

SEMI-ANNUAL PERFORMANCE REPORT for the period ending September 30, 2020

October 1, 2020

Reclamation Agreement No. R16AP00035

ASU Topminnow Holding

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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 many years as part of on-going 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 milestones for the reporting period were successfully met.

The following seven stocks are currently being held: Gila topminnow: Bylas Springs, Cienega Creek, Monkey Spring (now known to be contaminated by and hybridized with Yaqui topminnow and removed on September 30, 2020; see below) , Parker Canyon, Red Rock Canyon, Sharp Spring; Yaqui topminnow: North Pond and Tule Spring combined (San Bernardino National Wildlife Refuge [SBNWR]).

The following are the most recent augmentations from wild populations: Bylas Springs (July 12 and August 24, 2017; collected by U.S. Fish and Wildlife Service [FWS] and San Carlos Apache Tribal personnel); Cienega Creek (September 23, 2020), Monkey Spring (June 4, 2018), and SBNWR (Yaqui; September 30, 2020). Augmentations are scheduled at two-year intervals. The scheduled Bylas augmentation timeframes of spring-summer 2019 and 2020 were not met, but contact has been maintained with FWS personnel and fish could be available sometime during the next reporting period. A plan and schedule are to be developed for obtaining individuals from Monkey Spring to re-establish that captive population.

One Bylas stock Gila topminnow had a moderate-size, dorsal ulceration and was euthanized on June 5, 2020; a necropsy revealed no other lesions or signs of infectious agents.

No transfers of Gila topminnow or Yaqui topminnow from ASU stocks were made for stocking or other management purposes during the reporting period. However, remaining Monkey Springs fish were transferred to multiple entities for various uses (see below).

The Monkey Spring stock of Gila topminnow is contaminated by and hybridized with Yaqui topminnow according to a June 2020 preliminary draft report by FWS Southwestern Native Aquatic Resources & Recovery Center, Dexter, NM. The PI and DACT have been directed to destroy this stock. Potential sources of contamination and remedies to prevent future contamination of any kind will be discussed with DACT staff after the report is finalized. FWS and Reclamation have approved re-establishment with fresh, wild-caught fish of a captive stock of Monkey Spring Gila topminnow at ASU.

On September 29, 2020, a sample of 125 Monkey Spring stock of mixed size, age, and sex was removed from raceway #5 and euthanized with an overdose of MS-222. Seventy-five (75) of the 125 fish were fixed in 10% buffered formalin and will be rinsed in freshwater and transferred to 70% ethanol on October 2; these specimens will be transferred to Dr. Peter N. Reinthal, University of Arizona, for museum study. Fifty (50) of the 125 fish were preserved in 95% undenatured ethanol and will be transferred to Dr. Mariana Mateos, Texas A&M University for genetic evaluation. On September 30, most Monkey Spring stock Gila topminnow were removed from raceway #5 and transferred alive to David Ward, US Geological Survey, Flagstaff, for experimental use. Any fish remaining in raceway #5 were euthanized and fixed in 10% buffered formalin and will be rinsed in freshwater and transferred to 70% ethanol for deposition into the ASU Collection of Fishes.

The FWS draft report also found that the Parker Canyon Gila topminnow stock held at ASU clustered genetically with samples from Santa Cruz River, a result that according to the report “conflicts with management records.” Implications of this result are unclear and disposition of the Parker Canyon stock at ASU will be at the direction of FWS.

No additional technical issues other than minor matters arose during the reporting period and all were dealt with effectively and successfully by DACT staff.

A detailed account of project activities is provided in the accompanying Table and comprehensive “Histories” narrative that follow below.

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 September 30, 2020.

Wild Stock/Source	Original ASU Acquisition	Most Recent Augmentation	Next Augmentation	Location/Comment
Bylas Springs	June 24, 1994; N=20 Roper Lake State Park (via Bylas Springs in 1988) May 1997; N=17 Bylas Springs	July 12, 2017; N=45 fish captured; 23 into Tank 6 and 22 into Tank 9 August 24, 2017; N=54 captured; 27 into Tank 6 and 27 into Tank 9.	Spring-Summer 2020	Tanks 6 and 9. Tank 7 population was lost on November 19, 2007. Access to fish dependent on San Carlos Apache Tribe.
Cienega Creek	June 20, 1994; N=20	Sep 23, 2020; N = 111 mixed age/size/sex (coll. PCM & RWC)	Spring 2022	Tank 2. Fish collected at “Head-cut” site where they were abundant.
Monkey Spring	September 1993; N=10 (not confirmed) June 29, 1994; N=20	June 4, 2018; 122 mixed age/size/sex (coll. PCM & PAW)	Unknown – at direction of FWS	Tank 5. Determined in a June 2020 draft FWS report to be contaminated by and hybridized with Yaqui topminnow; destroyed autumn 2020 (see summary text above).
Parker Canyon	November 20, 2015; N=48 (not confirmed)	None	n/a	“New” Tank 1. Temporary holding only; no future augmentations planned.
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.	Tank 10. AZGFD withdrew 251 fish to support stocking July 27, 2016.
Sharp Spring	July 20, 1994; N=18	None	Extirpated in the wild.	Tanks 4 and 8. Tank 8 stock split from Tank 4 (original) as insurance against catastrophic loss. AZGFD withdrawal from Tank 4 of ca 150 fish and from Tank 8 of ca 400 fish to support stocking.

Yaqui topminnow	June 1998; N=39 Tule Spring and North Pond (SBNWR)	September 30, 2020; ca. 100 mixed age/size/sex (North Pond, SBNWR; coll. PCM & PAW)	Autumn 2022	Tank 3.
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Histories of Captive Stocks of *Poeciliopsis occidentalis* and *P. sonoriensis* at Arizona State University – Inception through September 30, 2018

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 Springs¹ 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 Ranch².

On July 12, 2017, N = 45 mixed age/size/sex Gila topminnow were captured from Bylas Spring (location redacted) by U.S. Fish and Wildlife Service and San Carlos Apache Tribe personnel and transferred and transported to the Department of Animal Care and Technologies at Arizona State University, Tempe. Fish were treated, held in quarantine for 30 days, and 23 individuals were added to existing stock in Tank 6; the other 22 individuals were added to the existing stock in Tank 7 (see below).

On August 24, 2017, N = 54 mixed age/size/sex Gila topminnow were captured from Bylas Spring (location redacted) by U.S. Fish and Wildlife Service and San Carlos Apache Tribe personnel and transferred and transported to the Department of Animal Care and Technologies at Arizona State University, Tempe. Fish were treated, held in quarantine for 30 days, and 27 individuals were added to existing stock in Tank 6; the other 27 individuals were added to the existing stock in Tank 7 (see below).

On April 5, 2018 a sample of 50 relatively small fish was captured and placed into individual 1.5-ml snap cap tubes containing approximately 1.0 ml of 85% ethanol. Tubes were stored vertically in a 9 x 9 slotted box and transferred to Reclamation on April 25, 2018 for delivery to USFWS for genetic studies.

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 at a nominal frequency of every two years.

¹ 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.

² 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)

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, 2006¹. Approximately 320-350 fish were removed from Tank 7 on October 3, 2007 and stocked on the Muleshoe Ranch². 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 Ranch².

On July 12, 2017, N = 45 mixed age/size/sex Gila topminnow were captured from Bylas Spring (location redacted) by U.S. Fish and Wildlife Service and San Carlos Apache Tribe personnel and transferred and transported to the Department of Animal Care and Technologies at Arizona State University, Tempe. Fish were treated, held in quarantine for 30 days, and 23 individuals were added to existing stock in Tank 9; the other 23 individuals were added to the existing stock in Tank 6 (see above).

On August 24, 2017, N = 54 mixed age/size/sex Gila topminnow were captured from Bylas Spring (location redacted) by U.S. Fish and Wildlife Service and San Carlos Apache Tribe personnel and transferred and transported to the Department of Animal Care and Technologies at Arizona State University, Tempe. Fish were treated, held in quarantine for 30 days, and 27 individuals were added to existing stock in Tank 9; the other 27 individuals were added to the existing stock in Tank 9 (see below).

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 at a nominal frequency of every two years.

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.

On November 11, 2017 Cienega Creek was visited by P.C. Marsh and B.R. Kesner at the same site as immediately above and attempts were made over a period of two hours to capture Gila topminnow with dip nets and seine; no fish were collected or observed. Another attempt will be made in spring 201 after consultation with D. Duncan (U.S. Fish and Wildlife Service) and R. Timmons (Arizona Game and Fish Department) to determine an optimal site to acquire fish.

On April 5, 2018 a sample of 50 relatively small fish was captured from this raceway and placed into individual 1.5-ml snap cap tubes containing approximately 1.0 ml of 85% ethanol. Tubes were stored vertically in a 9 x 9 slotted box and transferred to Reclamation on April 25, 2018 for delivery to USFWS for genetic studies.

On May 30, 2018 119 Gila topminnow were collected from the wild at Cienega Creek by P.C. Marsh and R.W. Clarkson and transferred to ASU (Marsh email); there were no mortalities. Fish were placed in approximately equal numbers (59 and 60) into two, quarantine for 30 days, after which they were added to the stock in the raceway.

On September 23, 2020 111 Gila topminnow were collected from the wild at Cienega Creek "Head-cut" site by P.C. Marsh and R.W. Clarkson and transferred to ASU (Marsh email); there was one (1) mortality. Fish were placed in approximately equal numbers (55 and 56) into two, quarantine for 30 days, after which they will be 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 spring 2020.

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.

On April 5, 2018 a sample of 50 relatively small fish was captured and placed into individual 1.5-ml snap cap tubes containing approximately 1.0 ml of 85% ethanol. Tubes were stored vertically in a 9 x 9 slotted box and transferred to Reclamation on April 25, 2018 for delivery to USFWS for genetic studies.

On June 4, 2018 122 Gila topminnow were collected from the wild at Monkey Spring by P.C. Marsh et al. and transferred to ASU (Marsh email); there were two (2) mortalities during transport of small (ca 12 mm TL) fish. The remaining 120 fish were placed into approximately equal numbers into two, quarantine for 30 days, after which they were added to the stock in the raceway.

The Monkey Spring stock of Gila topminnow was found contaminated by and hybridized with Yaqui topminnow according to a June 2020 preliminary draft report by U.S. Fish and Wildlife Service (FWS) Southwestern Native Aquatic Resources & Recovery Center, Dexter, NM. 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 was nominally scheduled for summer 2020. The stock is slated to be destroyed in its entirety and replaced by fresh, wild-caught individuals. Implementation of these actions is underway at this writing and will be documented in the next semi-annual report.

On September 29, 2020, a sample of 125 Monkey Spring stock of mixed size, age, and sex were removed from raceway #5 and euthanized with an overdose of MS-222. Seventy-five (75) of the 125 fish were fixed in 10% buffered formalin and will be rinsed in freshwater and transferred to 70% ethanol on October 2; these will be distributed to Dr. Peter N. Reinthal, University of Arizona, for museum study. Fifty (50) of the 125 fish were preserved in 95% undenatured ethanol and will be transferred to Dr. Mariana Mateos, Texas A&M University for genetic evaluation. On October 30, most Monkey Spring stock Gila topminnow were removed from raceway #5 and transferred alive to David Ward, US Geological Survey, Flagstaff, for experimental use. All fish remaining in raceway #5 were euthanized and fixed in 10% buffered formalin and will be rinsed in freshwater and transferred to 70% ethanol for deposition into the ASU Collection of Fishes.

Parker Canyon Creek

“New” Tank 1

Approximately 48 individuals (not enumerated) captured by AZGFD and USFWS personnel from Parker Canyon Creek (collection details available from those agencies) were transferred to ASU DACT on November 20, 2015 and placed into two, 40-L quarantine aquaria; later transferred to raceway (Tank) nos. 11 and 12 subsequently combined in “New” Tank 1. Fish in this tank are represented by abundant males and females plus smaller individuals all in apparent good health and condition. These fish are being held “temporarily” at the request of AZGFD and USFWS pending the outcome of genetic testing.

On April 5, 2018 a sample of 50 relatively small fish was captured and placed into individual 1.5-ml snap cap tubes containing approximately 1.0 ml of 85% ethanol. Tubes were stored vertically in a 9 x 9 slotted box and transferred to Reclamation on April 25, 2018 for delivery to USFWS for genetic studies.

The FWS draft report (see above) found that the Parker Canyon Gila topminnow stock held at ASU clustered genetically with samples from Santa Cruz River, a result that “conflicts with management records.” Implications of this result are unclear and disposition of the Parker Canyon stock at ASU will be at the direction of FWS.

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 251 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).

On May 10, 2018 a sample of 50 relatively small fish was captured from this raceway and placed into individual 1.5-ml snap cap tubes containing approximately 1.0 ml of 85% ethanol. Tubes were stored vertically in a 9 x 9 slotted box and transferred to Reclamation on May 31, 2018 for delivery to USFWS for genetic studies.

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.

On June 13, 2016 AZGFD (R. Timmons, et alia) removed 248 fish from raceway #4. Fish were stocked into Horseshoe Ranch Pond, Arizona.

On April 5, 2018 a sample of 50 relatively small fish was captured and placed into individual 1.5-ml snap cap tubes containing approximately 1.0 ml of 85% ethanol. Tubes were stored vertically in a 9 x 9 slotted box and transferred to Reclamation on April 25, 2018 for delivery to USFWS for genetic studies.

On August 29, 2018 AZGFD (T. Robinson) removed approximately 150 fish from raceway #4 (see also Tank #8 and “build up containers,” below. Fish were stocked into Pasture 9 Tank and Mud Spring, Cochise Co., AZ (see above and August 30, 2018 AZGFD distribution report).

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.

On August 29, 2018 AZGFD (T. Robinson) removed approximately 400 fish from raceway #8 (see also Tank #4, above, and “build-up containers,” below). Fish were stocked into Pasture 9 Tank and Mud Spring, Cochise Co., AZ (see above and August 30, 2018 AZGFD distribution report).

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.

Sharp Spring lineage number build up containers

In response to an initial request from Arizona Game and Fish Department (R. Timmons, emails September 13 and 29, 2017) and with approval of Reclamation (W.

Stewart) and U.S. Fish and Wildlife Service (D. Duncan), DACT personnel set up in winter 2017-2018 a bank of 10, 8-gallon satellite aquaria (polycarbonate boxes with a layer of gravel substrate and sponge filters) in which to produce additional Sharp Spring lineage Gila topminnow for stocking. Containers each were populated on November 13, 2017 with one breeding adult male and two female Gila topminnow and encouraged via thermal control to produce young. Approximately 150 fish were available for harvest from these containers, and an additional 500 are available from each of Tanks 3 and 4 (above) as of June 7, 2018 (DACT email to PCM). AZGFD was notified of this status and asked when they might come and take delivery. This work represents an additional, unbudgeted expense to the Topminnow at ASU grant for time, space, and other resources.

On August 29, 2018 AZGFD (T. Robinson) removed 91 fish from the 10 build-up containers, (see also Tank #4 and Tank #8, above. Fish were stocked into Pasture 9 Tank and Mud Spring, Cochise Co., AZ (see above and August 30, 2018 AZGFD distribution report).

The 10 build-up containers were broken down and taken out of service after all fish were removed on August 29, 2018.

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.

On April 5, 2018 a sample of 50 relatively small fish was captured and placed into individual 1.5-ml snap cap tubes containing approximately 1.0 ml of 85% ethanol. Tubes were stored vertically in a 9 x 9 slotted box and transferred to Reclamation on April 25, 2018 for delivery to USFWS for genetic studies.

On September 13, 2018, 179 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 into two, 10-gallon aquaria (89 fish in one and 90 in the other) for a minimum 30-day quarantine, which was in effect as of this writing. After any necessary treatments for parasites and/or disease and closure of the quarantine period these fish are to be 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 autumn 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.