the increased discharge. This resulted in a first treatment of Reach 3 at 35 ppb Fintrol. Backpack spraying and sand application to pools was again conducted as previously described. Reach 3 was divided into

sub reaches, A and B and two backpack spraying crews worked through the complex habitats of each sub-reach. Concurrently, one sand crew worked through the entire reach to apply Fintrol 15 sand to the previously measured pools.

During preparation of the Fintrol formulation the evening of November 8, complications arose again with an inability to get soy lipids to dissolve. Cold night time temperatures (~50 degrees F) and cool days, coupled with the large volume of Fintrol being used prevented lipids from dissolving, even in the presence of the additional surfactant and acetone. As a result, drip bucket performance was found to be very poor during the first treatment, most likely due to congealed soy lipids clogging the outflow valve of the buckets. In an attempt to alleviate this problem, crews were later instructed to only charge each bucket with ½ the provided Fintrol formulation, and set the drip rate to 160 ml/min so the buckets would drain in two hours, followed by refilling with the second half of formulation and drip rates set to drain in 2 hours. Working in this fashion decreased the amount of Fintrol formulation in each 19 L bucket in half and improved the ability to dissolve the soy lipids and improve flow rates.

On November 10, Reach 4 was treated for the first time. The formulation was prepared and placed in 4 L nalgene bottles on the evening of November 9 for transportation and distribution by



the field crews at an expected discharge of 12 cfs. However, discharge measurements made on the morning of November 10 showed discharge to be at 11 cfs. Again it was too late to modify the 4 L containers that the crews were using to transport the formulation into the field to the drip buckets, so the stream was treated with the same amount of Fintrol, 2440 ml. This resulted in a first treatment of Reach 4 at 54 ppb Fintrol. We again conducted backpack spraying and sand application to pools as previously described.

Figure 9. Fossil Creek Native Fish Restoration crews assisting with assembly of drip buckets for placement along the stream.

On the evening of November 10, discharge was again measured and found to be at 11.5 cfs. Fintrol was prepared and packaged with the expectation that discharge would again be near 12 cfs on the following day. Reach 3 was treated a second time with 2440 ml of Fintrol, plus additional surfactant and acetone on November 11. Backpack sprayers and sand were applied to appropriate habitats concurrently with drip bucket operation. During application, all crews were instructed to vigilantly search for live fish while hiking the creek and report any observations. On the morning of November 11, discharge was measured to be 11.2 cfs, resulting in an application concentration of 54 ppb.

Reach 4 was treated a second time on November 12. Each 4 L Nalgene bottle was filled with 2236 ml Fintrol plus appropriate amount of acetone and surfactant to reach a target concentration of 50 ppb at discharge of 11 cfs. Backpack spraying and sanding of the pools was accomplished again.

A neutralization station was established at the downstream end of Reach 4, at the newly constructed fish barrier. Liquid sodium permanganate was used as the oxidizing agent instead of powdered potassium permanganate because of previously stated properties. This allowed the two-man crew operating the neutralization station to apply the permanganate at a reduced rate. If 2.5% permanganate had been used, flow from the drum would have to be 2520 ml/min at the measured stream discharge of 12 cfs. This would have effectively drained the drum in 45 minutes and required around the clock mixing and re-filling the drum. Using the liquid permanganate allowed for a dispensing rate of 289 ml/min, the rate at which it was actually applied, to reach a concentration of 3 ppm permanganate in the stream at a discharge of 11 cfs.

The neutralization began on the afternoon of November 10 when the first Reach 4 treatment crew reached the barrier/neutralization site. It was not begun a day earlier when Reach 3 was treated because the neutralizing station was 3 miles below the last Reach 3 application point and



natural oxidation and travel time in the effects stream would prevent anv downstream of the target reach. Application of the permanganate was continued through noon of November 13, when it was discontinued. The neutralization crew, during the course of application, periodically hiked downstream to observe resident fishes for effectiveness of the neutralization process. There were no observable effects to fish below the neutralization station by either the piscicide or the permanganate application. One mitigating factor that helped prevent downstream effects was the onset of a severe rainfall event on the afternoon of November 12. Heavy rainfall throughout the night served to increase discharge in the creek well above anything measured during the week, likely to or above 20 cfs. The increased flow diluted any residual Fintrol in the stream.

Figure 10. Fossil Creek Native Fish renovation, neutralization station at downstream end of targeted reach, Fossil Creek Fish Barrier.

#### RESULTS

Fossil Creek Reach 1 and 2 were renovated at 0.5 cfs with 44 units of Fintrol (21.12 L) and 50.8 kg of Fintrol-15 antimycin coated sand. It took a field crew of approximately 30 people to

conduct the first phase of renovation that covered approximately 4.6 km of stream. Reaches 3-4 were treated with 750 units of Fintrol (360 L) and 136.5 kg of Fintrol-15 sand at a discharge ranging from 11-17 cfs. This phase of renovation involved about 40 people and covered 9.3 km. Personnel numbers do not include camp support staff, others that helped in salvage operations or Hatchery personnel involved in care of fish temporarily held in captivity.

Neutralization of the piscicide at the downstream end of the targeted reach involved application of 605 L of 20% liquid sodium permanganate. Sodium permanganate was applied from November 10 at 1200 hours until 1700 hours on November 11, then again from November 12 at 1200 hours until November 13 at 1200 hours.



Figure 11. Drip station placement and pool locations for renovation of Fossil Creek, Gila and Yavapai counties, AZ, Reach 3, November 2004.



Figure 12. Drip station placement and pool locations for renovation of Fossil Creek, Gila and Yavapai counties, AZ, Reach 4, November 2004.



Figure 13. Fossil Creek Fish Barrier as constructed.

In spite of several unexpected weather events, complicating environmental factors and unanticipated piscicide formulation problems, the Fossil Creek Native Fish Restoration Project was completed on November 17, 2004 with the repatriation of fish to Reaches 3-4. The total number of fish salvaged from the stream during the course of this operation was previously provided in Table 2. Observations made by field application crews during treatment of all Reaches resulted in no reports of live fish seen anywhere. In addition, follow-up electrofishing monitoring effort in Reaches 1-2 and hoopnetting in Reaches 3-4 failed to turn up any of the targeted nonnative species of green sunfish or smallmouth bass (Reid 2004).

# FOSSIL CREEK POST-RENOVATION MONITORING

#### INTRODUCTION

A meeting on February 23, 2005 was held at the Coconino National Forest Supervisor's Office to discuss monitoring efforts in Fossil Creek subsequent to fall 2004 renovations. At this meeting, revisions were discussed on an earlier draft monitoring proposal resulting from a December 2004 conference call between the Fossil Creek fish restoration participants. Due to difficult terrain, need to reduce additional stress on remaining native fishes, and a finite amount of time before the return of full flows (June 18, 2005), the meeting participants identified 8 priority areas (Table 5) in which to focus monitoring efforts. Priority areas were identified based on proximity to human disturbance, areas that previously contained high numbers of nonnative fishes, and habitats that were difficult to chemically renovate due to water depth or habitat complexity. Boulder Canyon was added once monitoring efforts commenced in early April due to the concern that recent high flow events may have distributed nonnative fishes from stocktanks into the drainages connected to Fossil Creek. Appendix 7 includes maps showing specific locations of some priority areas.

Priority Area	Netting	Electrofishing	Snorkeling	Nonnatives
	(# nets)	(# stations)	(# sites)	seen/captured?
1 (Below Brown bridge)	✓ (6)	✓(5)	1	No
2 (Irving to Brown bridge)	✓ (69)	✓(11)	2	Yes (1 sunfish seen)*
3 (Linoleum Camp)	✓ (12)	✓(2)	4	No
4 (Black Pool)	✓ (5)	<b>√</b> (9)	1	No
5 (Childs/Irving Road Fork)	✓ (14)		4	No
6 (Stehr Lake and above		<b>√</b> (4)	6a=1	No
Barrier)			6b=0	
7 (Sunfish barrier)			3	No
8 (Above Irving)	✓ (8)		2	No
9 Boulder Canyon (Appendix	✓ (4)	<b>√</b> (27)	NA	Yes (2 sunfish captured)
8)				

Table 5. Priority areas within Fossil Creek identified in the Short-Term Monitoring Plan, and the amount of effort completed using netting, snorkeling, and electrofishing sampling methods.

\*One green sunfish was seen in pool 3 below Irving

#### METHODS

A variety of sampling methods were used during the monitoring effort to increase the probability of encountering rare fish such as green sunfish and smallmouth bass following their eradication. In addition, using a wide variety of gear helped ensure that the complex habitat in Fossil Creek would be adequately sampled. Electrofishing using a backpack-electroshocking unit was used primarily in shallow (> 1 meter deep) riffle areas in an upstream direction. Sampled habitats were randomly chosen and a minimum of 90 seconds or 150 meters were shocked in each distinct habitat type. We deployed baited miniature hoopnets (50 X 100 cm, 10 cm throat, 6 mm nylon mesh), within each priority area for separate ~24 hour hauls. Each net was baited near it's cod end by attaching a nylon mesh bag (30 x 30 cm, 6 mm mesh) containing AquaMax<sup>TM</sup> Grower 600 for Carnivorous Species (Purina Mills Inc., Brentwood, MO). All captured fish were identified to species and measured in mm for total length (TL). Snorkeling surveys were conducted using two individuals side by side in the water doing independent identification and counts. Counts were repeated three times, compared for consistency and averaged for a single value.

## RESULTS

We backpack electroshocked a total of 3,879 meters with >15,274 seconds effort in the six priority areas and Boulder Canyon. Only eight fish were captured, speckled dace (n=2), Sonora suckers (n=1), and small, unidentified suckers (*Catostomus* species n=5) for an average CPUE of 0.02 fish/minute.

Northern Arizona University (NAU) snorkeled 7 out of the 8 priority sites and saw no nonnative fishes during their surveys. In addition, NAU staff also snorkeled two sites between priority areas 8 and 7 (behind the base camp) and one site between priority areas 4 and 7. No nonnatives were seen. As requested by Pam Sponholtz, they also sampled the isolated pool area above the springs (outside the Priority areas), and found one dead desert sucker and no nonnatives. All snorkeling surveys were between 65 and 240 meters long and replicated three times. Total effort was approximately 30 hours in the water for all of the sites including those above the Fossil

Springs diversion dam. USFS snorkeled four pools within Priority Area 5 on May 3-4 2004. No nonnative fishes were seen, although several pools held large numbers of crayfish.

Hoop (n=117 sets) and trammel netting (n=11 sets) efforts sampled six priority areas plus Boulder Canyon, comprising a total of 1,546 hours of fishing effort with an average in water net time of 20.05 hours. We captured a total of 56 fish during hoop and trammel netting efforts, comprised of roundtail chub (n=40), desert sucker (n=6), and Sonora sucker (n=9). Two green sunfish (less than 80mm) were also captured in Boulder Canyon. Average catch-per-unit-effort (CPUE) was 0.02 fish/hour.

## DISCUSSION

As designed, the monitoring effort organized for Fossil Creek post renovation was a success. A single green sunfish, although disappointing to find, was encountered during the effort. Sampling all the priority areas using three different methods was an extremely aggressive plan, yet nearly all the sampling was accomplished within the allotted time frame. The monitoring effort was also an excellent example of Federal, state, academic and volunteer participation and cooperation.

Despite the visual sighting of one green sunfish, the consensus of the Fossil Creek planning group was to not initiate a second round of treatment. This decision was made due to the successful spawning of the repatriated native fishes. Also, only one nonnative was found and the chance of re-establishment of green sunfish through natural reproduction was deemed to be unlikely. Only future monitoring (contracted by Bureau of Reclamation) will tell if our decision to not retreat was the right one (the recently completed 2005 monitoring effort did not detect nonnative fishes). The repatriated and immigrated (from upstream stocks) native fishes have dispersed into habitats beyond where they were released and successfully reproduced despite the stressful conditions of salvage and renovation this past fall. Even though Fossil Creek is closed to fishing for two years, greater public education needs to accompany management closures to help ensure that Fossil Creek remains free of nonnative fish for as long as possible.

USBR has contracted for five years of annual monitoring to detect presence of non-native fish in Fossil Creek. NAU continues to track changes in the restored fish and macroinvertebrate assemblages. APS is proceeding with removal of hydropower facilities.

## FOSSIL CREEK WATERSHED STOCK TANK RENOVATIONS

## INTRODUCTION

This chapter represents fulfillment of objective 7 under CAP Funds Transfer Program, Task 4-47. During the initial planning stages of the Fossil Creek Native Fish Renovation Project, biologists identified a need to address the impacts of non-native fish and crayfish populations at the watershed level and their potential to access Fossil Creek during runoff events. Stock tanks within the Fossil Creek drainage were identified as a potential source of non-native fish within the watershed. Although the primary concern was that non-native fish may enter Fossil Creek during high flow events, the removal of non-native fish from stock tanks occupied by the threatened Chiricahua leopard frog (*Rana chiricahuensis*) would also aid on-going recovery actions for the frog. An aerial flight conducted by the Bureau of Reclamation in June 2002 located 127 stock tanks in the Fossil Creek drainage. Sixty-five of the stock tanks were dry, 48 were holding water, and 14 were of undetermined status. Of the 62 stock tanks that were holding water or of undetermined status, 48 drained directly into Fossil Creek upstream of the proposed fish barrier and were identified as potential points of entry for non-native fish and crayfish into Fossil Creek.

In 2003, the Bureau of Reclamation, under the Central Arizona Project Fund Transfer Program, contracted Arizona State University to survey these 48 stock tanks to determine which tanks contained non-native fish and would need to be renovated prior to the restoration of Fossil Creek. During this study, each of the tanks was surveyed using several methods including seines, minnow traps, and gill nets; only five of the tanks contained non-native fish (Cashins 2003). The five tanks (Soldier Mesa, Mack's, Divide, Middle, and Black) are all located on the Coconino National Forest, east of Camp Verde off Highway 260 (Figure 7). Soldier Mesa, Divide, Middle, and Black tanks all contained green sunfish (*Lepomis cyanellus*) and Mack's Tank contained goldfish (*Carassius auratus*).



Figure 14. Fossil Creek watershed stock tank names and locations treated with rotenone during 2005. Yellow diamonds indicate location of stock tanks. Arrow indicates start of Boulder Canyon, which was also treated downstream to the confluence of Fossil Creek.

In June 2004, the U.S. Fish and Wildlife Service's Ecological Services Flagstaff Suboffice and the Arizona Fishery Resources Office were contracted under the CAP Fund Transfer Program to remove non-native fish from the five tanks and complete monitoring prior to the return of full flows to Fossil Creek, which was in June 2005. The objective of the project was to eliminate non-native fish from these stock tanks to remove a potential source of contamination to Fossil

Creek and promote further conservation of Gila River basin native fishes. The contract specified completion of the following tasks: (1) pre-treatment coordination with the Coconino National Forest and livestock permittees, (2) renovation using pumps and a chemical piscicide, accompanied by (3) detoxification, (4) removal of sediment and sealing of Black Tank, (5) post-treatment monitoring to assess treatment effectiveness, (6) re-treatment if necessary, followed by post-treatment monitoring, and finally (7) preparation of a final report that documented project completion.

#### METHODS

## Coordination

We completed the renovation as a cooperative project among the U.S. Fish and Wildlife Service, Arizona Game and Fish Department, Bureau of Reclamation, and Coconino National Forest. We met with Coconino National Forest Service staff and the grazing permittees for the Fossil Creek, Thirteen Mile, and Hackberry-Pivot Rock allotments on March 3, 2004. The permittees did not oppose the proposed action and agreed that if we planned the project so that no livestock were in the pastures during or immediately following the renovation, they would not have any issues with the project. We agreed to keep them informed throughout the project of our progress and any changes to the proposed renovation plan. During later discussions, the Forest Service expressed concern over pumping the stock tanks dry due to drought conditions and because the tanks might not re-fill. Therefore, we decided to use piscicide to remove non-native fish versus pumping the stock tanks dry.

## Plan Development and Consultation

Originally we planned to complete the project during the spring and summer of 2004, prior to the chemical renovation of Fossil Creek. The original proposed action included pumping the tanks dry when they were low (prior to monsoon season) and removing sediment from Black Tank to increase its ability to hold water. However, as noted above, the Forest Service did not want to pump the tanks dry during a drought. Therefore, we determined that the best alternative would be to chemically renovate the stock tanks with piscicide. During the NEPA process, only antimycin was analyzed and approved for use on Fossil Creek. However, we were concerned about the effectiveness of antimycin due to the high pH environment of these stock tanks. Based on several years of water quality data collected by the U.S. Fish and Wildlife Service and Arizona Game and Fish Department, the mean pH of the five stock tanks ranged from 8.4 to 9.8, which could render the antimycin ineffective. We worked with the Forest Service to complete an amendment to the Fossil Creek Native Fish Restoration Environmental Assessment. This document analyzed whether the effects of using rotenone in the stock tanks would be the same as using antimycin. The effects were determined to be similar, so we received approval from the Forest Service to use rotenone. However, we did not receive this approval until the renovation of Fossil Creek was underway, so the stock tanks were treated after Fossil Creek was chemically renovated. In addition, a very wet winter in 2004 resulted in the tanks overflowing into Boulder Canyon and an additional stock tank (Antelope Tank), so we also treated these areas.

We developed a pesticide use plan (PUP) in cooperation with the U.S. Forest Service and Arizona Game and Fish Department to guide the application of the chemical renovation of the stock tanks. This task was completed in early December 2004 and the PUP was signed by U.S.

Forest Service's Regional Office on December 17, 2004. We conducted informal section 7 consultation on potential effects from the project on the Chiricahua leopard and received a concurrence letter from the Fish and Wildlife Service dated March 21, 2005. NEPA compliance was completed via the Final Environmental Assessment for Native Fish Restoration in Fossil Creek (Bureau of Reclamation 2004) and amendment.

RESULTS

## Renovation

Renovation of all five tanks was completed by March 29, 2005. The Bureau of Reclamation provided the rotenone (Prentox®) in late January 2005. The U.S. Fish and Wildlife Service and the Arizona Game and Fish Department completed all renovations as a cooperative effort. Prior to treatment, we calculated the volume of each tank and the appropriate amount of rotenone to treat to the label specifications. The four stock tanks containing green sunfish were treated with rotenone at 1 part per million (ppm) and Mack's Tank, which contained goldfish, was treated at 4 ppm (per the label specification for goldfish). Rotenone was applied using backpack sprayers along shorelines and small boats to cover deeper sections of each tank. We used electric boat motors to mix the chemical into the water column. In all tanks containing green sunfish, distressed fish began appearing in approximately 15 minutes after chemical application, and fish were dying or dead within one hour of application. Mack's Tank contained goldfish; it took approximately three hours before fish began dying.

In addition to the five stock tanks, we treated a sixth stock tank (Antelope Tank) and portions of Boulder Canyon (a tributary to Fossil Creek that drains Divide, Antelope, Middle, and Black tanks) with rotenone on March 23, 30, and April 13, 2005. Although we had not originally planned to treat Antelope Tank or Boulder Canyon, high winter precipitation caused the stock tanks to overflow into the Canyon and green sunfish were able to move down drainage from Middle and Black tanks. We coordinated the treatment of these additional areas with the Arizona Game and Fish Department and Forest Service. Antelope Tank and Boulder Canyon were both treated at approximately 1 ppm. We used 2.83 gallons of rotenone to treat Boulder Canyon and gallons of rotenone used.

Table 6. Amount of Prentox® used to treat stock tanks and Boulder Canyon in the Fossil Creek drainage.

Stock Tank Name	Volume	Gallons of Prentox®	Gallons of Prentox®
	(acre/foot)	needed to treat at 1ppm	needed to treat at 4ppm
Soldier Mesa Tank	2.84	0.95	
Divide Tank	8.45	2.82	
Middle Tank	6.25	2.08	
Black Tank (including	5.61	1.87	
arm)			
Mack's Tank	4.83		6.44
Antelope Tank	2.2	0.73	
Boulder Canyon	8.49	2.83	

We did not use potassium permanganate to detoxify the rotenone following renovation since livestock would not use these tanks for several months following treatment. In addition, the organic load in the tanks helped detoxification to progress without the use of additional chemical. We signed all stock tanks during piscicide treatment and announced treatment dates and locations in the local newspapers to ensure that the public was aware of temporary closures at the stock tanks. All access points were also posted with this information.

#### Monitoring

We began monitoring the five tanks on August 9, 2005. We sampled tanks using monofilament sinking experimental gill nets (100ft x 6ft) set for approximately 24 hours. No fish were captured during this monitoring effort. In addition, crayfish densities in Soldier Mesa and Divide Tank appeared reduced. These stock tanks are very simple habitats and as such, this monitoring protocol was determined to be adequate to determine whether fish were present within the tanks. Cashins (2003) used the same sampling technique when he originally surveyed the tanks. Because of their potential use for ranid frog recovery, these tanks will continue to be monitored into the future.

#### DISCUSSION AND RECOMMENDATIONS

As described above, the original methods for accomplishing the objectives were modified extensively. The overall objective was met with these modifications and the non-native fish have been removed from the stock tanks and drainages that could act as source areas for contamination of Fossil Creek. The project was successful due to effective communication among the agencies involved and the flexibility of the program and the agencies to respond to changing conditions. For long-term success, we need to continue to actively deter the public from stocking fish in these stock tanks and be prepared to chemically renovate these tanks again if non-native fish are detected. Therefore, we offer the following recommendations:

- ∞ We recommend that agency law enforcement personnel assist biologists working in this area to ensure that non-native fish are not re-stocked in these tanks. In conjunction with our Chiricahua leopard frog recovery work, the U.S. Fish and Wildlife Service Ecological Services Flagstaff SubOffice and Arizona Game and Fish Department (Region 2) intend to continue to monitor the six treated stock tanks for the presence of non-native fish. We know that Divide Tank was re-stocked with green sunfish following the 2002 drought, and we expect that this may happen again. We are working with the Arizona Game and Fish Department and U.S. Forest Service to post signs reminding the public that it is illegal to move live fish, crayfish, and bullfrogs into these stock tanks. However, all agencies should target enforcement and education to keep these species out of the area.
- ∞ We recommend that all agencies including Arizona Game and Fish Department, U.S. Fish and Wildlife Service, U.S. Forest Service, and Bureau of Reclamation develop a plan for periodic monitoring of Boulder Canyon and all of the stock tanks that drain into Fossil Creek above the barrier. In order to keep non-native fish out of the renovated portion of Fossil Creek, we need to ensure that potential sources of contamination are identified and dealt with rapidly. Currently there are a number of stock tanks on the

Coconino National Forest side of Fossil Creek that are monitored by the Fish and Wildlife Service, Arizona Game and Fish Department and Forest Service. However, there are many stock tanks that are not visited, particularly on the Tonto National Forest that should be periodically monitored. The plan should identify a schedule for surveying the tanks that are not visited regularly, as a part of ranid frog or other surveys.

- $\infty$  We recommend that agencies and cooperators meet annually to discuss the results of ongoing monitoring in Fossil Creek and the surrounding areas (including the stock tanks and Boulder Canyon) and identify tasks each agency can complete to ensure we are actively working to keep non-native fish out of Fossil Creek.
- ∞ We recommend that the Arizona Game and Fish Department, Fish and Wildlife Service, Forest Service, and Bureau of Reclamation work with other interested parties to develop a method of controlling crayfish. Currently, crayfish occur in Fossil Creek and in many stock tanks in the drainage. Control of this non-native species would benefit all native fish and ranid frogs. Three of the six stock tanks that were chemically renovated cannot be used by Chiricahua leopard frogs due to the presence of crayfish.

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#### APPENDIX 1. FOSSIL CREEK RENOVATION FIRST BIOASSAY

On August 3-5, 2004 a bioassay was conducted on Fossil Creek, Gila and Yavapai Counties, Arizona. Participants included Dave Weedman and Kirk Young (AGFD), Rob Clarkson (USBR), Jerry Ward (USFS, State and Forest Service Certified Pesticide Applicator), Paul Marsh (ASU) and Allen Haden (NAU).

The project area is located on Fossil Creek, in the Mazatzal Mountains of central Arizona. Fossil Creek forms the boundary between Yavapai and Gila Counties, as well as Tonto and Coconino National Forests over most of its course. Fossil Creek is one of Arizona's rare warmwater perennial streams, flowing from a complex of springs, known as Fossil Springs, 14.3 miles through rugged and isolated terrain before entering the Verde River. Fossil Springs produces a constant water temperature of approximately 70 degrees Fahrenheit and flow of 43 cfs, most of which is captured by APS at the 25- foot high Fossil Springs diversion dam located 0.3 mile downstream of the springs. Base flows below the diversion dam vary between 0.4 cfs and 5 cfs, although episodic flows of much higher magnitude are possible from rainfall, snowmelt or temporary closure of the diversion at the Fossil Springs dam.

The area chosen for the bioassay is located approximately 0.5 miles below Fossil Springs Dam (Figure 1.). A five-foot waterfall exists at this point which appears to have acted as a barrier to the upstream migration of green sunfish, which are present below the fall but not known to be present above. There were two objectives to the bioassay:

- 1. Conduct application of Antimycin to determine concentrations effective at complete elimination of the fish assemblage,
- 2. Use Antimycin as a survey tool to determine if sunfish are present above the waterfall.



Figure 1. Overview of Fossil Creek Bioassay, August 4-5, 2004.

# Methods

On August 3, a hip chain was used to measure the stream course (following the thalweg) up and down stream from the falls (Figure 2). Flags were placed at 20m increments. In order to accomplish both objectives, two drip stations were established in Fossil Creek. Drip Station 1 (DS1) was established at 150 m above the falls in a riffle just above a large pool. Drip Station 2 (DS2) was set at the falls. A detoxification station was established 160 m downstream of the fall. Stream discharge was measured downstream of the falls using a flowmeter. It resulted in a calculation of discharge=0.4 cfs. A second method was employed at the falls to verify discharge. A 19 L bucket was used to capture the entire stream flow as it dropped over the fall. Fill rate for the bucket was measured 7 times and averaged, resulting in a discharge rate of 2.4 gal/sec. This converts to 0.32 cfs. The two values were close, so we decided to treat with both drip stations at 0.4 cfs.

During this same afternoon, we used rhodamine dye to measure travel time from the upper drip station to the waterfall, through one large and several smaller pools. Total distance traveled was 150 m in 2 hours 25 minutes. However, the first pool was only 30 m long and took 55 minutes for the dye to reach the outflow. This pool was not exceptionally large and below average for the system. There are many pools in Fossil Creek that would be expected to have much longer flushing rate and flow time. As soon as dye was detected at the outflow, an additional pulse of dye was added to increase detectability further downstream. Total travel time was calculated at 62 m/hour. Subtracting the flow time and distance for the aforementioned pool, travel time was 80 m/hour. Not including the large pool. Due to the extensive travel time through the upper reach, Drip Station 1 was scheduled to run for two 3-hour charges. Fintrol dose for each 3 hour bucket, to achieve a 25 ppb in-stream concentration was calculated to be 30 ml. The discharge rate for the bucket to drain in the 3 hours was calculated to be 106 ml/min.

On August 4, 2004 the two drip stations were assembled, placed, charged and begun. Drip Station 1 was to discharge at 26 ml/15 sec (~106 ml/min.). Drip Station 2 was set the same. Water temperature was measured just below the falls and was 15 degrees Celsius at 9:15 am.

Three mesh bags containing sentinel fish were placed in the stream. Fish were collected by backpack shocking and seining downstream of the drip stations. One mesh bag containing 4 green sunfish was placed at the lower end of DS2, just above the detoxification station. One bag containing only native species was placed about 100 meters below the detoxification station to confirm its effect. One mesh bag containing only native species was placed in a pool just above the waterfall to confirm toxicity 150 m below DS1.

## Results

Drip stations were checked and adjusted and observations made on August 4, 2004 as follows:

8:30 -DS1 started
9:00 -DS2 started
9:50 -DS1 running at 25.5 ml/15s—OK
10:00 -DS2 increased from 23 ml/s to 26 ml/s DETOX station also started and set to 84 ml/min with 2.5% solution KMnO4 for 3 ppm concentration.
11:40 -DS1 recharged with 30ml Fintrol and reset to 26ml/15s.

- 12:30 -DS2 stopped and remaining 1 inch of charge slowly drained into creek
- 12:45 -DS1 checked-OK
- 1:00 -No observed effect to any fish, either in live car or in stream
- 2:20 -DS2 moved to 20 m above falls in riffle and recharged to 100 ppb for 3 hour run (see further discussion below), live car with sunfish moved to pool just below fall, ~25 m downstream of new DS2 running at 100 ppb.
- 3:15 -DS2 checked --flow rate increased from 23ml/15s to 28 ml/15s
- 3:25 observed ~10 dead fish in head of pool immed below DS1 (all small bodied RHOS and chub. DS1 drained of about 1 inch of chemical and dismantled
- 4:30 Detox station stopped. No apparent effect to fish in pool downstream of it. Many sunfish and chub and sucker observed swimming normally
- 5:00 -No observed mortality in 70 meter stretch between Detox and 80 m below falls. Some small bodied mortality between fall and 80 m below. Observed sunfish swimming freely. In pools just below fall, chub and dace appear stressed, gulping air and lack of fright response. Sunfish in live car lively when lifted from water. Live car with native suckers, chub and dace immed above falls lively. Between falls and 150 m upstream were many live large chub and suckers, but also many dace mortalities.
- 5:20 DS2 drained of 1 inch chemical and dismantled.

Due to lack of visual effect on fish immediately below the two drip stations 5.5 hours later, a discussion was held and a decision was made to move DS2 upstream 20 meters above the fall and recharge to 100 ppb. This was done as a contingency experiment in the off chance we weren't effective at a complete kill at 25 ppb. The first 130 m below DS1 received the planned application of 25 ppb for 6 hours, and would provide needed info. However, there were no sunfish expected in this area and effectiveness on them could not be gauged. There were sunfish present in pools below the fall, as well as the 4 in the live car that were moved to the pool 5 m below the fall. I returned to the treatment area on August 5 to assess the effects of the bioassay and made the following observations from 9:30 until 10:45 am:

Pool just below DS1 and riffle below it down to 40 m above fall suffered a complete fish kill. No live fish were observed and many size classes of RHOS, PACL and chub were floating or sunk on bottom. There was one isolated backwater on river right below the pool that had live chub swimming. No sunfish were seen in this pool.

Pool #7 (which had previously been measured at 20 m long) began 40 m above falls and had many dead fish of all species/sizes, but I also observed 8 chub and 1 PACL alive located at the inflow to the pool. They appeared stressed, lethargic and lacked a fright response.

The pool just above the fall and below the 100 ppb DS2 was totally dead, no live fish seen. Live car with natives just above falls was all mortality. Below the fall was a live car with 4 sunfish. Two had expired and were less than 3" long but the two larger sunfish 3-5" long were still alive although lethargic.

Another live car with only native species was located 50 m below the falls and suffered 100% mortality. There were scattered mortalities between the falls and here, although none were identified as sunfish. A small pool located 80 m below the fall had many morts, none identified as sunfish and one live sunfish was observed, although it was lethargic and allowed itself to be picked up by hand. Pool 8 began just below here.

From 100 m to 140 m below the fall many live sunfish (25+) and 3 live chub were observed behaving normally, but there was apparent mortality of all other native species observed. These sunfish had easy access to the area from a large pool located below the detox station.

At 180 m below the fall began another pool where 1 live chub was observed, but many other native mortalities were seen. No dead sunfish were seen. Some native mortalities were seen in Pool #9, no sunfish mortalities but many live chub and sunfish were observed.

Some small bodied natives were observed down to the head of Pool #10, but no sunfish mortalities were seen. In and below Pool 10, no mortality of any species was observed.



Figure 2. Fossil Creek Bioassay, August 4-5, 2004 Drip station locations, pools

Discussion

It appeared that the application of antimycin to Fossil Creek during this bioassay may not be 100% effective on green sunfish at the concentrations identified for application (25 ppb) and over the expected distance effectiveness required. The greatest possible exposure time of the 4 green sunfish held in the live car between first exposure and last observation is about 18 hours.

This may not be long enough to confirm 100% mortality, even at 15 degrees C. It is also possible that water quality of Fossil Creek is affecting the toxicity, persistence in the environment or susceptibility of the species to Antimycin. Additional studies are definitely needed to determine if the nature of the calcium carbonate and  $CO_2$  laden water of Fossil Creek is affecting efficacy of the Antimycin. In addition, observation of delayed mortality out to 48-72 hours post-treatment is needed. It is possible that 25 ppb over a 6 hour exposure time is sufficient, but that 100% mortality won't be observed in less than 24 hours, and may not extend for the required 150 m between recharge buckets.

# APPENDIX 2. FOSSIL CREEK RENOVATION SECOND BIOASSAY, CONTROLLED EXPERIMENT

On August 10-12, 2004 experimental applications of Fintrol (Antimycin) were conducted to determine effective dosage rates and mortality time at varying dosages. Our objective was to determine 100% mortality for each species at differing concentrations of Fintrol. This experiment was conducted at the APS Irving power plant using water available from the penstock pipe running the Irving Turbine. This water is transported via the flume directly from Fossil Springs diversion dam 4 miles upstream of Irving.

#### Methods

Four tanks were treated with Fintrol and two control tanks were maintained. Fish species available for testing were green sunfish *Lepomis cyanellus* and smallmouth bass *Micropterus dolomieu* collected from Fossil Creek, and yellow bullhead *Ameiurus natalis* collected from a stock tank and supplied to us by Page Springs State Fish Hatchery. Basic water quality parameters (DO, pH, specific conductance, temperature, oxidation-reduction potential) were measured prior to and periodically throughout the treatment (Table 1). In addition, these basic paramaters and additional components were measured below Fossil Springs, below the diversion dam, at Irving, and below Sally Mae Wash for comparative and planning purposes (Table 2.).

The treatment tanks available for use were insulated fiberglass transport tanks (Figures 1 and 2). Control tanks used were plastic un-insulated water troughs (Figure 3), approximately the same capacity as the larger treatment tanks (volume not calculated). Treatment tanks were measured to determine volume (for calculating Fintrol application doses). Tanks were then filled from the pipeline and water quality parameters measured. Sunfish and smallmouth bass were collected from Fossil Creek by backpack shocking (sunfish) and angling (smallmouth). Ten sunfish, 5 bullhead and 4 smallmouth were placed in each treatment tank. Ten sunfish, 2 smallmouth and 5 bullhead were placed in each control tank. Final counts and measurement of sunfish mortalities from the treatment tanks may not equal 10 due to predation by yellow bullhead. Supplemental oxygen was used in the tanks as determined by observation and behavior. Diffusers (4) were limited and thus shared. Control tanks shared one diffuser and one was shared between Tank 3 and 4. Care was taken to rinse diffusers between movement from Tanks 3 and 4. All mortalities removed from the tanks during observation were measured to the nearest millimeter (data available).

## Results

Fintrol dosages were calculated and applied as follows: Tank 1=25 ppb, Tank 2=50 ppb, Tank 3=100 ppb and Tank 4=200 ppb. Tanks 5 and 6 were control tanks. Time to mortality for individuals was not recorded, but observations were made hourly for the first six hours, then as indicated in Table 3.

Table 1. Water quali	ity parameters record	ed periodically	during Fintrol	bioassay in	tanks at Fossil
Creek, August 2004					

Parameter	Tank 1	Tank 2	Tank 3	Tank 4	Tank 5	Tank 6
Pre-Treatmen	nt Measuremer	nts 8/10/04				
DO	6.7	N/A	6.5	N/A	24.2	N/A
PH	7.3		7.4		7.5	

Fossil Creek Native Fish Restoration Project
November 2005

SPC	740		742		750			
ORP	343		339		332			
Temp (C)	23.2		25.2		25.9			
2:30 pm post Fintrol application								
DO	5.79	6.0	4.5	6.2	22.9	12.9		
PH	7.33	7.3	7.4	7.5	7.5	7.4		
SPC	747	747	748	745	755	752		
ORP	316	315	304	298	320	317		
Temp (C)	23.5	23.4	25	23.6	26.2	26.2		
8:00 pm (pre	-flushing)							
DO	13.9	9.7	12.5	12.5	19.1	11.5		
PH	7.5	7.5	7.5	7.6	7.6	7.5		
SPC	743	743	743	741	751	747		
ORP	315	314	315	314	318	316		
Temp (C)	23.3	23.3	24.5	23.3	25	25.2		
8:00 am 8/11	/04 (pre applie	cation of 400 p	opb, 800 ppb)					
DO		18.7	7.4					
PH		7.7	7.6					
SPC	N/A	733	734	N/A	N/A	N/A		
ORP		308	306					
Temp (C)		20.9	20.6					

Table 2. Fossil Creek water quality measurements using hydrolab Surveyor 4, August 10 and 11, 2004.

	Spring	Reach 1	Irving	Irving	Road	Reach	~ .5 mi	~ .5
	Dam	above	Road	Road	below	3 Pool	Below	mi
	(a)	sunfish	upstream	upstream	Irving		Sally	Below
	trash	barrier	Pool	Run			Mae -	Sally
	rack						Pool	Mae -
								Riffle
Temp C	21.7	19.7	22.5	23.2	22.5	23.2	24.1	24.3
Cond.	740.5	617.7	564	564	710	559	523	522
PH	7.0	7.63	7.8	8.1	7.9	8.03	8.24	8.25
DO	7.6	5.9	5.5	7.34	8.1	8.1	8.0	8.2
% Sat.	91	68.7	67	90	98.4	99.7	100.6	103
Turb	16.9	1.4	32.1	38.7	5.4	1.6	10.1	9
Redox	327	306	337	316	197	276	299	311
TDS	.4741	.3954	.3605	.3611	.4558	.3580	.3349	.3346
(gr/l)								

	Tank 1	Tank 2	Tank 3	Tank 4	
Interior					
dimension	54 x 18 x 18	54 x 18 x 18	21.5 x 30.75 x 18	21.5 x 30.75 x 18	
(inch)					
Volume	$10.125 \text{ ft}^3$	$10.125 \text{ ft}^3$	$6.89 \text{ ft}^3$	$6.89 \text{ ft}^3$	
Capacity @18 inch depth	75.7 gallons	75.7 gallons	51.5 gallons	51.5 gallons	
Acre/feet	.0002324	.0002324	.0001581	.0001581	
Target dosage	25 ppb	50 ppb	100 ppb	200 ppb	
Fintrol needed	0.07 ml	0.14 ml	0.19 ml	0.39 ml	
10% Fintrol					
concentration	0.7 ml	1.4 ml	1.94 ml	3.88 ml	
applied					
	LECY=10	LECY=10	LECY=10	LECY=10	
Fish added	MIDO=4	MIDO= 4	MIDO= 4	MIDO= 4	
	AMNA=5	AMNA=5	AMNA=5	AMNA=5	
Application	2.20 mm	2:20 mm	2:20 mm	2.20 mm	
Time	2.20 pm	2.20 pm	2.20 pm	2.20 pm	
August 10 Observ	vations				
			3 MIDO dead	AMNA normal	
			AMNA normal	4 MIDO dead	
3:20 pm	Normal	Normal	LECY 1 dead	LECY=lethargic,	
-			others	3 dead	
			disoriented		
	AMNA normal	AMNA normal	AMNA normal	ANDIA normal	
4:20 pm	MIDO&LECY	MIDO lethargic	1 MIDO dead	AMINA HOI Hai	
_	lethargic	LECY lethargic	7 LECY dead	LEC I – / dead	
		AMNA normal			
5.20 mm	Sama	MIDO= 4 dead	AMNA normal	Sama	
5.20 pm	Same	LECY =1 dead	LECY=1 dead	Same	
		others lethargic			
6:20 mm	Sama	AMNA normal		Sama	
0.20 pm	Same	LECY=1 dead	Alvina lioilliai	Same	
	AMNA normal	AMNIA normal		ANNIA display	
7:20 pm	MIDO=3 dead	LECV-4 dood	Same	AlvinA display	
				some remargy	
	AMNA normal				
8:20 pm	MIDO=1 dead	AMNA normal	Same	Same	
	LECY=2 dead				
8:25 pm	All tanks flushed v	with fresh water to e	liminate presence of	Fintrol	
August 11 Observ	vations				

Table 3. Dimensions, treatment and observation of test tanks for Fintrol dosage evaluation at Fossil Creek. AMNA=yellow bullhead, MIDO= smallmouth bass, LECY= green sunfish.

	Tank 1	Tank 2	Tank 3	Tank 4
2:30 am	LECY= 4 dead	AMNA normal	AMNA normal	AMNA normal
6:20 am	AMNA normal LECY 1 barely alive	Same	Same	Same
8:00 am	Same	400 ppb = 1.14ml Fintrol	800 ppb =1.56 ml Fintrol	Same
10:20 am	AMNA normal LECY=1 dead	Same	AMNA less active	Same
12:20 pm	AMNA normal	AMNA Normal	AMNA Normal	AMNA Normal
2:20 pm	Normal	Normal	Lethargic, 1 w/labored gill movement	Normal
4:20 pm	Normal	Normal	Lethargic, 1 upside down	Normal
6:30 pm	Normal	Normal	AMNA active except 1dead	Normal
7:20 pm	Normal	Normal	Added 4 MIDO and 9 LECY from Control Tank	Normal
7:30 pm	Drained Tank	Drained Tank		Normal
8:00 pm			MIDO= 4 dead LECY= stressed AMNA= 4 normal, 1 dead	Normal
8:40 pm			LECY=7 dead AMNA= 4 normal	Normal
11:10 pm			AMNA=4 normal	Normal
Aug. 12, 6:40 am			AMNA= 4 normal	Normal
6:40 am	End of Test			

At 25 ppb, 100% mortality of green sunfish was achieved 20 hours after beginning. Smallmouth bass 100% mortality was achieved at 6 hours after beginning. Yellow bullhead showed no affects after 29 hours, when the 25 ppb test was ended.

At 50 ppb, 100 % green sunfish mortality was achieved after 4 hours exposure. Smallmouth bass mortality equaled 100% after 3 hours. There was no affect to yellow bullhead after 17 hours 20 minutes, when that test was ended and 400 ppb Fintrol was applied to the 5 bullheads remaining in that tank.

At 100 ppb, 100% green sunfish mortality was achieved 3 hours after beginning, although 89% were dead within 2 hours. Smallmouth bass 100% mortality occurred 2 hours after starting treatment. Yellow bullhead remained unaffected after 17 hours 20 minutes, when they were retreated with 800 ppb Fintrol.

At 200 ppb, green sunfish mortality was accomplished at 2 hours after beginning treatment, with 30% of them dead after the first hour. Smallmouth bass all died within 1 hour of treatment start.

At 400 ppb with a total exposure time 22 hours 40 minutes, there was no apparent affect to yellow bullhead over the same time frame.

At 800 ppb, exposure time was 22 hours 40 minutes with 20% mortality observed to yellow bullhead. However, the other 4 bullhead showed no signs of stress, loss of equilibrium or other behaviors indicating they were affected. We assume there were complicating or compounding factors that resulted in this mortality. No external physical wounds or abnormalities were observed, however.

# Discussion

Under optimum controlled conditions, Fintrol appears to cause mortality of smallmouth bass and green sunfish adequately and within an expected timeframe at both 25 and 50 ppb. However, previous instream tests resulted in less than optimum performance. During those tests, application of Fintrol at 100 ppb with a 3 hour exposure time only achieved 50% mortality of green sunfish at 20 hours following exposure. The other 50% were, however, on their way to expiring.

There are many possible reasons that Fintrol use in the stream bioassay was less effective than in static tanks. Fintrol used in that assay may have been compromised in some way. Natural biological activity within the stream may have oxidized with or otherwise bound up the antimycin molecule rendering it ineffective. Water chemistry in stream reaches below Fossil Springs diversion dam may be different than that used for the tank tests. Water chemistry is currently being analyzed to rule out or support this possibility.

Additional instream bioassay was needed to confirm results of the first instream bioassay, to test applicability and efficacy under the proposed treatment regime, and to confirm application rate that will be sufficient with existing conditions to accomplish our objectives for the Fossil Creek Restoration project.

Figure 1. Trailer test tank.



Figure 2. Smaller test tank.



Figure 3. Control tanks.



#### APPENDIX 3. FOSSIL CREEK RENOVATION THIRD BIOASSAY

#### Introduction

On August 24-26, 2004 another bioassay was conducted at Fossil Creek. This bioassay was initiated approximately 300 meters above the wet crossing at the APS Irving Power Plant and continued to just below the wet crossing at a cattail thicket above the Irving diversion dam. This area was chosen due to ease of access and presence of existing nonnative green sunfish instead of repeating the previous bioassay, where nearly all existing fishes were already removed. The objective of this bioassay was to determine the minimum effective concentration of Antimycin (Fintrol and Fintrol-15 sand) necessary to produce complete mortality of nonnative fish populations within the creek.

## Methods

The area to be treated was measured using a hip chain with flagging placed at 25-meter increments from the wet crossing at Irving upstream 300 meters. Green sunfish (*Lepomis cyanellus*) were collected from Fossil Creek and placed in live cars (5-10 fish per live car) in the creek at each 25-meter flag. The sunfish were collected from Fossil Creek using a backpack shocker and dipnets and were not measured for total length. Discharge of the creek within the test reach was determined by measuring cross sectional area with a meter tape and depth rod, then flow velocity was measured with a Marsh-McBirney flowmeter at 0.6 of the depth at 10 points across the stream. Discharge was calculated to be 1.7cfs at a narrow channel upstream form the wet crossing.

Two drip buckets for antimycin application were assembled and placed at 150 meters and 300 meters above the crossing. A neutralization bucket applying potassium permanganate for neutralizing the piscicide was placed on the creek just downstream of the wet crossing. There was a diversion canal leading from the dam to the Irving power plant, which was also measured for total volume. These measurements were used to calculate the amount of Fintrol-15 sand applied to treat the standing water.

Drip station 1 (DS1) was placed 300 meters above the wet crossing at Irving and was charged to 50ppb with 260ml of antimycin. The second drip station (DS2) was placed 150 meters below DS1 and 150 meters above the wet crossing. DS2 was charged to 100 ppb with 520ml of Antimycin. The two drip buckets were scheduled to run for three hours, then re-charged for another 3-hour application, for a total of 6 hours. DS2 was only charged with 360ml for the second 3-hour period due to a lack of available Fintrol. Six hours was chosen as the application time to increase the exposure time available and improve probability for complete mortality. A backpack sprayer was also utilized for this bioassay to assess its effectiveness on treatment of stream margins and backwaters similar to the proposed treatment for the complete renovation. It was charged with 110ml of Antimycin for the first 75 meters. It was then charged with 220ml of Antimycin for the next 150 meters then 110ml for the final 75 meters. The backpack sprayer was used to treat the margins and slack water of the creek. Backpack charging rates were determined by making assumptions on the area to be treated: over a 75 meter distance, a width of 4 feet from each bank and average depth of 2 feet would be treated by spraying. This yielded a volume of 3936 cubic feet to be treated at 100 ppb. There were also two large pools within the test reach that were treated with Fintrol-15 sand to achieve an instantaneous concentration of 50 ppb.

During the last bioassay, AGFD Water Quality specialists collected samples from 4 areas of Fossil Creek to assess existing water quality parameters. The areas were: above Fossil Springs Diversion dam, at Irving wet crossing, at bridge about 1.2 miles below Irving and at Sallie Mae confluence. Water samples were collected at various times throughout August 10, 2004.

Results

The Bioassay was conducted on August 25, 2004. Application start times, observations on effects and other pertinent information were recorded and reported in the timeline below.

8:05am started DS1 300m above the road crossing at 50ppb with 260ml.
8:30am started DS2 150m above the road crossing at 100ppb with 520ml.
10:00-11:30am backpack sprayer applied Antimycin to margins and slackwater.
10:30-11:30am sand was applied to both pools.
11:05 DS1 recharged with 260ml.
11:30 DS2 recharged, but with only 360 ml due to running out of Fintrol.
12:30-1:00 sand reapplied to pools.

Later in the day, an observation of the sentinel fish within the live cars was made. The observations begin in the middle of the reach, proceeded upstream, then from the middle downstream to the road crossing between 4:15 and 4:45 pm.

0m50 ppb drip station25m4:26 pm 5 fish alive50m4:24 pm 5 fish alive75m4:23 pm 10 fish alive100m4:21 pm 1dead 4 alive125m4:20 pm 4 fish alive150m4:15 pm 6 fish alive155mLocation of 100 ppb drip station175m4:15 pm 4 sunfish all dead200mno live car225m4:31 pm 9 sunfish all dead250m4:33 pm 8 sunfish all dead275m4:36 pm 9 sunfish all dead300m4:37 pm 6 sunfish dead 3 alive

At 7:45 am on August 26<sup>th</sup> live car observations were repeated for the entire 300m stretch. The observations were initiated from the top of the reach down to the crossing at Irving. The observations for the live cars were:

0m50 ppb drip station25m5 sunfish total= 3 dead, 2 alive50m4 dead fish 1 nearly dead75m3 nearly dead 13 dead

100m	1 alive, 4 dead
125m	4 alive, no dead fish, all swimming actively
150m	4 alive, 2 dead
155m	Location of 100 ppb drip station
175m	4 dead
200m	no live car
225m	9 sunfish all dead
250m	8 sunfish all dead
275m	9 sunfish all dead
300m	8:15am, 9 fish all dead

## Conclusion and Discussion

After the live car observations were conducted a brief visual observation of the canal running into the powerplant was conducted. Many dead fish and no live fish were observed. Observations show the test reach treated with 50ppb (0-150m) resulted in most fish dead but some of the fish still alive, especially at the end of the treated reach, where all 4 fish located 125 m from the drip station showed no ill effects. Fish observed in the live cars in the section treated at 100ppb indicated a complete kill with no live fish detected within the live cars.

Water quality samples taken August 10, 2004 weren't fully analyzed prior to this bioassay, unfortunately. High concentrations of dissolved iron were discovered in water samples taken just above Irving (Fossil Creek Restoration Project Report, Table 1). These concentrations were over 5 times higher than upstream levels near the spring source. Below Irving, the return of diverted flows to the stream channel served to dilute the high concentrations of iron, returning them to lower levels. Also noted within the area of higher iron concentrations was increased turbidity resulting from inflow from an off-channel spring. This spring contributes little flow to the stream, less than 0.1 cfs, but was surmised to be the source for the iron.

According to the Fintrol manufacturer, Nick Romeo, increased iron concentrations have an effect on Fintrol toxicity to fish. The iron is taken up by hemoglobin within the fish and transported to cells, where it partially blocks the transfer of antimycin to the mitochondria, thus reducing the effectiveness of the fish toxicant. High iron concentrations in flowing surface water may require higher Fintrol concentrations to achieve the desired effect. This situation may help explain why not all sunfish were affected at 50 ppb but 100 ppb was 100% effective. Although the previous bioassay conducted upstream failed to effect 100% mortality of sunfish at 25 ppb over 6 hours or at 100 ppb over a 3 hour application, there were complicating factors in the design of that bioassay. They included presence of large deep pools that weren't treated with sand or back-pack spraying, resulting in delays in downstream movement of the toxicant while oxidation of it was occurring in transit.

# APPENDIX 4. FOSSIL CREEK RENOVATION FOURTH BIOASSAY

On September 7, 2004 a final bioassay on Fossil Creek was conducted. This bioassay was designed to confirm the application rate for Fintrol needed to affect complete mortality of green sunfish, which had proven to be more hardy than smallmouth bass during the second bioassay controlled experiment. Participants included Arizona Game and Fish Department, Bureau of Reclamation, ASU, and NAU biologists.

The area for this bioassay began at the sunfish barrier, which is a large waterfall that acts as an upstream barrier to the movement of sunfish (Figure 1). At the recommendation of the manufacturer of Fintrol (Nick Romeo, pers. comm.) additional surfactants (Nonoxynyl-9) were added to the Fintrol to improve solubility and dispersal of the active ingredient, Antimycin, throughout the stream channel. Per recommendations, the additional surfactant was added at a ratio of 65 ml/480 ml of Fintrol, or 13.5%. This bioassay was designed to occur over a 4 hour exposure time, thus 5 gallon drip buckets would be filled and set at an outflow rate of 79 ml/min.

#### Methods

On September 7, the project reach was measured using a hip chain with flags placed every 25 meters, down to 275 meters below the barrier. At each flag a live car was placed in the creek. Discharge was measured at 1.2 cfs using a flow meter, depth rod and meter tape at a confined channel area.

On September 8, drip station 1 (DS1) was assembled and placed at the top of the test reach just below the sunfish barrier and drip station 2 (DS2) was placed 150 m downstream from DS1. Sentinel sunfish were captured several kilometers downstream using traditional backpack shocking techniques. These fish were placed in the live cars (4-6 each livecar). Based on previous discharge measurement, DS1 was charged to 50ppb by adding 244ml of Fintrol and 33 ml of Nonoxynyl 9. DS2 was charged to 75ppb by adding 366ml of Fintrol and 50ml of additional detergent. DS1 was started at 2:25pm and DS2 at 2:35 pm. Each drip station was set to apply over a 4 hour period. Spray and sand crews started application at 3:00 pm and completed at 4:00 pm. Volume measurements made July 8, 2004 of the three pools within the bioassay reach (pools 8,9,10) were used to calculate amount of sand applied, which was 24, 46, and 37 ounces respectively. Smaller pools not measured within the reach were also lightly treated with the remaining 5 oz. of sand from the container. Backpack sprayers charged with 200ml of Antimycin and 35ml of detergent were used to treat isolated pools, slack water and the stream margins.

#### Results

Visual observations of fish response within the livecars were begun at 6:00 pm at drip station 1. Observations progressed downstream to the end of the reach about 6:45 pm. Observations of fish health made were:

## **Beginning at 50ppb drip station**

0 11	-
25meters	6 fish total, all dead
50meters	6 fish total, all dead
75meters	6 fish total, all dead
100meters	6 fish total, all dead

125meters	4 fish total, all dead
150meters	4 fish total, all dead
At 75ppb drip stati	on
175meters	4 fish total, all dead
200meters	4 fish total, all dead
225meters	4 fish total, all dead
250meters	4 fish total, all dead
275meters	8 fish total, all dead

After each live car observation was made the fish were removed and the live cars pulled from the creek. The drip stations were rinsed and disassembled following the observations. All equipment was packed up and loaded. All observations were based on fish held in live cars. Observations of fishes living in the creek were not made or recorded.

#### Conclusion and Discussion

Observations of response by green sunfish to Fintrol application were very positive. At 50 ppb over a four-hour exposure time, green sunfish mortality was observed to be 100%. Undoubtedly, the use of Antimycin-coated sand and backpack sprayers helped. By bringing large pools to a concentration of 50 ppb instantly with the sand, they serve as an additional drip station and oxidation of the antimycin by natural factors while traversing the pool becomes less of an issue. Additionally, treatment of slow-moving side channels and backwaters helped to maintain the target concentration of 50 ppb. Based on the results from this bioassay and the previous ones, Successful renovation of Fossil Creek should be possible with the application of 50 ppb of Fintrol, with the treatment of the area of high iron concentration requiring application at a rate of 100 ppb.

REACH	POOL#	U Coor	TM dinates	WIDTH FT	LENGTH FT	MEAN DEPTH FT	VOLUME ft <sup>3</sup>	Flush hours @.5cfs	acrefeet	Sand in oz. for 50ppb
1A	01	447139	3808808	32	99	9	28512	16	0.65	63
1A	02A	447114	3808787	25	34	5	3910	2	0.09	9
1A	02B	447114	3808787	66	67	8	35376	20	0.81	78
1A	03	447002	3808726	23	61	3	4209	2	0.10	9
1A	04	446882	3808697	28	21	4	2352	1	0.05	5
1A	05	446870	3808690	42	173	7	50862	28	1.16	112
1A	06	446626	3808638	35	85	3	8925	5	0.20	20
1A	07	446531	3808857	25	75	5	9375	5	0.21	21
		Rea	ch 1A Subto	otal			143521		3.29	316
1B		446498	3808867			Sunfis	sh Barrier			0
1B	8	446457	3808865	23	57	4	5244	3	0.12	12
1B	9	446323	3808779	19	91	4	6916	4	0.16	15
1B	10	446258	3808746	32	245	4	31360	17	0.72	69
1B	11	446161	3808705	14	56	3	2352	1	0.05	5
1B	12	446106	3808703	26	130	4	13520	8	0.31	30
1B	13A	445903	3808814	31	66	4	8184	5	0.19	18
1B	13B	445903	3808814	31	96	3	8928	5	0.20	20
1B	14	445633	3808838	34	102	3	10404	6	0.24	23
1B	15	445357	3808754	25	92	3	6900	4	0.16	15
1B	16	445166	3808659	23	87	4	8004	4	0.18	18
1B	17	444737	3808482	40	147	4	23520	13	0.54	52
1B	18	444619	3808457	38	86	4	13072	7	0.30	29
1B	19	444448	3808329	34	28	4	3808	2	0.09	8
1B	20	444440	3808172	34	20	6	4080	2	0.09	9
1B	21	444440	3808172	30	114	4	13680	8	0.31	30
Re	each 1B subt	otal					159972	89	3.66	352
2		444456	3808113			wa	terfall			0
2	22	444431	3808087	71	76	8	43168	24	0.99	95
2	23	444468	3807957	43	89	3	11481	6	0.26	25
2	24	444504	3807849	63	59	3	11151	6	0.26	25
2	25	444468	3807768	44	42	5	9240	5	0.21	20
2	26	444363	3807716	45	69	4	12420	7	0.28	27
2	27	444232	3807675	36	84	3	9072	5	0.21	20
2	28	444206	3807661	47	84	4	15792	9	0.36	35
2	29	444148	3807633	25	31	4	3100	2	0.07	7
2	30	444046	3807441	44	83	7	25564	14	0.59	56
2	31	444000	3807278	32	140	4	17920	10	0.41	39
2	32	443687	3807083	31	152	3	14136	8	0.32	31
2	33	443553	3807095	32	87	3	8352	5	0.19	18
2	34	443402	3806974	30	112	3	10080	6	0.23	22
R	each 2 Subto	otal					191476	106	4.38	421
Reach 1B and 2 T				otals			351448	195	8.05	773

APPENDIX 5. FOSSIL C	CREEK POOL VOLUME CALCULATIONS FOR REACHES 1-4.

REACH	POOL#	UTM Coordinates		WIDTH FT	LENGTH FT	MEAN DEPTH FT	VOLUME FT <sup>3</sup>	acrefeet	Sand in oz. for 50ppb
3A	1a	443275	3806820	53	105	6.3	35060	0.80	77
3A	1b			35	96	4.3	14448	0.33	32
3A	2	443287	3806766	52	98	3.3	16817	0.39	37
3A	3	443300	3806723	40	250	3.3	33000	0.76	73
3A	4	443125	3806561	41	102	4.3	17983	0.41	40
3A	5	443099	3806555	40	75	4.7	14100	0.32	31
3A	5b			46	120	6.0	33120	0.76	73
3A	6	443072	3806543	60	150	4.0	36000	0.83	79
3A	7	442985	3806536	21	131	3.3	9078	0.21	20
3A	8	442941	3806528	35	100	4.7	16450	0.38	36
3A	9	442890	3806525	40	120	3.0	14400	0.33	32
3A	9b			30	100	4.0	12000	0.28	26
3A	10	442800	3806549	35	100	5.3	18550	0.43	41
3A	11	442755	3806532	28	195	9.3	50778	1.17	112
3A	12	442625	3806478	50	46	3.7	8510	0.20	19
3A	12b			37	140	3.7	19166	0.44	42
3A	13	442516	3806308	40	321	4.7	60348	1.39	133
3A	13b			33	140	4.0	18480	0.42	41
3A	14	442222	3806220	25	190	3.3	15675	0.36	35
3A	14b			35	230	4.7	37835	0.87	83
3A	15	442066	3806025	28	400	4.0	44800	1.03	99
3A	16	442145	3805851	45	350	9.3	146475	3.36	323
Re	ach 3A Sub	total					673072	15.45	1483
3B	17	441826	3805717	35	206	3.0	21630	0.50	48
3B	18	441690	3805699	40	175	8.3	58100	1.33	128
3B	19	441096	3805877	28	144	3.3	13306	0.31	29
3B	20	440909	8305596	29	250	3.3	23925	0.55	53
3B	21	440623	3805735	32	250	4.0	32000	0.73	71
Re	ach 3B Sub	total					148961	3.42	328
3 (4A)	22	440471	3805582	46	140	5.3	34132	0.78	75
3 (4A)	23	440384	3805380	23	135	4.3	13352	0.31	29
3 (4A)	24	440270	3805346	35	147	4.5	23153	0.53	51
3 (4A)	25	439878	3805201	30	176	6.3	33264	0.76	73
4A	26	439432	3804985	32	152	3.6	17510	0.40	39
4A	27	439497	3804323	30	250	3.7	27750	0.64	61
4A	28	439514	3804218	25	318	3.7	29415	0.68	65
Re	ach 4A Sub	total					178575	4.10	394
4B	29	439525	3803725	29	120	4.3	14964	0.34	33
4B	30	439525	3803542	53	99	6.7	35155	0.81	77

# Pool locations, measurements and calculations for reaches 3-4

Fossil Creek Native Fish Restoration Project	
November 2005	

REACH	POOL#	UTM Coordinates		WIDTH FT	LENGTH FT	MEAN DEPTH FT	VOLUME FT <sup>3</sup>	acrefeet	Sand in oz. for 50ppb
4B	31	439505	3803360	31	150	3.0	13950	0.32	31
4B	32	439586	3802836	20	105	4.3	9030	0.21	20
4B	33	439403	3802623	35	151	3.3	17441	0.40	38
4B	34	439403	3802438	20	75	3.3	4950	0.11	11
4B	35	439450	3802354	27	200	3.3	17820	0.41	39
4B	36	439035	3801649	32	110	3.3	11616	0.27	26
4B	37	438951	3801543	25	125	3.7	11563	0.27	25
4B	38	438903	3801427	35	225	2.7	21263	0.49	47
Reach 4B Subtotal							157750	3.62	348
Reach 3 Total							822033	19	1812
Reach 4 Total						336326	8	741	

# APPENDIX 6. A MODIFIED BUCKET SYSTEM FOR DISPENSING CHEMICALS INTO STREAMS.

A 19 L bucket was modified to provide a constant outflow rate for dispensing piscicides into streams for the removal of unwanted fishes. The design was modified from that provided by Stefferud and Propst (1996). The modification herein described reduces the need for silicon sealant in the field, and airtight seals can be accomplished using mechanical means (o-rings and nuts). The physics involved in the operation remain the same as other mariotte bottles.

<u>Materials: (Figure 1)</u> five gallon bucket airtight bucket lid with screw cap access 1 - 1/4x1/8 threaded brass straight valve

- (or needle valve) 1 - 1/8 x 1/8 adapter (not needed if use
- 1 1/8 x 1/8 adapter (not needed if use needle valve
- 2 3/8"o-rings
- 2 brass nuts
- 1 –1/4 x 1/8 90 degree compression elbow
- 16" of 1/4" copper tubing
- 1 compression nut
- 1 roll of teflon tape



Figure 1. Parts used to assemble Mariotte Bottle.



Figure 2. Hole placement on bucket.

# Assembly Steps:

- 1. Drill two holes in the bottom of the bucket with a 3/8 " drill bit, being careful not to wobble and accidentally enlarge the hole. One hole  $\frac{1}{2}$ " above the bottom of the bucket and one hole 1" above the bottom of the bucket (Figure 2).
- 2. Wrap threads of needle/straight valve with teflon tape and screw into lowermost hole of the bucket. Place one o-ring over the threads and install and tighten brass nut on threads.
- 3. Wrap threads of 90 degree elbow with teflon tape and screw into hole in bucket 1" up from bottom. Place second o-ring over threads, install second brass nut and tighten.

- 4. Install <sup>1</sup>/<sub>4</sub>" copper tubing with compression nut onto 90 degree elbow extending upwards above the top of the bucket (Figure 3).
- 5. Fill bucket full with water, place airtight lid on securely and add piscicide through removable screwcap lid. Shake thoroughly to mix piscicide and set dispensing rate.

#### Comments:

I used parts locally purchased at True Value for about \$15. The buckets, lids and brass fittings could likely be purchased at lower prices if bought in bulk. They did not have the needle valves, thus my need to buy a straight valve and add the adapter to extend it far enough from the bucket to allow adjustment of the valve. It would be quite simple to add an additional piece of copper tubing to the valve outflow, which came with a compression fitting, thus allowing the drip from the bucket to extend further out into the stream, likely improving mixing.



Figure 3. Assembled bucket





Fossil Creek post-renovation monitoring priorities for selected areas in Reaches 1-2.



Fossil Creek post-renovation monitoring priorities for selected areas in Reach 3.



Fossil Creek post-renovation monitoring priorities for selected areas in Reach 4.