

FINAL PERFORMANCE REPORT for the period January 19, 2015 to January 18, 2016, with updates through August 31, 2016.

September 2, 2016

U.S. Fish and Wildlife Service Agreement No. F11AC00131/F15AP00984

CAP Gila River Basin Native Fishes Conservation Program financial support for the maintenance of Gila and Yaqui topminnow refuge stocks at ASU

Paul C. Marsh  
School of Life Sciences  
Arizona State University  
Tempe, Arizona 85287-4501

Gila topminnow *Poeciliopsis occidentalis* and Yaqui topminnow *P. sonoriensis* are two, federally-listed endangered fish species that are native to Arizona. Several stocks have been held in protective custody by Arizona State University (ASU) for a number of years as part of ongoing conservation and recovery actions in the species' behalf. Stocks are housed in facilities of the Department of Animal Care & Technologies (DACT) on the ASU Tempe campus, and DACT staff provides routine maintenance and care of these animals.

All established project goals for the reporting period were successfully met.

Activities reported below that were implemented after closure of the performance period through August 31, 2015 were in behalf of the same program but without the benefit of FWS funding.

The following stocks are currently being held: Gila topminnow: Bylas Springs, Cienega Creek, Monkey Spring, Red Rock Canyon, Sharp Spring; Yaqui topminnow: North Pond and Tule Spring (SBNWR), combined.

The following augmentations from wild populations have taken place or are planned: Cienega Creek (autumn 2016), Monkey Spring (July 16, 2014), and SBNWR (Yaqui; November 6, 2014). Other stocks are extirpated in the wild (Red Rock Canyon, Sharp Spring) or have otherwise been unavailable (Bylas Springs). Annual requests for augmentation stock from Bylas Springs were made through 2014 to US Fish and Wildlife Service, without result, and no additional requests are anticipated at this time.

The following transfers of Gila topminnow were made: On February 2, April 27 and May 11, 2015, 60, 500, and 30, respectively, Gila topminnow (Red Rock lineage, Tank 10) were removed by BR/FWS and shipped to USGS/FWS for disease testing, research, and *Gyrodactylus* studies (see BR/USGS correspondence). On August 26, 2015 AZGFD personnel removed approximately 200 Gila topminnow (Sharp Spring lineage, Tanks 4 and 8) for transport and stocking the same day into Stop Sign Tank and Swimming Pool Tank, Robbins Butte Wildlife Area, Arizona (see AZGFD trip report).

Problems and solutions: No other issues other than minor matters arose during the reporting period and all were dealt with effectively and successfully.

A more detailed account of project activities is provided in the accompanying Table and comprehensive "Histories" narrative that follow below, updated through August 31, 2016

Current status of Gila topminnow (*Poeciliopsis occidentalis*) and Yaqui topminnow (*P. sonoriensis*) stocks held and maintained in protective captivity in the Animal Resources Center at Arizona State University, Tempe, as of August 31, 2016.

<b>Wild Stock/Source</b>	<b>Original ASU Acquisition</b>	<b>Most Recent Augmentation</b>	<b>Next Augmentation</b>	<b>Location/Comment</b>
Bylas Springs	June 24, 1994; N=20 Roper Lake State Park (via Bylas Springs in 1988)  May 1997; N=17 Bylas Springs	May 23, 2006; N=150-175 Fish stocked in Tank 6.  June 13, 2007; Unknown number of young-of-year fish moved from Tank 6 to Tank 9.	No response from San Carlos Tribe or results from multiple follow-up inquiries with FWS.	<b>Tanks 6 and 9.</b> Tank 7 population was lost on November 19, 2007.
Cienega Creek	June 20, 1994; N=20	September 10, 2015; N ca. 110 mixed age/size/sex (coll. PCM)	Spring 2017	<b>Tank 2.</b> 2015 augmentation delayed until autumn 2015
Monkey Spring	September 1993; N=10 (not confirmed) June 29, 1994; N=20	June 20, 2016; 157 mixed age/size/sex (coll. AZGFD)	Summer 2018	<b>Tank 5.</b>
Red Rock Canyon	May 25, 2006; N=166; 119F, 47M From Desert Harbor Elementary School (via Red Rock Canyon in February 2002)	None	Extirpated in the wild.	<b>Tank 10.</b> BR/USFWS withdrawals April and May 2015 to support research.
Sharp Spring	July 20, 1994; N=18	None	Extirpated in the wild.	<b>Tanks 4 and 8.</b> Tank 8 stock split from Tank 4 (original) as insurance against catastrophic loss. AZGFD withdrawals from both tanks August 2015 to support stocking.
Yaqui topminnow	June 1998; N=39 Tule Spring and North Pond (SBNWR)	August 17, 2016; ca. 110 mixed age/size/sex (North Pond, SBNWR; coll. PCM)	Autumn 2018	<b>Tank 3.</b>

# **Histories of Captive Stocks of *Poeciliopsis occidentalis* and *P. sonoriensis* at Arizona State University – August 31, 2016**

## **Bylas Springs**

Twenty (20) adult females were collected by Marsh et al. from Roper Lake State Park near Safford, AZ, on June 24, 1994 and transported alive to ASU (ASU Federal Endangered Species Permit report to USFWS, Albuquerque, MN; Marsh unpublished field notes). The source of the Roper Lake stock was 300 fish removed from Bylas Middle Spring (S2) on September 26, 1988 (Weedman 1998). Seventeen (17) fish were collected from Bylas Springs and moved to ASU via USFWS in May 1997 via San Carlos FAO (R. Scheffer, personal communication in Weedman 1998). It is unknown if these fish augmented or replaced those acquired in 1994.

### **Tank 6**

A past Animal Resource Center tank label indicated the original captive Bylas Springs topminnow stock was potentially moved into Tank 6 on October 8, 2000 (ARC notes). Approximately 50-100 topminnows collected by Paul Marsh from Bylas Springs<sup>1</sup> were infused into Tank 6 on June 12, 2006 and again on July 25, 2006 (J. White-James email). Approximately 320-350 fish were removed from the tank on October 3, 2007 and stocked on the Muleshoe Ranch<sup>2</sup>.

Fish in this tank are represented by adult male and females plus smaller individuals all in apparent good health and condition. Augmentation from wild stock will be implemented whenever authorization can be obtained.

### **Tank 7**

The origin of this tank is unknown, though it is thought to originate from some of the topminnows collected by Paul Marsh on May 23, 2006<sup>1</sup>. Approximately 320-350 fish were removed from Tank 7 on October 3, 2007 and stocked on the Muleshoe Ranch<sup>2</sup>. An extensive fish die-off due to unknown reasons occurred during October and November 2007. All fish in Tank 7 were dead by November 19, 2007.

### **Tank 9**

Progeny of “20 F backup” were moved from Tank 6 into Tank 9 on July 23, 2005 as insurance against catastrophic loss of the original (Tank 9) Bylas Springs stock (White-James [ASU] email). Approximately 320-350 fish were removed from Tank 9 on October 3, 2007 and stocked on the Muleshoe Ranch<sup>2</sup>.

Fish in this tank are represented by adult male and females plus smaller individuals all in apparent good health and condition. Augmentation from wild stock will be implemented whenever authorization can be obtained.

---

<sup>1</sup> On May 23, 2006, 150-175 fish (mixed Bylas S1 and S2) from Dewey San Carlos Apache Tribe & M. Brouder USFWS Pinetop were used to augment the ASU captive fish stock (P. Marsh email). Fish were quarantined in three aquaria upon arrival at the ARC and later distributed into tanks.

<sup>2</sup>Fish were transported by vehicle to the TNC San Pedro Preserve, held overnight, then transported via helicopter on October 4, 2007 to several sites on the TNC Muleshoe Ranch (PCM field notes; Mary Richardson, USFWS, Phoenix)

## **Cienega Creek**

### ***Tank 2***

Twenty-three (23) adult females were collected by Marsh et al. on June 29, 1994 from Cienega Creek near Mattie Canyon (ASU permit report; PCM unpublished). Weedman (1998) erroneously reports the date as July 1994. It is unknown if these fish augmented or replaced those reportedly acquired in 1993. There is a record of Carla Hurt moving 86 fish into Tank 2 on December 5, 2002 (C. Hurt email), but the history of these Cienega Creek topminnow is unknown.

Eighty-one (81) individuals (72F and 9M) were collected from Cienega Creek by Abe Karam (via USFWS, Tucson) on June 1, 2006 (A. Karam memo). Those fish were quarantined in aquaria for a month, and then transferred to Tank 2 on July 25, 2006 (J. White-James email). During October 2007 approximately 77 individuals were found dead due to unknown causes and the remaining fish from Tank 2 were quarantined in aquaria (J. White-James emails).

On January 23, 2008, the 18 remaining healthy individuals were returned to Tank 2. Abe Karam (via USFWS, Tucson) collected 166 individuals (99F and 67 M) from Cienega Creek on June 4, 2008 (A. Karam memo). These fish were quarantined in aquaria, and over the following days, approximately 32 individuals were found dead due to unknown causes. After one month in quarantine and no further mortalities, 134 fish were added to Tank 2, which contained 18 individuals (J. White-James email).

On April 19, 2011, A. Karam and K. Patterson collected 165 individuals (112 Females, 53 males) from Cienega Creek. Those fish remained under quarantine for the next 90 days (A. Karam memo), then were infused into the Tank 2 population.

On July 29, 2013, P.C. Marsh and J.C.G. Marsh collected about 200 individuals (equal numbers of male and female) from Cienega Creek on the Empire Ranch, Pima Co., Arizona (approximate UTM 12R 538985, 3516964 NAD83) and transported them to ASU where they were transferred in approximately equal numbers into quarantine aquaria (see trip report transmitted August 12, 2013) where they remained quarantined for 30 days prior to infusion into the stock in raceway 2.

On September 10, 2015, P.C. Marsh, B.R. Kesner, and J.B. Wisenall collected 110 individuals (mixed age, size, and sex) from Cienega Creek near the Gardner Canyon confluence (approximate UTM coordinates 539021 E, 3516559 N; 12R, NAD83) and transported them to ASU where they were transferred in approximately equal numbers into quarantine aquaria for 30 days prior to being added to the stock in raceway 2.

Fish in this tank are represented by abundant adult male and females plus smaller individuals all in apparent good health and condition. The next augmentation from wild stock is nominally scheduled for summer 2017.

## **Monkey Spring**

### ***Tank 5***

During September 1993, 10 individuals were collected from Monkey Spring and brought into captivity, though this stock acquisition could not be validated from collection and permit reporting records available at ASU (P. Marsh, personal communication). Approximately 25 individuals were collected by Marsh et al. on June 29, 1994 at Monkey Spring (ASU permit report; PCM unpublished). Carla Hurt

moved 168 fish derived from the original stock in aquaria to Tank 5 on November 1, 2002 (C. Hurt email). The email implies there was already a population of Monkey Spring topminnows established in Tank 5 when these additional fish were added, so the exact inception date of Tank 5 is unknown. Marsh et al. augmented the population with 100 individuals collected from Monkey Spring on May 30, 2007 (P. Marsh email). Karam and Adelsberger augmented the population with 115 individuals (45 females, 47 males, 23 juveniles) collected from Monkey Spring on February 3, 2010 (A. Karam memo). Those individuals were quarantined at ASU, and then infused into the Tank 5 population. Karam, Massure, and Koebele augmented the population with 162 individuals (104 F, 58M) collected from Monkey Spring on June 13, 2012 (A. Karam memo). Those fish were quarantined at ASU then infused into the stock in raceway 5.

On July 16, 2014 approximately 125 Gila topminnow were collected from the wild at Monkey Spring by AZGFD personnel (R. Timmons, et alia) and transferred to ASU. These fish were placed into quarantine during which time there were "a few" mortalities, and an absolute count of 137 individuals was made on August 28, 2014 when fish were transferred to the parent stock in the raceway.

On June 29, 2016 157 Gila topminnow were collected from the wild at Monkey Spring by AZGFD personnel (R.W. Timmons, et alia) and transferred to ASU. These fish were placed into quarantine for 30 days, after which they were added to the stock in the raceway.

Fish in this tank are represented by abundant adult male and females plus smaller individuals all in apparent good health and condition. The next augmentation from wild stock is nominally scheduled for summer 2018.

### **Red Rock Canyon**

Eighty (80) topminnows were collected by AZGFD from Red Rock Canyon (Falls enclosure & Cott Tank drainage) on February 19 and 20, 2002 and held in aquaria, awaiting completion of a refuge pond at Desert Harbor Elementary School (J. Voeltz email). On June 13, 2002 AZGFD released 60 (54 F, 6 M) of those individuals into the refuge pond at Desert Harbor Elementary School (J. Voeltz email).

### **Tank 10**

Arizona Game and Fish Department collected 166 individuals (119 F, 47 M) from Desert Harbor Elementary School on May 25, 2006 and transported those fish to ASU where they were quarantined in aquaria (J. Voeltz email). All individuals were moved into Tank 10 on June 12, 2006 (J. White-James email). Approximately 447 fish died of unknown causes between November 14 and December 2, 2007 (J. White-James emails). One hundred individuals (79 F, 21 M) were collected via AZGFD from Desert Harbor Elementary School on January 29, 2008 and were quarantined in aquaria at ASU for 30-d (P. Marsh email). Those fish were added to Tank 10 on February 29, 2008. The wild stock of topminnows in Red Rock Canyon has since been extirpated. On October 15, 2010, approximately 1,500 topminnow were removed from Tank 10 by Jeff Sorenson (AZGFD) and Abe Karam (M&A). Those fish were transported by AZGFD to a pond at The Audubon Center at Rio Salado in Phoenix. On July 24, 2013 AZGFD personnel removed 350 fish from this tank for transport and stocking into Walnut Spring, Arizona the same day.

On February 2, 2015 Robert Clarkson (Reclamation), Doug Duncan (Fish and Wildlife Service) and I removed sixty (60) mixed age/size/sex (mostly small) Gila topminnow *Poeciliopsis occidentalis* from raceway 10 (Red Rock stock) in LSD

104. Fish were euthanized in a high concentration solution of MS-222 then placed in a plastic bag submersed in slushy ice. Fish were shipped the same day to a US FWS facility for examination and disease free certification. On April 27, 2015 Robert Clarkson (Reclamation) and I removed approximately 500 live, mixed size/age Gila topminnow *Poeciliopsis occidentalis* (Red Rock stock) from Raceway 10 in the DACT facility at Arizona State University in Tempe. Fish were shipped live via Federal Express for priority overnight shipment to Steve Redman (USGS Upper Midwest Environmental Sciences Center in La Crosse, Wisconsin). Additional details regarding use of these fish are available from Reclamation. On May 1, 2015 we were notified by UMESC that these last fish were found to have a *Gyrodactylus* infestation. Notably, this infestation was not identified during the disease certification process, which passed the fish as “clean.” There have been no reports from professional staff with DACT of any issues with the stock at ASU. On May 11, 2015, thirty (30) live, mixed age/size/sex Gila topminnow *Poeciliopsis occidentalis* from the Red Rock Canyon stock in Raceway 10 in LSD104 were collected and shipped via Federal Express overnight for experimental work on *Gyrodactylus* at the U.S. Geological Survey laboratory in La Cross, Wisconsin.

On July 27, 2016 personnel from AZGFD (R.W. Timmons, et alia) removed 250 Redrock lineage Gila topminnow from this raceway and transported and stocked them into a private, Safe Harbor Agreement pond (G. Nabhan) in Patagonia, Arizona (see August 1, 2016 email from RTimmons@azgfd.gov).

Fish in this tank are represented by abundant adult male and females plus smaller individuals all in apparent good health and condition. There can be no future augmentation from the wild population because the source is extirpated.

#### **Tank 7**

Tank 7 was set up as a temporary topminnow holding facility while infrastructure repairs were made to the refuge facility at Desert Harbor Elementary School (R. Timmons email). Approximately 340 topminnows were collected by AZGFD from Desert Harbor Elementary School during March 2008. Those fish were added to Tank 7 on March 28, 2008, but 40-45 individuals had jumped out of the tank or died by the next morning (J. White-James email). Ross Timmons removed 200 individuals from Tank 7 on July 3, 2008, and 100 individuals on August 8, 2008 and transported them back to the repaired refuge facility at Desert Harbor School (P. Marsh emails). The remaining fish (N=259) were collected by AZGFD employees on September 3, 2008 and were stocked into a private pond in Amado, AZ (A. Karam email). Tank 7 was subsequently drained, cleaned, and remains fishless.

#### **Sharp Spring**

##### **Tank 4**

Eighteen (18) adult females were collected by P. Marsh on July 20, 1994 (ASU permit report; PCM unpublished). The wild stock of fish in Sharp Spring has since been extirpated.

On August 26, 2015) AZGFD (T. Robinson, et alia) removed approximately 100 fish, mostly sub-adult and adult females, from raceway #4. Fish were stocked in unknown proportion into Stop Sign Tank and Swimming Pool Tank at Robbins Butte Wildlife Area, Arizona. Fish in this tank are represented by abundant adult male and females plus smaller individuals all in apparent good health and condition. There can be no future augmentation from the wild population because the source is extirpated.

### **Tank 8**

Carla Hurt (ASU) moved 20 fish derived from the original stock of Sharp Spring fish in Tank 4 to Tank 8 on December 10, 2002 (C. Hurt email). These fish were split off as insurance against catastrophic loss of the original stock because of concerns about viability of the wild source, which is now extirpated. On June 3, 2013 approximately 250 fish were removed from this tank by AZGFD (Lara Upton) for stocking the following day into Pasture 2 Tank, San Raphael Valley, Arizona.

On August 26, 2015) AZGFD (T. Robinson, et alia) removed approximately 100 fish, mostly sub-adult and adult females from raceway #8. Fish were stocked in unknown proportion into Stop Sign Tank and Swimming Pool Tank at Robbins Butte Wildlife Area, Arizona.

Fish in this tank are represented by abundant adult male and females plus smaller individuals all in apparent good health and condition. There can be no future augmentation from the wild population because the source is extirpated.

### **Super Topminnow**

Topminnow in Tank 1 consisted of a combination of Bylas, Cienega, Monkey and Sharp stocks which were acquired for graduate student research under Phil Hedrick. During November 13-16, 2007, approximately 132 individuals died of unknown causes. The stock in its entirety was destroyed on March 24, 2009 because it had no practical value and its research purpose had expired.

### **Yaqui Topminnow**

#### **Tank 3**

Original acquisition of this stock took place during June 1998 when 39 mixed sized individuals were collected from North Pond and Tule Spring (genetically identical) on the USFWS San Bernardino National Wildlife Refuge (SBNWR) near Douglas, Arizona. Fish were transferred alive by Kevin Cobble (USFWS), less one mortality, to ASU (ASU permit report). Marsh et al. collected 100 individuals from Twin Pond on San Bernardino NWR on June 27, 2007 (P. Marsh email) to augment Tank 3. Those fish were quarantined in aquaria for ~45-d during which time 60 individuals died of unknown causes. The remaining 40 individuals were infused into Tank 3 on August 15, 2007.

Karam and Behrstock (via SBNWR) collected 178 individuals (78 F, 100 M) from North Pond and Tule Spring (A. Karam memo) on June 25, 2008 to augment Tank 3. Some of the fish were infected with yellow grub. Fish were quarantined in aquaria for 30-d during which time ~ 40 individuals died due to unknown causes.

On April 21, 2011 A. Karam and K. Patterson collected 104 Yaqui topminnow from SBNWR. Collections were made with the help of FWS staff. 50 males and 23 females were collected from North Pond, and 19 males and 12 females were collected from Hay Hollow. All fish were transported to ASU and remained under quarantine for the 90 days. In a post-collection email from Bill Radke (refuge manager at SBNWR), it was determined that the Hay Hollow fish had undergone a genetic bottleneck and the entire April 21, 2011 collection should be euthanized (A. Karam memo). Prior to their destruction, 25 females were removed from quarantine and frozen in the ultra-cold -80°C freezer at ASU. That sample was transferred to Dr. Nathaniel Jue at the University of Connecticut for genetic analysis. The remaining 79 fish were euthanized on June 21, 2011.

On May 23, 2012 A. Karam and G. Ley collected 145 Yaqui topminnow from SBNWR (A. Karam memo). One hundred (100) females and 45 males were collected from North Pond. Most fish were infested with yellow grub (infestations ranged from mild to severe). Prior to transport fish were sorted to insure infested topminnow were not included in the collection. Fish were quarantined at ASU, treated for parasites, and infused into the captive population. Fish in this tank are represented by adult male and females plus smaller individuals all in apparent good health and condition.

On November 5, 2014 approximately 160 mixed age/size/sex Yaqui topminnow *Poeciliopsis sonoriensis* were captured from Urquides Pond (UTM Nad83 Zone 12R 665218 E 3468152 N; SBNWR) to augment the existing captive stock at Arizona State University (ASU) in Tempe. Fish were quarantined until December 9, 2015 when they were added to the refuge population in raceway 3.

On August 17, 2016 approximately 110 mixed age/size/sex Yaqui topminnow were captured from North Pond UTM 12N 665182, 3470130) to augment the existing stock at ASU. Fish were placed in approximately equal numbers into two, 10-gallon aquaria for a 30-day quarantine during which they were treated for an outbreak of "ich," which was well controlled with malachite green, and several individuals infected with a parasitic oligochaete work were removed and euthanized. After quarantine fish were added to the stock of Yaqui topminnow in raceway 3.

Fish in this tank are represented by abundant adult male and females plus smaller individuals all in apparent good health and condition. The next augmentation from wild stock is nominally scheduled for summer 2018.

### References

Marsh, P. C. 1994. Report for 1994 activities and 1995 permit application for U.S. Fish and Wildlife Service Endangered Species Subpermit PRT 67681. Report, in part, to U.S. Fish and Wildlife Service, Albuquerque, NM.

Weedman, D. A. 1998. Gila topminnow, *Poeciliopsis occidentalis occidentalis*, revised recovery plan. U.S. Fish and Wildlife Service, Albuquerque, NM. 86 pages. Draft document.