Bonita Creek Native Fish Restoration: Native Aquatic Species Salvage, Chemical Renovation, and Repatriation of Native Aquatic Species

May, 2009

Anthony T. Robinson
Codey Carter
David Ward
Arizona Game and Fish Department
5000 W. Carefree Highway
Phoenix, AZ 85086

Heidi Blasius
U.S. Department of Interior
Bureau of Land Management
Safford Field Office
711 14th Avenue
Safford, Arizona 85546

Arizona Game and Fish Department Mission

To conserve, enhance, and restore Arizona's diverse wildlife resources and habitats through aggressive protection and management programs, and to provide wildlife resources and safe watercraft and off-highway vehicle recreation for the enjoyment, appreciation, and use by present and future generations.

The Arizona Game and Fish Department prohibits discrimination on the basis of race, color, sex, national origin, age, or disability in its programs and activities. If anyone believes they have been discriminated against in any of AGFD's programs or activities, including its employment practices, the individual may file a complaint alleging discrimination directly with AGFD Deputy Director, 5000 W. Carefree Highway, Phoenix, AZ 85086, (623) 236-7290 or U.S. Fish and Wildlife Service, 4040 N. Fairfax Dr., Ste. 130, Arlington, VA 22203.

Persons with a disability may request a reasonable accommodation, such as a sign language interpreter, or this document in an alternative format, by contacting the AGFD Deputy Director, 5000 W. Carefree Highway, Phoenix, AZ 85086, (623) 236-7290, or by calling TTY at 1-800-367-8939. Requests should be made as early as possible to allow sufficient time to arrange for accommodation.

ACKNOWLEDGEMENTS

The restoration of native aquatic species to Bonita Creek was a multi-agency effort: Codey Carter of Arizona Game and Fish Department led overall activities and the chemical renovation, Heidi Blasius of U.S. Bureau of Land Management of led the aquatic species salvage and restocking efforts, and David Ward of Arizona Game and Fish Department set up and ran the on-site fish holding facility. Other individuals from these and other agencies and entities that provided invaluable support were: Chris Cantrell, Jamie Evans, Amy Bailey, Tony Robinson, Ross Timmons, Suzanne Ehret, Jason Kline, Tim Grosch, Mike Lopez, and Jeff Sorensen of Arizona Game and Fish Department; Mary Richardson, Chuck Minckley, Glen Knowles, Jeremy Voeltz, David Smith, and Jennifer Johnson of U.S. Fish and Wildlife Service; Jeff Simms, Jeff Conn, and Joy Price of U.S. Bureau of Land Management; Rob Clarkson and Andrew Ashby of U.S. Bureau of Reclamation; Lisa Brooke McDonough and Peter Reinthal of University of Arizona; Matt O'Neill and Cassie Ka'apu-Lyons of Northern Arizona University, Paul Marsh and Jeremy Pennel of Arizona State University; and volunteers Kyle Simms, Sally Stefferud, Elizabeth Stewart, Dara Stewart, and Jamie Ashby. The Sonora mud turtle salvage crew consisted of Arizona Game and Fish Department personnel Tom Jones, Cristina Jones, Audrey Owens, and Christina Akins, and Mike Barker a volunteer. Special acknowledgement is also given to the City of Safford, who both supported the project goals and provided access to their Bonita Creek water supply facility that facilitated the native fish salvage and holding efforts.

Recommended Citation:

Robinson, A. T., C. Carter, D. Ward, and H. Blasius. 2009. Bonita Creek native fish restoration: native aquatic species salvage, chemical renovation, and repatriation of native aquatic species. Arizona Game and Fish Department, Phoenix.

Bonita Creek Native Fish Restoration: Native Aquatic Species Salvage, Chemical Renovation, and Repatriation of Native Aquatic Species

INTRODUCTION

Southwestern native fishes are among the most imperiled group of aquatic species in the United States (Rinne and Minckley 1991, Minckley and Deacon 1991). Of 18 freshwater native fish species in the Gila River Basin (Minckley 1999; not including the extinct Santa Cruz pupfish *Cyprinodon arcuatus*), 12 (67%; as of December 2008) are listed as federally threatened or endangered. Reasons for decline of native fishes include habitat loss and destruction, and negative interactions (predation, competition, hybridization, parasite transmission) with non-native aquatic organisms (Miller 1961, Minckley and Deacon 1991). Six native fish species (Gila chub *Gila intermedia*, longfin dace *Agosia chrysogaster*, speckled dace *Rhinichthys osculus*, desert sucker *Catostomus clarki*, Sonora sucker *Catostomus insignis*, and razorback sucker *Xyrauchen texanus*, but the later was stocked in 1987) have been documented in Bonita Creek, a tributary to the Gila River near Safford, Arizona (Minckley 1973; U.S. Bureau of Reclamation and U.S. Bureau of Land Management 2007). In addition several nonnative species invaded the lower ~6 km of Bonita Creek and include green sunfish *Lepomis cyanellus*, smallmouth bass *Micropterus dolomieu*, fathead minnow *Pimephales promelas*, red shiner *Cyprinella lutrensis*, common carp *Cyprinus carpio*, mosquitofish *Gambusia affinis*, channel catfish *Ictalurus punctatus*, black bullhead *Ameiurus melas*, and yellow bullhead *Ameiurus natalis* (U.S. Bureau of Reclamation and U.S. Bureau of Land Management 2007).

A portion of the Gila River and Bonita Creek northeast of Safford, Arizona were designated the Gila Box Riparian National Conservation Area (RNCA) in 1990. Bureau of Land Management (BLM), who administers the land, developed a comprehensive management plan for the Gila Box RNCA (U. S. Bureau of Land Management 1998). A principal objective of the RNCA Management Plan is to maintain or enhance populations of threatened, endangered, and other priority species. Management actions prescribed in the RNCA Management Plan include reintroduction of native fishes and construction of a fish barrier in Bonita Creek. Species under consideration for reintroduction included razorback sucker, spikedace *Meda fulgida*, loach minnow *Rhinichthys cobitus*, desert pupfish *Cyprinodon macularius*, and Gila topminnow *Poeciliopsis occidentalis*. The biological opinion issued by the U. S. Fish and Wildlife Service (USFWS) for construction of the barrier, chemical treatment of the stream, and reintroduction of native fishes (U.S. Fish and Wildlife Service 2007) concluded the project was not likely to jeopardize the continued existence of Gila chub, Gila topminnow, desert pupfish, spikedace, and loach minnow, and no destruction or adverse modification of critical habitat was anticipated. The Bureau of Reclamation (Reclamation) issued a Finding of No Significant Impact, and BLM issued a decision notice that construction and operation of the fish barrier and other proposed efforts to restore a native fishery in Bonita Creek would not be major Federal actions that would significantly affect the quality of the human environment.

The Reclamation-funded fish barrier was completed in September 2008. Restoration of the native fish assemblage began soon afterwards, and is the subject of this report. The objectives

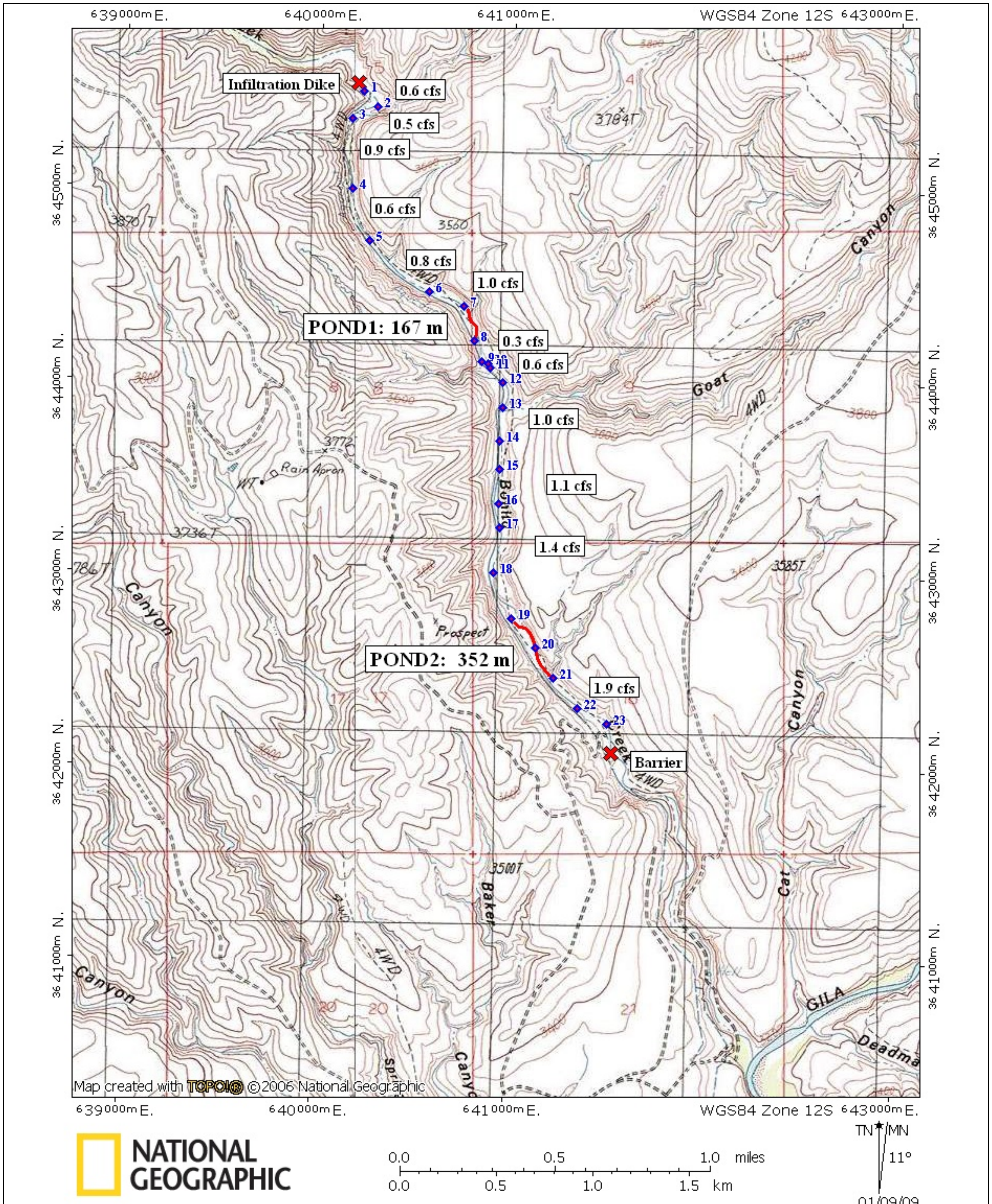

Figure 1. Map showing location of project area (Bonita Creek, Arizona), two beaver ponds in the project area, estimated locations of drip stations derived from a dye test during June 2008, and discharge measured during June 2008.

were to salvage native aquatic vertebrate species (Sonora mud turtles and native fishes) from a 2.6 mile reach of Bonita Creek above the barrier (Figure 1), eradicate nonnative fishes, and then to restock the salvaged species and repatriate several threatened or endangered fish species. The restoration activities were accomplished jointly and cooperatively by Arizona Game and Fish Department, BLM, Reclamation, USFWS, University of Arizona, Arizona State University, Northern Arizona State University, and volunteers.

SITE DESCRIPTION

Bonita Creek is a south flowing tributary to the Gila River, northeast of Safford, Arizona (Figure 1). Bonita Creek originates in highlands (e.g., Ash Flat, Nantac Rim, Gila Mountains) on the San Carlos Apache Reservation and drains southeast to the Gila River. The majority of the ~22 km stretch from the confluence with the Gila River upstream to the reservation boundary is on BLM lands. The City of Safford has an infiltration gallery dike across the streambed about 6.3 km upstream from the confluence; a pipeline from the dike delivers water to the city. Upstream of the dike, only native fishes are present; downstream of the dike both native and nonnative fishes are present. A Reclamation-funded fish barrier was built approximately 2 km upstream of the confluence during the summer of 2008. The reach targeted for renovation (project area; Figure 1) was approximately 4.3 km long and was located between the infiltration gallery dike and the constructed fish barrier.

There were two large beaver pond complexes in the project area (Figures 1 and 2) during June-September 2008. One was approx 167 m long and the other was 352 m long with an average depth of approximately 1 m in both. There were a lot of stagnant areas, backwaters and undercut banks associated with these slack waters that would have been difficult to treat. The beaver

Figure 2. A portion of the 167 m long beaver pond complex in Bonita Creek during June 2008.

dams were breached before salvage of native fishes then breached again immediately before the chemical renovation. Though the size of the ponds was reduced by breaching and draining, there were still considerable amounts of slack-water habitat remaining.

AQUATIC SPECIES SALVAGE AND HOLDING OPERATION

Methods

Salvage

Native fish were salvaged from Bonita Creek during October 5-7, 2008. To facilitate the salvage, the reach targeted for renovation was delineated into three manageable (approximately 1.4 km each) sub-reaches and 46 beaver dams were temporarily breached to reduce water volume and backwater habitat. A salvage crew led by an experienced fisheries biologist was assigned to each of the three sub-reaches and was responsible for salvaging native fish within their assigned reach. To capture as many native fish as possible all major habitats (*i.e.*, riffles, runs, and pools) were sampled using a variety of different methods including backpack electrofishing (Smith-Root Inc. models LR24 and 12-B), seining (straight seines with 3 mm mesh), minnow traps (cylindrical metal traps with 3mm mesh and Promar® collapsible minnow traps 0.46 m long x 0.3 m wide, with 2 mm mesh), and hoop-nets (1.4 m long x 0.5 m wide, with 25 mm mesh and 1.7 m long x 0.8 m wide, with 25 mm mesh). Each reach was electrofished throughout its entirety at least once and if native fish were abundant within the reach (i.e., upper and middle reaches) it was electrofished in its entirety three times over the three days. On the third day of the salvage all crews targeted habitat of the least-commonly captured native fish and focused efforts within the upper two reaches to capture and salvage as many native fish as possible; the upper two reaches supported the majority of native fish. Salvage efforts focused on all native fish species with an emphasis on acquiring as many endangered Gila chub as possible. Nonnative fishes that were captured during the salvage operations were euthanized with tricaine methanosulphonate (MS-222). Salvaged native fish were placed into five gallon buckets that were equipped with battery-powered aerators and transported to the temporary holding facility.

Sonora mud turtle *Kinosternon sonoriense* were also salvaged from Bonita Creek during October 5-7, 2008. Sonora mud turtles were captured using hoop nets (dimensions) and Promar® collapsible minnow traps (0.46 m long x 0.3 m wide, with 2 mm mesh) that were baited with a 0.25-0.5 full can of sardines. Six to eight traps and 26 hoop nets were set around noon on October 5, and checked during the morning of October 6, then reset and checked again during the morning of October 7. Hoop nets and minnow traps were set in the vicinity of 12 of the 15 road and creek crossings within the treatment area and near the barrier; 1-4 hoop nets and 0-2 minnow traps were set at each location, usually half downstream and upstream of each road crossing. Turtles from all nets and traps at a crossing or pair of crossings were combined and processed because of limited number of 5-gal buckets. We measured and recorded midline carapace length (mm), plastron length (mm), and mass (g) of each turtle. We marked each turtle with a unique number by filing notches into a combination of marginal scutes (i.e., each marginal scute represents one number, so filing a notch in marginal scute 40, 2, and 6 represents turtle 48). After processing, turtles were transported in 5-gal buckets to the temporary holding facility.

Invertebrates were salvaged from throughout the target reach of Bonita Creek on October 6 -7, 2008. Invertebrates on the surface of the water or in the water column were captured using dip and sweep nets. Invertebrates found attached to rocks or other substrates were hand captured.

Captured invertebrates were held in 5-gal buckets until they could be transported to the temporary holding facility.

Holding

We developed a portable fish holding system that could be easily transported and set up at Bonita Creek for maintaining native fish salvaged during the chemical renovation (Figure 3). The holding tank system consisted of six circular, 6-foot-diameter (500 gallon) collapsible tanks made of 22 oz. Polyester Scrimmed Vinyl, manufactured by Pearls of Paradise®. Each tank comes with a custom ¼ inch mesh net cover to prevent fish from jumping out. Two air stones provided aeration to each tank. Air was supplied by a Sweetwater® linear piston air pump (9 watts) that operated using a 12 volt deep cycle battery. The battery was recharged every 48-hours with a small portable generator. Each tank was fitted with a standpipe made of slotted 1 ½ inch well pipe to maintain consistent water level and allow water changes while preventing escapement of fish. We fitted each tank outlet to a common 3 inch PVC drain that extended down slope using Uniseals®, so that water could flow through each tank. One to three gallons per minute of fresh water was continuously piped to each tank from the nearby City of Safford water line; this prevented buildup of fish waste and maintained water temperatures at or near 20°C. One of the major difficulties with maintaining wild caught fish during salvage operations is the high likelihood of parasite and disease outbreaks. *Ichthiopthirus multifillis* or Ich is prevalent on wild fish and commonly causes high mortality when fish are confined at high densities. To prevent Ich outbreaks we treated fish with salt at 3 ppt every night for 8-12 hours. A low-range digital salt meter was used to verify salinities in each tank. Salt treatments were done at night because daytime temperatures were too warm to stop water flow during the day. Salt at 3ppt interrupts the life cycle of Ich (Selosse and Rowland 1990, Mifsud and Rowland 2008), reduces fish stress, and decreases nitrite, a byproduct of fish waste. Ammonia and nitrite levels were checked every 4-6 hours in each tank to verify that fish waste products were not becoming toxic during salt treatments. Fish were fed once daily a combination of freeze dried bloodworms and small sinking pellets. All fish were sorted by size when placed into the tanks to prevent larger fish from eating or damaging smaller fish. Each tank was filled with several large rocks and root wads to provide places for fish to hide.

Figure 3. Collapsible tank setup used for holding native fish during the Bonita Creek renovation.

Turtles were kept in hard plastic 'kiddie' pools (~1.5 m diameter and 0.3 m deep) adjacent to the fish holding tanks. Turtles from each station or pair of stations were kept in separate kiddie pools (labeled with station numbers) so that they could be returned to their respective areas of capture after holding. Chicken wire was attached over the top of the pools to keep predators out.

Invertebrates were held in an oval shaped 200-gal plastic tank, adjacent to the fish holding tanks. Prior to invertebrate collection, rocks and branches of various sizes were placed into the tank and it was filled with water piped from the City of Safford water line.

Results

Two-hundred and one adult Sonora sucker, 335 juvenile Sonora sucker, one desert sucker, 53 adult Gila chub, 180 juvenile Gila chub, 107 longfin dace, and 25 speckled dace were salvaged from Bonita Creek. These native fish were held for up to 12 days and then returned to the stream. One adult Sonora sucker and three small Gila chub died during holding; the Sonora sucker died shortly after being placed in the holding facility. No parasites or pathogens were observed on the fish during holding, but a few small chub had signs of fungus (likely a result of skin injuries during capture that had become secondarily infected in the holding tanks) before they were released back into Bonita Creek.

We captured 32 turtles over the 2 days of trapping, all in hoop nets (Table 1). In addition, two turtles were captured by hand and eight were captured by fish biologists. Forty-one of the captured turtles were adults (17 females 24 males), and one was a juvenile. There were no mortalities of turtles during the salvage or the holding period. We noted old injuries on several turtles: two were missing a hind foot, one had a bulging eye, one turtle had a scar indicating a crushing injury, and one had a wart-like growth on a hind leg. All of these turtles were otherwise healthy.

Table 1. Locations and numbers of Sonora mud turtle captured during October 5-7, 2008. Road crossings were numbered from upstream to downstream (number 1 was the road crossing just downstream of the infiltration gallery and number 15 was the crossing just upstream of the barrier). Coordinates are NAD 27.

	Upper end coordinates		Lower end coordinates		
Road Crossings	Easting	Northing	Easting	Northing	# Turtles
1 and 2					5
3 and 4			640126	3644952	1
5 and 6	640694	3644428	640793	3644244	5
7 and 8	640982	3643972	640946	3643886	6
9 and 10	640952	3643635	640940	3643194	10
11 and 12	640945	3643194	640939	3643023	3
13 and 14	640952	3642890	641225	3642428	6
15 and barrier	641448	3642295	641555	3642068	4
Unknown					2
Total					42

A variety of invertebrates were collected and held during the salvage. Taxa included: Gastropoda (snails), Hirudinea (leeches), Amphipoda (amphipods), Arachnida (fishing spiders), and various Insecta including Diptera larvae, Odonata larvae, Coleoptera (diving beetles), and Hemiptera (e.g., water boatmen, back swimmers, and giant water bugs).

CHEMICAL RENOVATION

Methods

A chemical treatment plan was developed in June 2008, and modified based on reviewer comments and conditions in Bonita Creek the week before renovation. The plan provided a rough estimate of chemical and personnel needs, and basic treatment strategies for the renovation. A commercial formulation of rotenone (CFT Legumine™ Prentiss Inc.; 5% rotenone) was chosen to kill fish in the stream. For initial planning purposes, the maximum concentration specified by the label (2ppm) was used because yellow bullhead and common carp were present in the stream, both of which have high tolerances to rotenone (CFT Legumine™ label). The project area was visited in early June 2008 and October 2008 and discharge and chemical travel times were measured, and visual assessments of stream geomorphology (tributaries, springs, beaver ponds, backwaters) were made. A visual survey of the watershed was made during June 2008 to determine potential sources of re-invasion of nonnative fishes (i.e. stock tanks, tributary canyons). Two consecutive days of chemical treatment (two treatments) were planned.

Chemical Application

Estimation of Discharge, CFT Legumine Concentration, and Chemical Travel Time

Discharge (i.e., water flow, measured in ft^3/sec) and current velocity (ft/sec) were measured during June 2008 and again in October 2008 immediately before the renovation to estimate the amount of chemical necessary to treat the stream at the desired concentration (2 ppm, but see below), and to determine amount of chemical needed in each drip station. Discharge was measured at multiple cross-sections along the length of the treatment area according to standard methods and calculated using the following equation:

$$Q = \sum (V \cdot D \cdot W)$$

where Q = stream discharge (ft^3/s), V = current velocity of cell (ft/s), D = depth of cell (ft), and W = width of cell. Current velocity was measured with a Marsh-McBirney Flow-Mate™ model 2000 flow meter. Discharge in Bonita Creek ranged from 0.06 in a beaver pond in the middle of the project area to 2.16 ft^3/s at the bottom of the reach during June 2008.

Five-gallon buckets (drip stations), modified according to Stefferud and Propst (1996) so that they would continuously release liquid, were used to dispense chemical into the stream; the buckets were originally used for the Fossil Creek renovation (Weedman et al. 2005). Buckets had a small brass valve close to the bottom, and another fitting, also close to the bottom, with an elbow on the outside facing up with a thin (~5 mm diameter) brass pipe that extended upwards past the top of the bucket lid. Rubber gaskets sealed the valve, pipe fitting, and the bucket lid to the bucket so that water would not leak out, and when the bucket was filled and the valve turned on, a vacuum would form, and hence allow liquid to be dispensed at a constant rate. Amount of chemical needed in each drip station was calculated based on the formulae given on the CFT Legumine™ label:

$$X = Y(102\ F)H$$

Table 2. Estimated amount of CFT Legumine (ml) needed for each drip station to achieve 2 ppm formulation for a six hour treatment at the discharge measured in June2008.

Station	Discharge (cfs)	Volume (ml) of CFT Legumine added to drip station for a 2 ppm concentration for a 6 hr treatment
1	0.6	734.4
2	0.5	612
3	0.9	1101.6
4	0.6	734.4
5	0.8	979.2
6	1	1224
7	1	1224
8	0.3	367.2
9	0.6	734.4
10	0.6	734.4
11	0.6	734.4
12	1	1224
13	1	1224
14	1	1224
15	1.1	1346.4
16	1.4	1713.6
17	1.4	1713.6
18	1.4	1713.6
19	1.9	2325.6
20	1.9	2325.6
21	1.9	2325.6
22	1.9	2325.6
23	1.9	2325.6
Total		30967.2 ml (8.2 gal)

where X = the ml of CFT Legumine™ added to the drip station, Y is the desired concentration (ppm) of CFT Legumine, F is the water flow (ft^3/sec), and H is the duration of the application in hours. For example, for a six hour treatment at 2 ppm and 1 ft^3/sec flow rate the amount of chemical added to the drip station would be:

$$X = 2(102*1)6 = 1224 \text{ ml}$$

Estimated volume of CFT Legumine for each treatment day (Table 2) was based on discharge measurements taken during June, 2008.

Rate of application (drip rate) of diluted CFT Legumine into the stream was determined based on Finlayson et al. (2000). We planned on a treatment time of 6 hours, which is the mid-point of the 4-8 h treatment period recommended in Finlayson et al. (2000) and on the CFT Legumine™ label. An approximately six hour duration drip was accomplished by maintaining a 53 ml/min (13 ml/15 s) drip rate for 5 gallons (18.927 L) of liquid; at a constant rate of 53 ml/min, 5 gallons would drain from the bucket in 5 hours and 57 minutes.

Drip stations were spaced at intervals equal to the distance dye traveled in 1 hr (Finlayson et al. 2000; Figure 1). Rhodamine dye was applied to the stream and followed downstream for 1 hour (referred to as a dye test) in June 2008 before the beaver dams were breached, and again during October immediately before the renovation and after the beaver dams had been breached. Results of the June 2008 dye test indicated that 23 drip stations were needed, spaced an average of 173 m apart (range of 20m to 420m apart). However, this dye test was done with the two large beaver dams intact (Figure 1). The number of stations was revised to 25 after the October 4, 2008 dye test done immediately before the renovation and after the beaver dams were breached (Figure 4); some stations were close to each other but in different channels (if a station was needed in a location where the stream was divided, a bucket was placed in each channel).

We planned to treat the stream at the maximum concentration specified by the label (2 ppm CFT Legumine™ in streams) because of presence of yellow bullhead and carp in the stream (see above). Bioassays were done on September 3, 2008 to determine concentration of CFT Legumine™ and duration of application needed to kill the target species in the stream (Table 3). Bioassay results indicated that at 2 ppm CFT Legumine™, 100% of green sunfish died within 34 minutes, and 100% of the yellow bullhead died within two hours and four minutes. Therefore, with a concentration of 2 ppm CFT Legumine™, a 6 h treatment time was estimated to be more than enough time to effectively kill all fish within the project area.

Treatment Operations

Stream discharge was measured at several cross-sections one day before treatment to ensure that the appropriate concentration of chemical was applied to the stream. The stream was treated twice, one six-hour treatment per day over two consecutive days. Eight people were needed to operate the 25 drip stations each treatment day (3-4 stations per person). Drip station operators began the drip stations in the morning (approximately synchronously), monitored drip rate approximately every half hour, and adjusted flow as necessary to keep a drip rate of 13 mm/15 seconds. Stream areas that were largely disconnected from the flow of the stream (backwaters, springs, seeps, heavily vegetated slack waters, etc.), and so unlikely to be influenced by piscicide from the drip stations, were treated using backpack sprayers containing a 10% concentration (1 L chemical in 10 L of water) of CFT Legumine™. Care was taken so that the total CFT Legumine™ applied did not exceed maximum dosage on the label. Sprayers worked together in teams of two (two teams on day 1 and three teams on day 2), one on each bank of the stream. The project area was divided into two reaches the first treatment day, each containing 13-14 stations, and three reaches each containing 8-9 stations on the second treatment day; one spraying crew was assigned to each reach. Several large pools and backwaters in the stream were treated (with sprayers) as if they were impoundments, as per instructions on the CFT Legumine™ label. Surface area and depth of these slack-water areas were measured, and the estimated acre-feet calculated. Based on the label, 1 gal of CFT Legumine would treat 0.75 acre-feet of impoundment. The volume in liters of 2 ppm concentration CFT Legumine needed for each slack-water area was calculated by dividing the acre-feet by 0.75 and then multiplying the quotient by 3.79 (L/gal). One-two people (rovers) walked back and forth throughout the project area during application of chemical each treatment day to ensure operations were going as planned, bring drip station operators or sprayers more chemical if needed, and to replace or repair any faulty equipment.

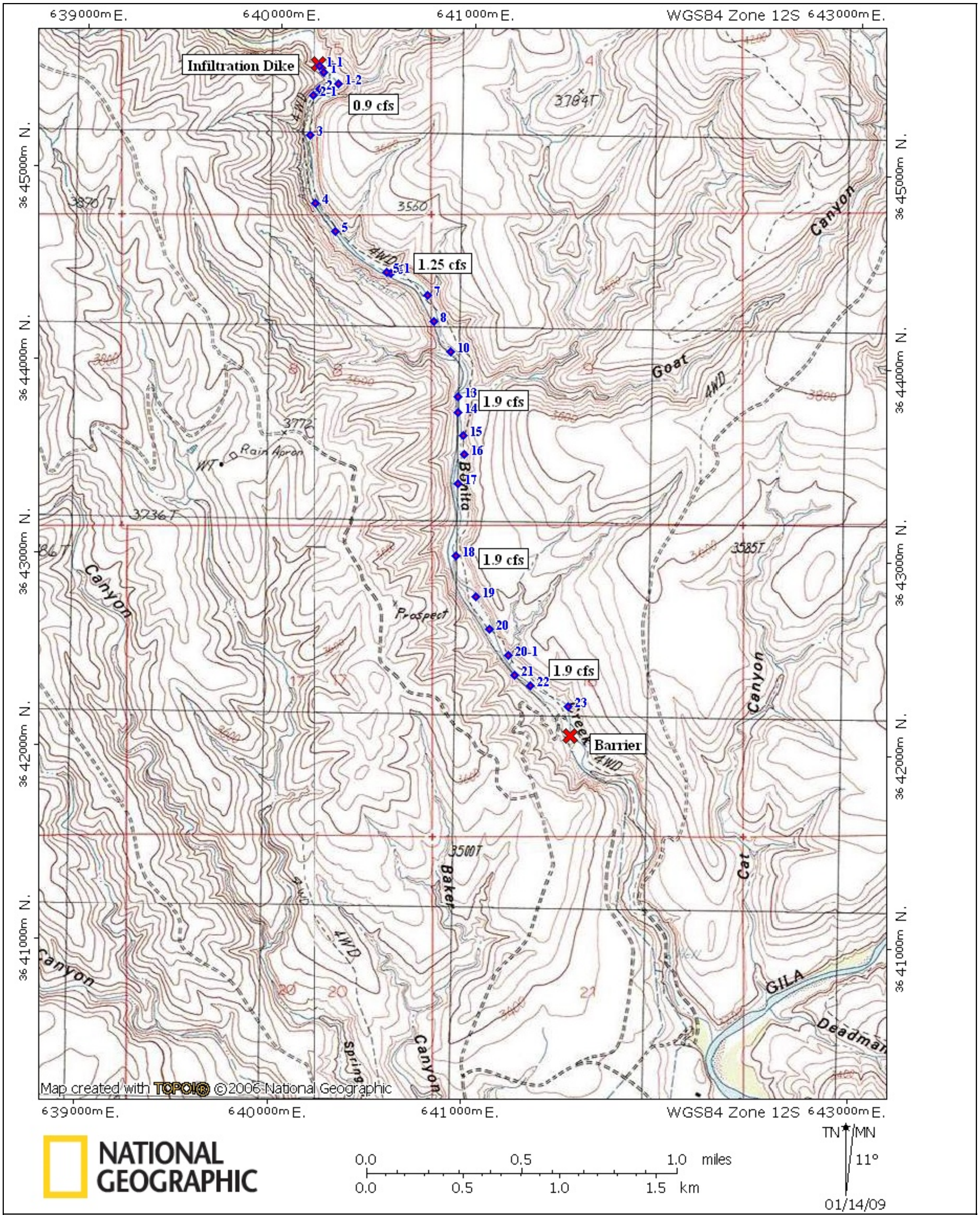

Figure 4. Map of project area showing final locations of chemical drip stations used during the October 8 and 9, 2008 treatments.

Table 3. Results of bioassays to test the effect of different CFT Legumine™ concentrations on mortality of green sunfish and yellow bullhead over a four hour period in Bonita Creek water on September 3, 2008. Water temperature in the buckets was 25°C.

Bucket number	Rotenone concentration (ppm)	Species	Number of fish	Start time	Time of final mortality	Number mortalities
1	0	Yellow bullhead	5	1250		0
2	0	Green sunfish	5	1250		0
3	0.5	Yellow bullhead	3	1252		0
		Green sunfish	3	1252	1408	3
4	1.0	Yellow bullhead	5	1252	1652	1
5	1.0	Green sunfish	5	1253	1409	5
6	2.0	Yellow bullhead	5	1250	1454	5
7	2.0	Green sunfish	5	1254	1328	5

Evaluation of Treatment Efficacy

During the chemical treatment of Bonita Creek, sentinel fish were held within cages (cylindrical live-nets with 60 mm diameter hoops, 1 cm mesh net, and floats attached to the top hoop) placed in the creek immediately upstream of each drip station (except for the uppermost drip station) to determine the efficacy of the treatment. Two to three yellow bullhead were used as sentinel fish in each live net.

After treatment on the first day crews walked the stream and removed as many dead fish as practicable and scanned the stream for any live fish. Sentinel fish were checked to see if the treatment effectively killed fish; if not corrective measures were taken (e.g., area with live fish was spayed again with chemical). After the first treatment, the drip buckets were rinsed, filled ~3/4 full with water from the stream, and set up for the second treatment. The drip stations were not charged with rotenone until immediately before the next treatment. A second set of sentinel fish were placed in the stream (same locations) immediately before the second treatment. After the second treatment, sentinel fish were checked, and crews looked for live (would indicate that neither treatment was 100% effective) and dead fish in the stream; dead fish were removed from the stream.

Beginning two days after the treatment, the stream was surveyed for any live fish for three days, using backpack electrofishers (Smith-Root Inc., models LR-24 and 12-B), Promar® collapsible minnow traps (0.46 m long x 0.3 m wide, with 2 mm mesh), and hoop nets (1.4 m long x 0.5 m wide, with 25 mm mesh and 1.7 m long x 0.8 m wide, with 25 mm mesh); if live fish were found, another chemical treatment was planned. For electrofishing, the entire treated reach was surveyed in a single pass, moving upstream from the barrier to the infiltration gallery. Hoop nets were set for 2-3 nights in three reaches (lower = from the barrier upstream to the campground, middle = from the campground upstream to the road crossing above station 7, and upper = from the road crossing to the infiltration gallery). Eight hoop nets were set in the upper reach for three nights (October 11-13), 14 hoop nets were set in the middle reach for two nights (October 12-

13), and 17 hoop nets were set in the lower reach for two nights (October 12-13); all were baited with either Aquamax pellets or dry dog food.

Sentinel fish (four Sonora sucker) were also placed into each reach of the project area on October 13, 2008 and held for 48 h to determine if the chemical was remaining and still toxic to fish. If the sentinel fish survived for 48 h, the stream was considered safe for restocking (Finlayson et al. 2000).

Neutralization

A neutralization station was set up immediately downstream of the fish barrier. Sodium permanganate ($NaMnO_4$) was used to neutralize the rotenone. The CFT Legumine™ label gives directions for use of a 2.5% solution of potassium permanganate at a resultant stream concentration of 2-4 ppm to detoxify CFT Legumine™. However, $NaMnO_4$ can also be used, and comes premixed in a 20% solution (8 times as concentrated as the recommended potassium permanganate solution). Application rate for $NaMnO_4$ was determined by calculating the application rate for potassium permanganate based on the formula given on the CFT Legumine™ label, and dividing the result by 8.

Application rate of potassium permanganate was determined by the formula $X = Y(70F)$; where X = ml of 2.5% permanganate solution per minute, Y = ppm of desired permanganate concentration, and F = cubic feet per second of stream flow. Our desired permanganate concentration was 6 ppm, which was the mid-point of the range of ratios of permanganate to rotenone (2:1 to 4:1) recommended in Finlayson et al (2000); i.e., a 3:1 ratio. Based on stream flow (2 ft^3/sec) measured at the lower end of the project area in June 2008 and a desired permanganate concentration of 6 ppm the estimated rate of application rate for potassium permanganate was:

$$X = 6*(70*2) = 840 \text{ ml/min} = .222 \text{ gal/min} = 13.32 \text{ gal/h}.$$

Dividing by 8 to determine the application rate for $NaMnO_4$ gives a result of 105 ml/min (52.5 ml/30 sec) or 1.66 gal/h. The solution was dispensed into the stream from 30-gal closed head drums; the drums used at the Fossil Creek renovation (Weedman et al. 2005). During the neutralization process, application rate was measured approximately every two hours, and adjusted to the desired rate (105 ml/min) if necessary. A dye test was done to determine chemical travel time and to determine distance downstream for a backup station. A second neutralization station was set up 1.5 h travel time downstream from the first (~150 m downstream at the first road crossing below the barrier), ready to be deployed if needed (was never run). Residual permanganate was measured with a colorimeter according to Engstrom-Heg (1971) at 30-min travel time downstream and at the first road crossing downstream of the neutralization station. Target residual was greater than 1 ppm permanganate at 30 min travel time downstream of the detox station.

Sentinel fish were used to determine if the $NaMnO_4$ applied was adequately neutralizing the CFT Legumine™, and to determine when to stop applying $NaMnO_4$ to the stream. Two cages of sentinel fish (3-5 green sunfish per net) were placed downstream of the upper neutralization station to insure that the rotenone was adequately neutralized. One live-net was placed immediately upstream of the downstream-most neutralization station. A second live-net of sentinel fish was placed approximately 30 min travel time downstream of the downstream-most neutralization station, in a large pool. Other sentinel fish (green sunfish) were used to determine when, after the end of the second treatment the CFT Legumine™ had dissipated enough to allow cessation of application of the $NaMnO_4$. Approximately 17 h after the end of the second rotenone treatment (minimum estimated flush time for the entire treated reach), water from the stream immediately above the fish barrier was placed into buckets outfitted with bubblers, and 4-5 green sunfish were added and observed. If mortality was observed, the process was repeated every 2 h during daylight hours until no mortalities were observed after holding fish for 4 h, at which time application of $NaMnO_4$ would be stopped.

Based on dye tests done in June 2008, the estimated maximum time for all water to flush out of the reach is 23 h. Adding 1 h for permanganate to oxidize initial organic demand in the neutralization contact zone at the initiation of the treatment, 6 h treatment time, and an additional 4 h added on at the end of the treatment to make sure all of the rotenone has been neutralized, equals 34 h. Since this is a back to back treatment on consecutive days the neutralization station will run continuously through both days and continue an additional minimum of 27 h after the second treatment is completed. Therefore, the estimated minimum time for operation of the detoxification station was 24 + 34 = 58 h. These numbers were for planning purposes because the actual cessation of neutralization was determined by status of sentinel fish held in buckets containing water from immediately upstream of the neutralization station (described above). The estimated minimum total amount of $NaMnO_4$ needed, based on June 2008 flow data and 58 h of application was:

$$105 ml/min * 60 min/h * 58\ h = 365{,}400\ ml = 96.5\ gal\ of\ 20\%\ NaMnO_4.$$

Safety

Applicator Safety

Before chemical application all personnel received a safety briefing. The briefing included: (1) the acute and chronic applicator exposure hazards, (2) routes of pesticide exposure, (3) symptoms of overexposure, (4) how to obtain emergency medical care, (5) decontamination procedures, (6) how to use the required safety equipment, (7) safety requirements and proper procedures for pesticide handling, transportation, storage, and disposal, and (8) environmental hazards. In cases of emergency, applicators were told to contact the emergency management system (dial 911), or the local hospital: Mt Graham Regional Medical Center , 1600 S 20th Ave, Safford, AZ (phone = 928-348-8777). A washing facility was available on site (soap and clean water) to wash off any chemical that might be spilled on the body, and emergency eye wash stations were placed at the chemical loading and neutralization stations.

Applicators were required to wear long sleeved shirts, long pants, boots, safety glasses, and chemical resistant gloves. Personnel working with undiluted CFT Legumine™ and $NaMnO_4$ were required to wear gloves and an appropriate respirator as described on the chemical labels.

Public Health and Safety

The City of Safford operates a municipal water system in Bonita Creek. Most of the water comes from the infiltration gallery upstream of the project area, but at least one well is located in the substrate underneath Bonita Creek in the project area. The EPA approved Restricted Pesticide Label directs that any water intakes located in the treatment area, or within one mile downstream of the treatment area, be closed during treatment and detoxification. Since rotenone is strongly bound to organic matter in the substrate it is unlikely that it would enter the groundwater (Dawson et al. 1991); however, we coordinated with the City of Safford to insure that chemical would not be released into the stream while the well was operating. There are no restrictions to swimming in water treated with rotenone once it is mixed into the stream; however since undiluted rotenone formulation will be present in the project area, entry into the stream in the project area was closed to the public during the treatment. Warning and public notification signs (Figure x) were placed at all entry points into the project area during the renovations.

Results

Chemical Application and Treatment Efficacy

The first chemical treatment (renovation) was completed on October 8, 2008. Twenty-five drip stations (Figure 4 and Table 4) were operated by eight people (3-4 per person) beginning at approximately 1000 h, and finishing at approximately 1600 h. A total of approximately 13 gal of CFT Legumine™ were dispensed from drip stations each treatment day (Table 4). Concurrent with drip station operation, two crews of two people each sprayed chemical along each shoreline and into slack-water areas between each station, beginning at approximately 0830 h. At six identified pools and backwaters, spraying crews applied a pre-determined amount of chemical (Table 5). Fish began showing signs of stress within a half hour after treatment began, and dead fish were observed within the first hour after the treatment began. At the end of the first treatment dead fish were observed throughout the stream, and were removed. Within the six experimental-abundance sections dead fish were also identified to species and counted. Also at the end of the first treatment all sentinel fish upstream of all stations had died, except at one station (20-1), where one yellow bullhead was still alive, but obviously stressed. Station 20-1 was in a long backwater-side channel, and it was likely that not enough chemical from the upstream station (19) entered the side channel. The entire side channel was sprayed thoroughly again, emptying the full contents of two backpack sprayers.

A second chemical treatment was completed on October 9, 2008. Chemical was applied in a similar fashion to the first treatment day. Eight people operated 25 drip stations (3-4 stations/person) beginning at approximately 0830 h and finishing at 1430 h. Concurrent with drip station operation, three crews of two people each sprayed chemical along each shoreline and into slack-water areas between each station (and into six identified pools), beginning at approximately 0830 h. No dying or newly dead fish were observed by the drip station operators or the spraying crews. Sentinel fish were placed into the stream upstream of each station immediately before the start of the second treatment, and all were dead by the end of the second treatment.

The entire project area was surveyed for live fish on October 11-14, 2008 to determine the efficacy of the chemical treatments. No fish were captured in any of the 39 hoop net sets; 86 net-nights of effort. On October 13, 2008 the entire project area was electrofished from the barrier upstream to the infiltration gallery and no live fish were captured or observed; electricity was applied to the stream for 6,899 seconds in the lower reach, 7,653 sec in the middle reach, and 10,340 sec in the upper reach. Although no fish were observed during electrofishing, 15 bull frogs were observed in the lower reach. Because no live fish were found after the two chemical treatments, the renovation was considered a success late on October 14, 2008.

Table 4. Approximate stream discharge (ft^3/s) and volume (ml) of CFT Legumine™ added at each drip station on Bonita Creek during each treatment, October 7 and 8, 2008.

Station	Discharge (ft^3/s)	Volume (ml) of CFT Legumine™ added per station
1	1	1,224
1-1	1	1,224
1-2	1	1,224
2	1	1,224
2-1	1	1,224
3	1	1,224
4	1	1,224
5	1.5	1,836
5-1	1.5	1,836
6	1.5	1,836
7	1.5	1,836
8	1.5	1,836
10	1.5	1,836
13	2.0	2,448
14	2.0	2,448
15	2.0	2,448
16	2.0	2,448
17	2.0	2,448
18	2.0	2,448
19	2.0	2,448
20	2.0	2,448
20-1	2.0	2,448
21	2.0	2,448
22	2.0	2,448
23	2.0	2,448
Total CFT Legumine™		48,960 ml (12.93 gal)

Table5. Location, area, depth, volume, and amount of CFT Legumine™ used to treat six different pools or backwaters in Bonita Creek during each renovation (October 7 and 8, 2008). Two of the locations were complex so 2-3 area and depth measures were taken.

Location	Area (ft^2)	Depth (ft)	Volume (acre-ft)	Liters CFT Legumine™
Pool between station 8 and 10	5,481	4.92	0.619066	3.13
Pool between stations 10 and 12	2,960	1.64	0.111442	0.563
Pool between station 12 and road crossing	1,620	1.31	0.091639	0.463
	1,140	1.64		
Backwater by station 7	1,536	3.28	0.115658	0.584
Pool near old station 12	1,679	6.56	0.252852	1.278
Barrier pool and backwater	14,280	1.0	1.210744	6.118
	9,563	3.28		
	4,325	1.64		

Post-treatment sentinel fish were put in holding nets and placed into the stream at 0900 within each of the three reaches mentioned above on October 13, 2008 and held for 48 h; four Sonora sucker were held in each net. All sentinel fish survived 48 h in the stream. The project area was considered ready to restock on October 15, because no live fish were found during the post-renovation survey, and all post-renovation sentinel fish survived for 48 h.

Neutralization

The neutralization station was operated from 0600 h on October 8 until approximately 1000 h on October 11, 2008. Initially, the operator could not establish a constant drip, so the drum was moved to the middle of the barrier spillway and the long delivery tube was cut off and at 0710 h the drip was set to 55 ml/30 sec. The $NaMnO_4$ application began before the rotenone treatment to allow the permanganate to disperse throughout the stream and ensure that the desired residual concentration (> 1 ppm) was achieved at the 0.5 h flow distance. The drip rate was adjusted down or up during the neutralization process to maintain a residual permanganate concentration of greater than 1 ppm. Sentinel fish (green sunfish) were set at the first road crossing and further down in the huge pool at 1315 h on October 8. Some mortality of sentinel fish at the first road crossing was observed during the neutralization process, and drip rates adjusted upward to minimize that mortality. Sentinel fish at the furthest downstream live net remained alive throughout the two piscicide treatments and neutralization process.

On October 10, the day after the two piscicide treatments, the neutralization station operator began evaluating if CFT Legumine™ had dissipated from the system by adding sentinel green sunfish to a bucket filled with water flowing over the barrier at 0615 h; all fish were dead by 0630 h indicating that the rotenone was still active. This process was repeated four more times at approximately 2 h intervals throughout the day using two to four green sunfish for all tests but one, for which 15 mosquito fish were used; all test fish died within an hour indicating that the rotenone was still active. Sodium permanganate was dispensed from the neutralization station for an additional day, until ~1030 h on October 11, 2009. Sentinel fish placed into the stream within the treatment area on October 13 (see above) survived for 48 h, so by October 15, the water within the treated reach was no longer considered toxic to fish.

POST-RENOVATION AQUATIC VERTEBRATE STOCKINGS

Methods

Native aquatic vertebrates salvaged from Bonita Creek and held on-site were stocked back into the stream following determination that no more live fish were present within the treatment area and CFT Legumine™ was neutralized within the treatment area. Native aquatic vertebrates salvaged and returned to the stream included speckled dace, longfin dace, Gila chub, desert sucker, Sonora sucker, and Sonoran mud turtle. Plans also included stocking several federally listed native fish species (spikedace, loach minnow, Gila topminnow, desert pupfish, and razorback sucker). The preferred lineages of spikedace and loach minnow to stock into Bonita Creek were from Eagle Creek, a tributary to the Gila River just east of Bonita Creek. However, Eagle Creek populations of these two species are extremely rare (spikedace were last captured in 1989 and loach minnow were last captured in 1997), therefore the second priority lineages were Blue River loach minnow and upper Gila River (Gila Birding Area) spikedace. Stocks of both the Blue River loach minnow lineage and upper Gila River spikedace lineage were available from Bubbling Ponds Hatchery Research Facility. Bylas lineage of Gila topminnow was selected to be stocked into Bonita Creek because is the preferred lineage to use for all upper Gila River Basin (above Coolidge dam) waters. We planned to stock a mixed lineage of desert pupfish. Both Gila topminnow and desert pupfish stocks were acquired from a pond on The Nature Conservancy's Lower San Pedro River Preserve. *Lernaea cyprinacea*, a parasitic copepod, was present in the larger pond where fish were collected, so individuals were carefully examined and only those with no visible *Lernaea* attached (the adult female stage of the parasite) to them were transported to Bonita Creek. *Lernaea* were already present in Bonita Creek (see fish salvage results above) so this precaution was to reduce stress on fish in their new environment rather than preventing an introduction of the parasite into a system where it was absent.

Spikedace, loach minnow, Gila topminnow, and desert pupfish were transported to Bonita Creek within insulated coolers or fish hauling tanks filled with well water at the source (Bubbling Ponds Hatchery Research Facility or The Nature Conservancy's Lower San Pedro River Preserve). Water in the containers was aerated using battery powered aerators or regulated oxygen tanks. To reduce stress while in transport and help kill external parasites (fish originating from TNC) non-iodized salt was added to attain a ~0.6% solution or Stress Coat® was added as per directions on the label. Upon arrival at the stream, fish and water were transferred into aerated 5-gallon buckets and walked to the stocking sites. At the stocking sites, fish were tempered to the stocking site water temperature and chemistry by exchanging a quarter of the water in each 5-gallon bucket with stream water every 10-15 minutes, until water temperatures in buckets were within 1°C of stream temperatures, after which fish were released into the stocking site. Fish were observed during the tempering process and after release, and behaviors (stressed or normal) noted.

Only the first post-renovation stockings of these species are reported in this report. Additional stockings are planned for up to five years (depending on when and if species establish self-sustaining populations).

Results

Salvaged Gila chub, longfin dace, speckled dace, desert sucker, and Sonora sucker were stocked back into Bonita Creek on October 15, 2008 (Table 6). All salvaged fish and spikedace and loach minnow were stocked into appropriate habitat in the upper portion of the treated reach (upstream of the location of drip station #5), because we assumed they would naturally disperse downstream as the populations grew. Desert pupfish and Gila topminnow were stocked into pool habitat a couple of weeks later. Desert pupfish and Gila topminnow were stocked into separate locations, approximately 3.3 km apart (Gila topminnow downstream of pupfish) to obviate any potential immediate competition between the two species. All fish of all species did not appear to be stressed (i.e., behaved normally) upon release.

All 42 salvaged Sonora mud turtles were released on October 16, 2008 near where they were captured (Table 1). All turtles behaved normally upon release.

Salvaged aquatic invertebrates were released back into the upper portion of the treated reach in Bonita Creek on October 15, 2008.

Table 6. Numbers, date, and location of native fish species stocked into the treatment area in Bonita Creek after the October 8-9, 2008 chemical renovation.

Species	Date stocked	Number stocked	Reach stocked	Reach coordinates (UTM, NAD 83) Upper end	Lower end
Gila chub	10/15/2008	230	Between stations 1 and 3	640196E 3645531N	640150E 3645172N
Longfin dace	10/15/2008	107	Between stations 2 and 3	640200E 3645413N	640150E 3645172N
Speckled dace	10/15/2008	25	Between stations 2 and 3	640200E 3645413N	640150E 3645172N
Desert sucker	10/15/2008	1	Between stations 1 and 5	640196E 3645531N	640288E 3644678N
Sonora sucker	10/15/2008	201	Between stations 1 and 5	640196E 3645531N	640288E 3644678N
Loach minnow	10/15/2008	678	Between stations 1 and 2	640196E 3645531N	640200E 3645413N
Spikedace	10/15/2008	448	Between stations 1 and 2	640196E 3645531N	640200E 3645413N
Gila topminnow	10/31/2008	975	Approximately between stations 20 and 20-1-1	641187E 3642504N	640973E 3642692N
Desert pupfish	10/31/2008	147	Near station 2-1	640185E 3645367N	

DISCUSSION

Evidence presented in this report indicates that the two chemical treatments successfully eradicated all fish from the project area in Bonita Creek, and therefore salvaged native aquatic species and other native fishes were subsequently stocked. Periodic monitoring (at least annually) will be necessary to evaluate the success of the renovation and the success of the subsequent native fish stockings, and a monitoring plan needs to be developed. The goal of the renovation was to eradicate nonnative fish from the project area. The goal of the subsequent native fish repatriations is to establish populations in Bonita Creek. A species will be considered to have established (a successful repatriation) when it is reproducing to the point where it is self-sustaining (Armstrong and Seddon 2007). Therefore the objectives of monitoring will be to: 1) detect any nonnative fishes, 2) verify persistence of native fish species since stocking, 3) detect recruitment of young (and hence reproduction) into the population, 4) evaluate if relative abundance changes over time (i.e., from one monitoring period to the next), and 5) determine if species have dispersed throughout the treated reach. If nonnative fishes are found during 2009 monitoring, it may indicate that the renovation was unsuccessful, particularly if large numbers of nonnatives are found, and another renovation may be needed. After monitoring, data should be evaluated and a decision made as to whether or not augmentation stockings are needed (if a population has established another stocking may not be necessary). Regardless of monitoring results, we plan on augmenting listed-fish populations at least once, during 2009, and stocking several of the listed species (e.g., spikedace and loach minnow) upstream of the project area. Additional desert sucker and speckled dace will definitely be stocked because the numbers stocked during 2008 were likely too few to establish populations. Relatively few longfin dace and desert pupfish were stocked, but given the reproductive capacity of these species, those numbers may have been sufficient to establish populations; again, monitoring data will allow us to evaluate whether or not to augment. We also expect that periodic downstream dispersal of native fishes from above the treatment area will naturally augment the repatriated populations. If habitat is evaluated and deemed suitable, razorback sucker could be considered for stocking in the future.

REFERENCES

Armstrong, D. P., and P. J. Seddon. 2007. Directions in reintroduction biology. Trends in Ecology and Evolution 23:20-25.

Engstrom-Heg, R. 1971. Direct measurement of potassium permanganate demand and residual potassium permanganate. New York Fish and Game Journal Vol. 18, No. 2, July 1971.

Finlayson B., R. Schnick, R. Cailteux, L. Demong, W. Horton, W. McClay, C. Thompson, and G. Tichacek. 2000. Rotenone use in fisheries management: administrative and technical guidelines. American Fisheries Society, Bethesda, Maryland.

Mifsud C., and S. J. Rowland. 2008. Use of salt to control ichthyophthiriosis and prevent saprolegniosis in silver perch, Bidyanus bidyanus. Aquaculture Research 39:1175-1180.

Miller R. R. 1961. Man and the changing fauna of the American Southwest. Papers of the Michigan Academy of Science, Arts, and Letters 46:356-404.

Minckley, W.L. 1973. Fishes of Arizona. Arizona Game and Fish Department, Phoenix.

Minckley, W. L., and J. E. Deacon. 1991. Battle against extinction: native fish management in the American West. The University of Arizona Press, Tucson, Arizona.

Minckley, W. L. 1999. Frederic Morton Chamberlain's 1904 survey of Arizona fishes, with annotations. Journal of the Southwest 41:177-237.

Prentiss Inc. No date. CFT Legumine Restricted Use Pesticide Label. http://www.prentiss.com/Products/Labels/Specimen_Labels/cft_spec.pdf

Rinne, J., and W. L. Minckley. 1991. Native fishes of arid lands: a dwindling resource of the desert Southwest. General Technical Report RM-206. U. S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins Colorado.

Stefferud, J. A., and D. L. Propst. 1996. A lightweight, constant-flow device for dispensing liquid piscicides into streams in remote areas. North American Journal of Fisheries Management 16:228-230.

U.S. Bureau of Land Management. 1998. Gila box riparian national conservation area management plan. Environmental Assessment/Finding of No Significant Impact and Decision Record. Safford Field Office. Safford, Arizona.

U.S. Bureau of Reclamation and U.S. Bureau of Land Management. 2007. Draft environmental assessment, native fish restoration in Bonita Creek, Gila Box Riparian National Conservation Area, Graham County, Arizona. U.S. Bureau of Reclamation, Phoenix Area Office, Phoenix, AZ.

U. S. Fish and Wildlife Service. 2007. Biological Opinion for the Restoration of Native Fishes in Lower Bonita Creek and Implementation of a Memorandum of Understanding and 10-Year Operation Plan between the Bureau of Land Management and the City of Safford.

Weedman, D.A., P. Sponholtz and S. Hedwall. 2005. Fossil Creek Native Fish Restoration Project. Arizona Game and Fish Department, Phoenix Arizona.