Capture and Transport of Eagle Creek Roundtail Chub to Bubbling Ponds Hatchery, with Genetic/Morphometric Characterization of Eagle Creek Chubs and Plans for Population Replication

Submitted by:

Robert W. Clarkson, Bureau of Reclamation
6150 West Thunderbird Road, Glendale, Arizona 85306

Paul C. Marsh, Marsh & Associates, LLC
5016 South Ash Avenue #108, Tempe, Arizona 85282

Thomas E. Dowling, Arizona State University
School of Life Sciences, Tempe, Arizona 85287

David L. Ward, Arizona Game and Fish Department
1600 North Page Springs Road, Cornville, Arizona 86325

Submitted to:

Gila River Basin Native Fishes Conservation Program
Completion Report for Fund Transfer Tasks 3-106 and 3-107

November 24, 2009

Abstract— A proposed native fish restoration project on Blue River (San Francisco drainage), AZ-NM, includes repatriation of roundtail chub *Gila robusta*. As part of that project, chub were captured from Eagle Creek, Greenlee Co., AZ, and transported to Bubbling Ponds State Fish Hatchery (BPH) at Cornville, AZ, in October 2009. Genetic and morphometric analyses confirmed the identity of these chub as *G. robusta*. The 73 chub successfully transferred to BPH will be prophylactically treated and transferred to one of two concrete ponds constructed specifically for this project. Due to rarity of Eagle Creek chub, propagation at BPH is necessary to obtain sufficient numbers to stock Blue River. Fish either will be allowed to spawn naturally or be injected with a synthetic hormone to induce spawning. Progeny will be separated from broodstock to enhance survival and growth over the summer, and the cohort will be translocated to Blue River downstream of Fritz Ranch (19 km above the mouth) in autumn 2010. Mechanical removal of large catfishes upstream of a to-be-constructed fish barrier near the mouth of Blue River will reduce predation pressure and enhance the probability of population establishment. Augmentation of both BPH and Blue River roundtail chub will occur via periodic additions of wild-caught Eagle Creek chub over time.

INTRODUCTION

The conservation status of lower Colorado River basin populations of roundtail chub *Gila robusta* has degraded significantly during the past half-century (Bezzerides and Bestgen 2002, Voeltz 2002, Desert Fishes Team 2003), and this population segment is now warranted for listing under the Endangered Species Act (Federal Register 74[128]:32352-32387). Habitat loss and introduced non-native fishes are believed to be the primary causes of decline. The Gila River Basin Native Fishes Conservation Program (GRBNFCP) is attempting to protect and recover warm-water native species in the Gila River sub-basin in part by replicating rare populations behind constructed fish exclusion barriers. Blue River (San Francisco drainage) has been proposed for emplacement of one such barrier, and habitats there are thought suitable for establishment of roundtail chub (Marsh and Clarkson 2001). Eagle Creek, the drainage immediately west of Blue River, supports a viable but declining population of roundtail chub, and thus is an appropriate source of chub for translocation to Blue River. Replication of the Eagle Creek population is of further significance in that it is the most upstream (eastward) population remaining in the basin following the species' extirpation from the mainstem Gila River.

Given the relative rarity of roundtail chub in Eagle Creek, capture of sufficient numbers to support a direct translocation of fish from Eagle Creek to Blue River was considered unlikely. Therefore, plans were made to propagate captured individuals at Bubbling Ponds State Fish Hatchery (BPH) at Cornville, Arizona, prior to stocking offspring into Blue River. In addition, the source site for chub collection on Eagle Creek (near Sheep Wash) was between areas of occupation known to harbor roundtail and Gila (*G. intermedia*) chubs, and it was uncertain which species inhabited the proposed source area.

Here we report the results of capture efforts for chub at Eagle Creek conducted October 27-29, 2009, and the transfer of captured fish to BPH on October 29, 2009.

We also describe the holding and propagation facilities at BPH that were constructed specifically for this task, present genetic and morphometric data that characterize Eagle Creek chub species, and discuss planned procedures for propagation and eventual stocking of roundtail chub to Blue River.

The field work and pond construction were funded by the GRBNFCP. Collection and transport of roundtail chub was authorized under Arizona Game and Fish Department (AZGFD) scientific collecting permit SP731787, facilitated by Chris Cantrell (AZGFD). The first three authors, plus Brian Kesner, Guillermo Ley, Lori McIntosh (Marsh & Associates), and Stephanie Coleman (U.S. Forest Service) conducted the field work. Dowling and Marsh characterized Eagle Creek chubs genetically and morphometrically, respectively. Ward designed the chub ponds and received and cared for the fish at BPH. Many unnamed but appreciated AZGFD employees also assisted with the construction of the ponds.

METHODS

Construction of propagation/holding ponds – Anticipating the need for holding/propagation space at BPH to facilitate the stocking of Eagle Creek roundtail chub into Blue River, two 18 ft x 21 ft x 5 ft-deep concrete-lined ponds were constructed at BPH during August-October 2009 (Figure 1). The ponds were plumbed to each receive approximately 114 Lpm (30 gpm) of artesian-supplied outflow from the spikedace/loach minnow circular raceways and artificial streams constructed for the GRBNFCP (Ward 2008, 2009). Outflow from the ponds is to an off-floodplain terrace that does not connect hydrologically to Oak Creek.

Figure 1. Photos of completed roundtail chub holding/propagation ponds at Bubbling Ponds State Fish Hatchery.

Identification of Sheep Wash chub –Chubs to be transported to BPH were captured on National Forest lands from a ~3.2 km reach of Eagle Creek located approximately 1.6 km upstream of the mouth of Sheep Wash (Figure 2). This area was selected because sampling at lower Eagle Creek (accessible from Lower Eagle Creek Road) by

Marsh & Associates over the past several decades determined that roundtails there were becoming increasingly rare, and the probability that capture of more than a handful of chubs was low. Similar sampling above Sheep Wash found chubs more plentiful, but the identity of those fish was uncertain given that Gila chub is found upstream. As it was unknown where the two species segregated geographically, and to minimize the possibility that hybrids were collected, a sample of 10 fish was collected from the site on June 16, 2009, by Marsh & Associates for a detailed characterization of their identity. Specimens were preserved in 10% formalin in the field following preservation of fin clips in 95% ethanol for genetic analyses. Formalin-fixed specimens were rinsed in fresh water for 72 hrs and transferred to 50% ethanol for 21 days prior to permanent storage in 70% ethanol and subsequent morphometric analyses.

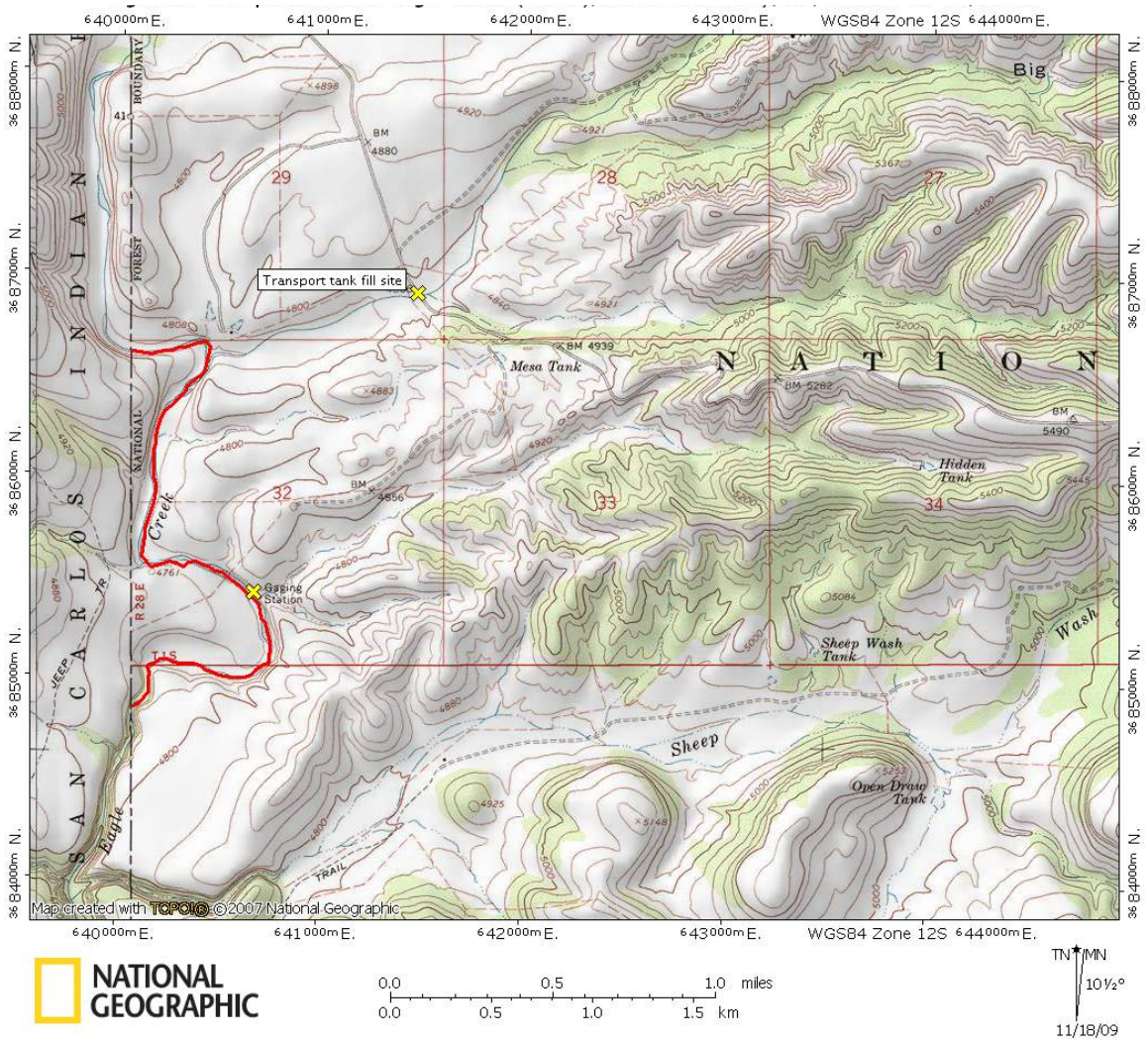

Figure 2. Reach of Eagle Creek, Greenlee Co., Arizona, where roundtail chub captures were made October 27-29, 2009 (in red).

Morphometric measurements – Standard length, head length, and caudal peduncle depth were measured on each whole specimen using Fowler Ultra-Cal Mark III digital

calipers, and the ratio of head length to caudal peduncle depth was calculated according to the primary dichotomous key distinguishing characteristics provided by Minckley and DeMarais (2000) and Minckley and Marsh (2009). Anal fin rays of each fish were counted with aid of a dissecting microscope.

Genetic analyses – Microsatellite structure of the 10 Eagle Creek fin clips from above Sheep Wash was compared to that of known *G. robusta* from Aravaipa Creek and lower Eagle Creek, to samples of known *G. intermedia* from upper Eagle Creek (near Honeymoon), lower Bonita Creek (immediately to the west of Eagle Creek), Black River (Salt River drainage), and to an undetermined sample from Eagle Creek collected near Fifteenmile Canyon approximately 14 km downstream from the Sheep Wash sampling area. Detailed methods of preparing and evaluating these samples for characterization of genetic variation were provided by Dowling et al. (2008).

Capture and transport of roundtail chub – Two teams participated in sampling: one crew deployed downstream of the USGS gage station above Sheep Wash (Figure 2), and a second sampled upstream. Primary capture gears were backpack electrofishers (Smith-Root models 12-A and 12-B) and hoop nets (2-ft diameter hoops, single 4-in diameter throat, 1/4-in mesh). Hoop nets were baited with Aquamax® 600 fish feed pellets, and were initially deployed on the afternoon of October 27. They were run and re-baited in the morning and late afternoon of October 28, and pulled in the morning of October 29. Net locations that did not capture chub were moved to new locations when appropriate. Electrofishing was performed during daylight on October 28, and typically used the setting "I-5" at 200 or 300 volts. Standard wire minnow traps of 1/4- or 1/8-in mesh and baited with Aquamax® were also deployed during October 27-28.

Captured chub were placed in 5-gal plastic buckets and transferred to centrally-located live cars in the stream until transferred to transport tanks. Non-chub fishes were identified and released alive at their point of capture. Crayfish *Orconectes virilis* were dispatched.

Twin transport tanks from BPH were each filled in the afternoon of October 28 with 132 L (35 gal) of water collected from Freeport Mac-Mo-Ran pump outflows discharged into Big Dry Creek immediately upstream of the capture reach (Figure 2). On the morning of October 29 following the final capture of chub and pulling of all hoop nets, water temperature of the transport tanks was matched to that of Eagle Creek by addition of ice to the tanks and mixture with Eagle Creek water. Approximately 3 cups of salt was added to each tank to achieve a salinity of 3 ppt to reduce stress during transport. Prior to the transfer of fish from live cars to the tanks, oxygen was supplied to each tank through diffusers. Oxygen supply and fish status was checked approximately every hour during the 7-hr transport from Eagle Creek to BPH on October 29.

RESULTS

Identification of Sheep Wash chub – Morphometric measurements of the 10 Eagle Creek chub taken from the Sheep Wash area in June 2009 are shown in Table 1. Individual and mean head length:caudal peduncle depths all were greater than 3.0, one of the main criteria rejecting a classification as *G. intermedia*. All specimens also had 9 anal fin rays, which further typifies *G. robusta* (*G. intermedia* usually has 8 rays).

The assignment probability plot of microsatellite data (Figure 3) identifies considerable diversity among samples. Plot k2 (2 groupings) clearly delineates the two species groups as predicted. Plot k4 aligns all samples of *G. robusta* from Eagle Creek and Black River, indicating greater similarity of these individuals relative to other samples examined. Plot k5 has the greatest similarity among runs (highest h'), and thus provides the most robust analysis. The five groupings show considerable diversity among *G. robusta* groups and the two *G. intermedia* groups, but there is no confusion between *G. robusta* and *G. intermedia* samples. Plot k5 of Figure 3 aligns Sheep Wash chub with known *G. robusta* from Black River and distinct from lower Eagle Creek. The previously-undetermined sample from Fifteenmile Canyon (between Sheep Wash and lower Eagle Creek) also is classified to *G. robusta* and shows some admixture between the two aforementioned groups. Most significantly, this analysis definitively rejects any alignment with known *G. intermedia* from upper Eagle Creek and Bonita Creek.

Table 1. Measurements of standard length (SL), head length (H), caudal peduncle depth (CP), the ratio of head length to caudal peduncle depth (H/CP), and anal fin ray (AFR) count for specimens of *Gila* collected from Eagle Creek above Sheep Wash, June 16, 2009.

no.	SL	H	CP	H/CP	AFR
1	62.8	18.05	5.74	3.1	9
2	67.0	20.00	5.57	3.6	9
3	77.5	22.97	6.20	3.7	9
4	72.5	20.70	6.18	3.3	9
5	75.2	23.20	6.38	3.6	9
6	59.8	16.80	4.79	3.5	9
7	71.0	20.20	5.68	3.6	9
8	75.8	21.31	5.81	3.7	9
9	66.1	19.91	5.49	3.6	9
10	73.1	21.26	6.00	3.5	9
sum	700.8	204.4	57.84	35.3	90
mean	70.08	20.44	5.784	3.5	9

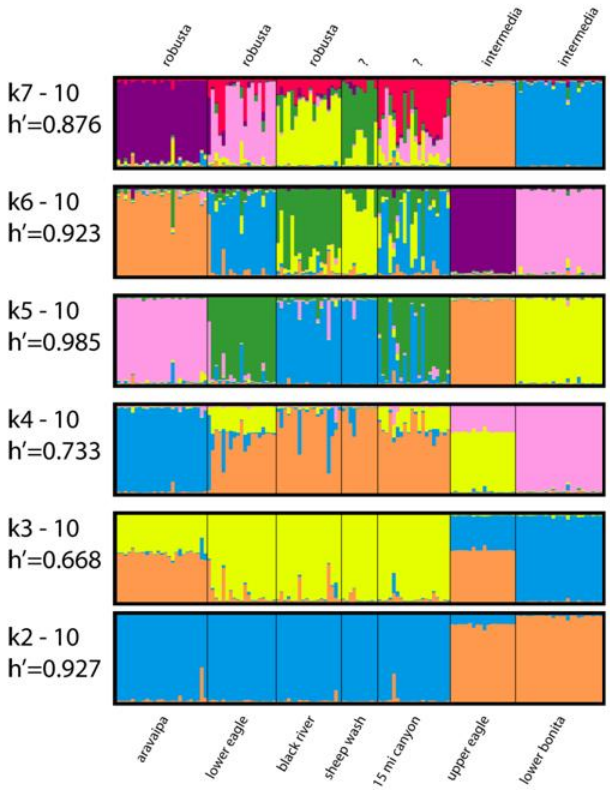

Figure 3. Assignment probability plots for samples of *Gila* from Eagle Creek and adjacent streams. k# indicates the assumed group size and h' the statistic measuring consistency across 10 replicate runs at that value of k; see text and Dowling et al. (2008) for additional explanation.

Capture of fishes – Twenty-eight hoop nets were deployed within the 3.2-km sampled reach of Eagle Creek. Each soaked for approximately 36 hours, and was run 3 times during that period at approximate 12-hr intervals. Twenty-four minnow traps were also set in shallower waters during this period. Electrofishing effort totaled 794 seconds between the two shockers.

A total of 79 roundtail chub was captured among these gears. Most (61) were in hoop nets; none were taken in minnow traps. Young-of-year fish comprised perhaps 20% of the catch. The remainder represented several larger size classes, although maximum length was <250 mm. All chub were successfully held alive in live cars and were transferred to the transport tanks with no mortalities.

Only native fishes were captured. All were common, but we did not record all numbers. In approximate order of numerical abundance, species included speckled dace *Rhinichthys osculus*, desert sucker *Pantosteus clarki*, roundtail chub, Sonora sucker *Catostomus insignis*, and longfin dace *Agosia chrysogaster*. Crayfish were commonly taken in hoop nets and readily observed during electrofishing.

Transport to BPH and prophylaxis – All 79 chub were successfully transported alive to BPH, where they were transferred at approximately 2000 hrs on October 29. Transport tanks were removed from the transport vehicle, and water temperature acclimated to BPH temperature (19 C). Fish were then transferred to two circular 500 gallon tanks in the BPH quarantine facility for prophylactic treatments. Netting was placed over the raceways, but on October 30 it was discovered that six fish jumped through small holes in the netting or onto the top of the filter apparatus and died. This problem was immediately fixed and there have been no further mortalities. Fish were treated for 14 days at a salinity of 3.5 ppt to remove *Ichthiopthirus multifillis,* followed by two consecutive 14-day treatments with Diflubenzuron (Anchors Away®) to remove *Lernaea*. These treatments were followed by two 24-hr treatments with Praziquantel at 1.5 mg/l to remove Asian fish tapeworm *Bothriocephalus acheilognathi* (Ward 2007). Fish are fed a combination of freeze-dried blood worms and commercial fish flakes and pellets twice daily.

DISCUSSION

As expected, we did not capture sufficient numbers of roundtail chub from Eagle Creek to support a direct translocation to Blue River. The plan therefore is to utilize progeny from captive propagation at BPH to stock Blue River. Following completion of prophylactic treatments, all of the wild-caught chub will be held in one of the BPH chub ponds, provided with appropriate substrate and cover, and allowed to either spawn naturally or aided by injections of the synthetic hormone Ovaprim®. Following spawning, adults will be separated from eggs/larvae by transferring one or the other group to the second pond. Progeny will be allowed to grow-out through the summer of 2010. In autumn, the cohort will be stocked to Blue River downstream of Fritz Ranch.

In summer 2010, another (the third consecutive year) mechanical removal of non-native fishes will be undertaken within the Blue River stocking reach, the intent being to remove channel and flathead catfishes and other species that periodically move into Blue River from San Francisco River (results of the 2008 effort are in Clarkson et al. 2008). This effort will reduce predation pressure on the stocked chub cohort and should increase odds of successful establishment. Construction of a fish exclusion barrier on Blue River is scheduled to begin in autumn 2010 and be completed by late winter 2011. Another intensive round of mechanical removal of non-natives will be undertaken in 2011 and thereafter as necessary.

To ensure that captive and translocated Eagle Creek roundtails reflect the maximum genetic variation present in the wild Eagle Creek population, Blue River will be augmented for several years with additional progeny from BPH propagation efforts. The BPH population will also be augmented with additional wild individuals captured from Eagle Creek, and additional wild fish from Eagle Creek will be stocked to Blue River periodically after the Blue River population is established and reproducing.

Replication of Eagle Creek roundtail chub to Blue River will help ensure against extinction in the wild, and can buy time for the declining source population until limiting factors can be removed from Eagle Creek.

LITERATURE CITED

Bezzerides, N., and K. Bestgen. 2002. Status review of roundtail chub *Gila robusta*, flannelmouth sucker *Catostomus latipinnis*, and bluehead sucker *Catostomus discobolus* in the Colorado River basin. Final Report to Bureau of Reclamation, Salt Lake City, UT. Larval Fish Lab Contribution 118.

Clarkson, R.W., P.C. Marsh, J.A. Stefferud, and B.R. Kesner. 2008. Fishery survey of lower Blue River, Greenlee County, Arizona, May 19-22, 2008. Unpublished report. Bureau of Reclamation, Phoenix, AZ, and Marsh & Associates, Chandler, AZ.

Desert Fishes Team. 2003. Status of federal- and state-listed fishes of the Gila River basin, with recommendations for management. Desert Fishes Team Report No. 1.

Dowling, T.E., P.C. Marsh, C.D. Anderson, M.S. Rosenberg, and A.T. Kelsen. 2008. Population structure in the roundtail chub (*Gila robusta* complex) of the Gila River basin as determined by microsatellites. Final report to Arizona Game and Fish Department, Contract AGR 4/21/04, through funding by U.S. Fish and Wildlife Service Agreement 20181-4-J824F and Bureau of Reclamation Agreement 1425-97-AA-32-00420. School of Life Sciences, Arizona State University, Tempe.

Marsh, P.C., and R.W. Clarkson. 2001. Management strategies for native fishes in Blue River, Arizona and New Mexico. Report prepared for U.S. Forest Service, Apache-Sitgreaves National Forest and Gila River Basin Native Fishes Conservation Program.

Minckley, W.L., and B.D. DeMarais. 2000. Taxonomy of chubs (Teleostei, Cyprinidae, Genus *Gila*) in the American southwest with comments on conservation. Copeia 2000:251-256.

Minckley, W.L., and P.C. Marsh. 2009. Inland fishes of the greater southwest: chronicle of a vanishing biota. University of Arizona Press, Tucson.

Voeltz, J.B. 2002. Roundtail chub (*Gila robusta*) status survey of the lower Colorado River basin. Final Report to Central Arizona Project Native Fish Conservation and Nonnative Aquatic Species Management and Control Program. Arizona Game and Fish Department Technical Report 186.

Ward D. 2007. Removal and quantification of Asian tapeworm from bonytail chub using Praziquantel. North American Journal of Aquaculture 69:207-210.

Ward, D. 2008. Bubbling Ponds native fish propagation and research facility status update – February 2008. Report to Bureau of Reclamation, CAP Program, Phoenix, Arizona. Arizona Game and Fish Department, Phoenix.

Ward, D. 2009. New artificial streams for spawning spikedace at Bubbling Ponds research facility. Report to Bureau of Reclamation, CAP Program, Phoenix, Arizona. Arizona Game and Fish Department, Phoenix.