

FINAL PERFORMANCE REPORT for the period June 1, 2016 to April 30, 2021

April 30, 2021

Bureau of Reclamation Agreement No. R16AP00035, as modified

ASU Topminnow Holding

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

Gila topminnow *Poeciliopsis occidentalis* and Yaqui (Sonoran) 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 on-going research, 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.

Viable populations of topminnow are housed in 188-gallon (712-L) circular, fiberglass tanks with continuous fresh water inflow and biological filtration. Viable populations are characterized by presence of multiple size and age classes of both males and females and natural reproduction in excess of mortality. Small numbers of fish also are held for a variety of reasons in standard 10-gallon (38-L) glass aquaria with continuous aeration and under-gravel filtration. Fish typically do not reproduce in aquaria and do not represent viable populations. All fish are fed daily, observed for population and individual health and condition, and diagnosed and treated as appropriate for pathogenic parasites or diseases. All stocks are maintained separately and do not share equipment or infrastructure of any kind.

All established project milestones for the performance period were successfully met through the first four years of the reporting period but were not successfully met during the final year of the agreement. Specifically, during the last year the Bylas Spring stock of Gila topminnow was lost due to unexplained mortality that exceeded recruitment, the Monkey Spring stock of Gila topminnow was intentionally eliminated because it was found to be contaminated by and hybridized with Yaqui topminnow (Marsh 2020, Mussmann et al. 2020), and the Sharp Spring stock was reduced by mortality to such a small number of fish that it no longer represents a viable population. Further information on demise of these three stocks is provided in the stock history accounts that follow below.

The following stocks currently (spring 2021) are being held as viable populations by ASU: *Gila topminnow* from Cienega Creek, Parker Canyon, and Red Rock Canyon; *Yaqui topminnow* combined samples from North Pond, Tule Spring, and 2PhD Pond (San Bernardino National Wildlife Refuge [SBNWR]) and House Pond (John Slaughter Ranch Museum). A small number of fish from Sharp Spring also are being held, but these do not represent a viable population.

During the five-year reporting period the following augmentations from wild populations have taken place at ASU: Bylas Springs (July and August 2017), Cienega Creek (May 2018, September 2020), Monkey Spring (June 2016, June 2018), and SBNWR (Yaqui; August 2016, September 2018, and September-October 2020). Other stocks are extirpated in the wild (Red

Rock Canyon, Sharp Spring) and would only be available for augmentation from other captive populations.

No transfers of Gila topminnow were made during the final semi-annual reporting period of the agreement from to October 1, 2020 through April 30, 2021. A complete accounting of transfers during the agreement period is provided in the stock history accounts that follow.

Minor matters arose during the reporting period and all were dealt with effectively and successfully. More serious issues that arose during the 5-year tenure of this agreement are detailed in the account of project activities provided in the accompanying Table and comprehensive stock histories narrative that follow below, updated through April 30, 2021.

Status of Gila topminnow (*Poeciliopsis occidentalis*) and Yaqui topminnow (*P. sonoriensis*) stocks held and maintained in protective captivity by the Department of Animal Care and Technologies at Arizona State University, Tempe, as of April 30, 2021.

Wild Stock/Source	Original ASU Acquisition	Most Recent Augmentations	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	Jul 12 & Aug 24 2017, 45 & 54 individuals from "S3" captured by USFWS and SCAT and transferred to ASU.	Undetermined	Stock lost to natural mortality in excess of recruitment December 2020.
Cienega Creek	June 20, 1994; N=20	Sep 22 2020 122 mixed age/size/sex (coll. P.C. Marsh)	Spring 2022	Tank 2. Viable population. Back-up in aquaria #18 & #19.
Monkey Spring	September 1993; N=10 (not confirmed) June 29, 1994; N=20	June 4, 2018 122 mixed age//size/sex (coll. P.C. Marsh)	Potentially Jun 2021	Stock determined contaminated/hybridized and destroyed/distributed Oct 2020. Back-up in aquarium #5A.
Parker Canyon	November 20, 2015; N = 48	None	None	Tank 1. Viable population. Back-up in aquaria #1A & #1B. Originally intended as a short-term hold pending genetic assessment.
Red Rock Canyon	May 25, 2006; N=166; 119F, 47M From Desert Harbor Elementary School (via Red Rock Canyon in February 2002)	None	None (extirpated in the wild).	Tank 10. Viable population.
Sharp Spring	July 20, 1994; N=18	None	None (extirpated in the wild).	Primary stock lost to mortality in excess of recruitment. Back-up in aquaria #4A & #8A.
Yaqui topminnow	June 1998; N=39 Tule Spring and North Pond (SBNWR)	Sep 30-Oct 1 2020 61 mixed (E 2PhD Pond SBNWR) plus 61 mixed (House Pond Slaughter Ranch); coll. P.C. Marsh	Autumn 2022	Tank 3. Viable population. Back-up in aquaria #20 & #21.

Histories of Captive Stocks of Gila topminnow *Poeciliopsis occidentalis* and Yaqui (Sonoran) topminnow *P. sonoriensis* at Arizona State University

Bylas Springs

Current status at ASU. No fish remain in captivity at ASU.

Stock history

Twenty (20) adult females were collected by P.C. 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 U.S. Fish and Wildlife Service (USFWS), Albuquerque, MN; P.C. 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 DACT tank label indicated the original captive Bylas Springs topminnow stock was potentially moved into Tank 6 on October 8, 2000 (DACT notes). Approximately 50-100 topminnows collected by P.C. 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, 45 individuals were captured from Bylas Springs "S3" by San Carlos Apache Tribe (SCAT) and USFWS biologists and transferred to P.C. Marsh for transport to DACT at ASU. Fish were quarantined in 10-gallon aquaria where they were treated preemptively for stress, parasites, and disease and in late September 2017 added in approximately equal numbers to the primary stocks in Tank 6 and Tank 9 (below).

On August 24, 2017, 54 individuals were captured from Bylas Springs "S3" by SCAT and USFWS biologists and transferred to P.C. Marsh for transport to DACT at ASU. Fish were quarantined in 10-gallon aquaria where they were treated preemptively for stress, parasites, and disease and in late September 2017 added in approximately equal numbers to the primary stocks in Tank 6 (above) and Tank 9.

Fish in Tank 6 were represented by adult males and females plus smaller individuals all in apparent good health and condition until mid-2020, after which numbers began to decline. Moribund individuals were subject to necropsy, remaining individuals were treated for with salt potential monogenean trematode infestation, and an ultraviolet sterilizer was placed on the tank. By November 2020, the stock was down to fewer than 2 dozen fish that were transferred to a 10-gallon aquarium for observation and treatment, but all fish were lost by December 9, 2020.

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.C. Marsh email). Fish were quarantined in three aquaria upon arrival at the ARC and later distributed into tanks.

²Fish were transported by vehicle to the TNC San Pedro Preserve, held overnight, and transported via helicopter on October 4, 2007 to sites on the TNC Muleshoe Ranch (P.C. Marsh field notes; M.E. 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 P.C. 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, 45 individuals were captured from Bylas Springs “S3” by SCAT and USFWS biologists and transferred to P.C. Marsh for transport to DACT at ASU. Fish were quarantined in 10-gallon aquaria where they were treated preemptively for stress, parasites, and disease and in late September 2017 added in approximately equal numbers to the primary stocks in Tank 6 (above) and Tank 9.

On August 24, 2017, 54 individuals were captured from Bylas Springs “S3” by SCAT and USFWS biologists and transferred to P.C. Marsh for transport to DACT at ASU. Fish were quarantined in 10-gallon aquaria where they were treated preemptively for stress, parasites, and disease and in late September 2017 added in approximately equal numbers to the primary stocks in Tank 6 (above) and Tank 9.

Fish in Tank 9 were represented by adult males and females plus smaller individuals all in apparent good health and condition until late-2017, after which numbers began to decline. Moribund individuals were subject to necropsy, remaining individuals were treated for with salt potential monogenean trematode infestation, and an ultraviolet sterilizer was placed on the tank. The population stabilized for a time, then began to decline again in spring 2019 at which time a prophylactic treatment with Clout³ was performed. Despite treatment all fish perished and the stock was lost.

Acquisition of fish from the wild to re-establish this captive stock is under discussion.

Cienega Creek

Current status at ASU. Viable population in Tank 2. Quarantine fish in two, 10-gallon aquaria #18 and #19.

Stock history

Tank 2

Twenty-three (23) adult females were collected by P.C. Marsh et al. on June 29, 1994 from Cienega Creek near Mattie Canyon (ASU permit report; P.C. Marsh 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 C.R. Hurt moving 86 fish into Tank 2 on December 5, 2002 (C.R. Hurt email), but the history of these Cienega Creek topminnow is unknown.

³ Clout (active ingredients metronidazole and trichlorfon) is a medication for fish that is used to treat external parasites such as leeches, lice, gill and skin flukes, “ich” (*Ichthyophthirius*), anchor worms (*Lernaea*), and other parasitic copepods and protozoa.

Eighty-one (81) individuals (72 females and 9 males) were collected from Cienega Creek by A.P. Karam (via USFWS, Tucson) on June 1, 2006 (A.P. 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. A.P. Karam (via USFWS, Tucson) collected 166 individuals (99F and 67 M) from Cienega Creek on June 4, 2008 (A.P. 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.P. Karam and K.R. Patterson collected 165 individuals (112 females, 53 males) from Cienega Creek. Those fish remained under quarantine for the next 90 days (A.P. 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 males and females) 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 Tank 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 in Tank 2.

On May 30, 2018. P.C. Marsh and R.W. Clarkson collected 119 mixed age, size, and sex individuals from Cienega Creek at Las Cienegas National Conservation Area near UTM 0538960, 357291 and transported them to ASU where they were transferred in approximately equal numbers into two, 10-gallon quarantine aquaria for 30 days of observation and prophylactic treatment prior to being added to Tank 2.

On September 22, 2020. P.C. Marsh and R.W. Clarkson collected 111 mixed age, size, and sex individuals from Cienega Creek at "Head-cut" near UTM 535470 E, 3541896 and transported them to ASU where they were transferred in approximately equal numbers (55 and 56 individuals, respectively) into two, 10-gallon quarantine aquaria for 30 days of observation and prophylactic treatment prior to being added to the stock in Tank 2.

The next scheduled augmentation of this stock is spring 2022.

Monkey Spring

Current status at ASU. Captive population functionally extirpated. Small population in 10-gallon aquaria # 5A.

Stock history

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.C. Marsh, personal communication). Approximately 25 individuals were collected by P.C. Marsh et al. on June 29, 1994 at Monkey Spring (ASU permit report; P.C. Marsh unpublished). C.R. Hurt moved 168 fish derived from the original stock in aquaria to Tank 5 on November 1, 2002 (C.R. 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. P.C. Marsh et al. augmented the population with 100 individuals collected from Monkey Spring on May 30, 2007 (P.C. Marsh email). A.P. Karam and C.A. Adelsberger augmented the population with 115 individuals (45 females, 47 males, 23 juveniles) collected from Monkey Spring on February 3, 2010 (A.P. Karam memo). Those individuals were quarantined at ASU, and then infused into the Tank 5 population. A.P. Karam, W.A. Massure, and C. Koebele augmented the population with 162 individuals (104 F, 58M) collected from Monkey Spring on June 13, 2012 (A.R. Karam memo). Those fish were quarantined at ASU then infused into the stock in Tank 5.

On July 16, 2014 approximately 125 Gila topminnow were collected from the wild at Monkey Spring by AZGFD personnel (R.W. Timmons, et al.) 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 Tank 5.

On June 29, 2016 157 Gila topminnow were collected from the wild at Monkey Spring by AZGFD personnel (R.W. Timmons, et al.) and transferred to ASU. These fish were placed into quarantine for 30 days, after which they were added to the stock in Tank 5.

On June 4, 2018 122 mixed age, size, and sex fish were collected by P.C. Marsh and P.A. Weathers from the wild population at Monkey Spring on the Rail-X Ranch, Patagonia, Arizona, and transported to ASU where they were separated into equal numbers and placed into two, 10-gallon quarantine aquaria. Fish in both aquaria appeared “sickly” and on June 20 two individuals were necropsied with negative results, and all were treated with Clout. Fish recovered and after an additional period of quarantine were added to the primary stock in Tank 5

Fish in Tank 5 were represented by abundant adult male and females plus smaller individuals all in apparent good health and condition. However, according to a draft USFWS report (Mussmann et al. 2020), the captive Monkey Spring stock of Gila topminnow was contaminated by and hybridized with Sonora (Yaqui) topminnow. The source of the contamination was unknown.

In autumn 2020 the stock in its entirety was dispersed. Samples of 50 preserved individuals each were provided to Dr. Peter N. Reinthal, University of Arizona as museum specimens and to Dr. Mariana Mateos, Texas A&M University for potential

genetic analysis. David Ward, U.S. Geological Survey, Flagstaff was provided with a large sample (100s of fish) for experimental work. Remaining fish were retained by DACT personnel for further health-related study, then euthanized.

Acquisition of fish from the wild to re-establish this captive stock is under discussion.

Parker Canyon Creek

Current status at ASU. Viable population in Tank 1. Small populations in 10-gallon aquaria #1A and #1B.

Stock history

On October 29, 2015, a team led by R.W. Timmons (AZGFD) captured 64 mixed age, size, and sex Gila topminnow from Parker Canyon Creek at a site approximately 4 miles (6.4 km) downstream from Parker Canyon Lake, Arizona. This population was unknown prior to autumn 2015 so specimens were preserved for genetic analysis and arrangements made to maintain live fish temporarily at DACT at ASU.

Tank 1

On November 20, 2015, 48 Gila topminnow collected from Parker Canyon Creek (see above) were transferred to DACT at ASU and distributed into two, 10-gallon quarantine aquaria. Fish cleared quarantine and were transferred to Tank 1 in early January 2016 where adult survival was stable. Genetic evaluation of the stock was reported by Mussmann et al. (2020).

Parker Canyon Gila topminnow will remain in captivity at ASU until a decision is made that determines their fate.

Red Rock Canyon

Current status at ASU. Viable population in Tank 10.

Stock history

Eighty (80) topminnows were collected by AZGFD from Red Rock Canyon (Falls enclosure & Cott Tank drainage) on February 19 & 20, 2002 and held in aquaria while 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 females and 47 males) 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 47 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.C. 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 J.R. Sorenson (AZGFD) and A.P. Karam. 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 Tank 10 for transport and stocking into Walnut Spring, Arizona the same day.

On February 2, 2015 R.W. Clarkson (Reclamation), D. Duncan (USFWS) and P.C. Marsh removed sixty (60) mixed age/size/sex (mostly small) Gila topminnow *Poeciliopsis occidentalis* from Tank 10 (Red Rock stock). 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 R.W. Clarkson (Reclamation) and P.C. Marsh removed approximately 500 live, mixed size/age Gila topminnow *Poeciliopsis occidentalis* (Red Rock stock) from Tank 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 Tank 10 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 al.) removed 250 Redrock lineage Gila topminnow from this tank 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.W. 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). R.W. 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.C. 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.P. Karam email). Tank 7 was subsequently drained, cleaned, and remains fishless.

Sharp Spring

Current status at ASU. Small populations in 10-gallon aquaria #4A and #8A.

Stock history

Tank 4

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

On August 26, 2015 AZGFD (A.T. Robinson, et al.) removed approximately 100 fish, mostly sub-adult and adult females, from Tank 4. Fish were stocked in unknown proportions into Stop Sign Tank and Swimming Pool Tank at Robbins Butte Wildlife Area, Arizona.

A request for 500 to 1000 Sharp Spring stock Gila topminnow to support augmentation/introduction at wild sites was received from AZGFD on October 13, 2017. In response DACT staff set up ten, 10-gallon breeding aquaria to produce additional individuals because the primary stock in Tank 4 and Tank 8 (below) was not considered large enough to support removal of the requested number of fish. Breeding aquaria were taken out of service after fish were removed (below).

On August 29, 2018 AZGFD (A.T. Robinson) removed 641 Gila topminnow, 150 from Tank 4, 400 from Tank 8 (below) and the remainder from the ten, 10-gallon breeding aquaria (see above). Of these 137 were stocked into Pasture 9 Tank on the San Rafael Cattle Company Ranch and 494 were stock into Mud Spring, Cochise Co.; see separate documentation provided by AZGFD.

Fish from Tank 8 (below) were combined into Tank 4. The primary population of this stock was lost early in 2021 to a combination of mortality in excess of recruitment, a persistent infestation of monogenean trematodes, potential effects of genetic a inbreeding due to small founder population size, and other, unknown factors. Nine (9) back-up fish are in a 10-gallon aquarium.

There can be no future augmentation from the wild population because the source is extirpated.

Tank 8

C.R. 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.R. 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 now is extirpated. On June 3, 2013 approximately 250 fish were removed from this tank by AZGFD (Lara Upton [McCall]) for stocking the following day into Pasture 2 Tank, San Raphael Valley, Arizona.

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

A request for 500 to 1000 Sharp Spring stock Gila topminnow to support augmentation/ introduction at wild sites was received from AZGFD on October 13, 2017. In response DACT staff set up ten, 10-gallon breeding aquaria to produce additional individuals because the primary stock in Tank 8 was not considered large

enough to support removal of the requested number of fish. Breeding aquaria were taken out of service after fish were removed (below).

On August 29, 2018 AZGFD (A.T. Robinson) removed 641 Gila topminnow, 400 from Tank 8 and the remainder from the ten, 10-gallon breeding aquaria (see above). Of these 137 were stocked into Pasture 9 Tank on the San Rafael Cattle Company Ranch and 494 were stock into Mud Spring, Cochise Co.; see separate documentation provided by AZGFD.

Fish in Tank 8 were combined with those in Tank 4 and the tank was taken out of service. Twelve (12) back-up fish are in a 10-gallon aquarium.

There can be no future augmentation from the wild population because the source is extirpated.

Super Topminnow

Topminnow in a separate 188-gallon (728-L) tank consisted of a combination in unknown proportions of Bylas Springs, Cienega Creek, Monkey Spring, and Sharp Springs stocks that were acquired for graduate student research under Professor Philip W. Hedrick at ASU. 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

Current status at ASU. Viable population in Tank 3. Quarantine fish in two, 10-gallon aquaria #20 and #21.

Stock history

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 on the USFWS San Bernardino National Wildlife Refuge (SBNWR) near Douglas, Arizona. Fish from these two sites were derived from the same source and thus were genetically identical (W.E. Radke, USFWS, personal communication). Fish were transferred alive by Kevin Cobble (USFWS), less one mortality, to ASU (ASU permit report). P.C. Marsh et al. collected 100 individuals from Twin Pond on SBNWR on June 27, 2007 (P.C. 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.

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

On April 21, 2011 A.P. Karam and K.R. Patterson with the help of USFWS staff collected 104 Yaqui topminnow from SBNWR. Fifty 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 90 days. In a post-collection email from W.E. 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.P. 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.P. Karam and G. Ley collected 145 Yaqui topminnow from SBNWR (A.P. 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 ensure infested topminnow were not included in the collection. Fish were quarantined at ASU, treated for parasites, and infused into the captive population.

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 Tank 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 unidentified parasitic oligochaete worm were removed and euthanized. After quarantine fish were added to the stock of Yaqui topminnow in Tank 3.

On September 13, 2018 179 mixed age, size, and sex fish were collected by dip net from North Pond on the SBNWR and transported to DACT at ASU where they were distributed in approximately equal numbers (89 and 90) into two, 10-gallon quarantine aquaria. These fish were infected with "Ich" and treated successfully with Clout, and were retained in the quarantine aquaria because of mortality issues that developed with the primary population in Tank 3 (see next).

In early October 2018, the fish in Tank 3 experienced significant mortality (27 fish) and were found to be infected with *Tetrahymena*, a pathogenic ciliate. The population was treated with multiple applications of Clout and water in the tank was exchanged multiple times but mortalities continued at an estimated rate of 15 to 30 fish per day. As a result a subpopulation was removed to an isolated 10-gallon aquarium for back-up and separate treatment. The primary population in Tank 6 was lost in its entirety by spring 2019 and the back-up subpopulation in the 10-gallon aquarium dwindled to 6 fish, which were euthanized.

Tank 3 was cleaned, sterilized, and restarted and in mid-March 2019 and the quarantine fish collected on September 13, 2018 were placed into the tank. After a successful re-set as evidenced by reproduction, population increase, and apparent high adult survival, a die-off of mostly small (young) fish began in early December 2019. The tank was treated with Clout and a UV filter was installed and the die-off ended.

On September 30 & October 1 2020, 61 mixed age, size, and sex fish were collected by minnow trap in East 2PhD Pond (UTM 12R 664400 E, 3468255 N) on the SBNWR and another 61 mixed age, size, and sex fish were collected by minnow trap in House Pond (UTM 12R 663740 E, 3468235 N) on the John Slaughter Ranch

adjacent to the refuge. Fish were combined in a single cooler and transported to DACT at ASU where they were distributed in equal numbers into two, 10-gallon quarantine aquaria.

Fish in Tank 3 are represented by abundant adult males and females plus smaller individuals all in apparent good health and condition. The next augmentation from wild stock is scheduled for autumn 2022.

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.

Marsh, P.C. 2020. Semi-annual performance report for the period ending September 30, 2020. Reclamation Agreement No. R16AP00035. ASU Topminnow Holding. Arizona State University, Tempe. 13 pages.

Mussmann, S., A. Harrison, P. Brown and W. Wilson. 2020. Genetic characterization of refuge, reestablished, and natural populations of the Gila topminnow (*Poeciliopsis occidentalis*) in Arizona. Preliminary Draft Report, U.S. Bureau of Reclamation. U.S. Fish and Wildlife Service Southwestern Native Aquatic Resources and Recovery Center, Dexter, New Mexico. 26 pages.

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