North American Journal of Fisheries Management

ISSN: 0275-5947 (Print) 1548-8675 (Online) Journal homepage: http://www.tandfonline.com/loi/ujfm20

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North American Journal of Fisheries Management 37:445–455, 2017

ISSN: 0275-5947 print / 1548-8675 online
DOI: 10.1080/02755947.2017.1280571

MANAGEMENT BRIEF

First Reproduction by Stocked Bonytail in the Upper Colorado River Basin

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Abstract
Bonytail *Gila elegans*, a large-bodied cyprinid that is endemic to the Colorado River basin of the American Southwest, was historically widespread and abundant in large warmwater streams but is now critically endangered. To increase recovery prospects, over 500,000 Bonytails have been stocked in the upper Colorado River basin since 2000, but adult survival has been low and reproduction has not been detected. We provide the first documented evidence of successful reproduction by stocked Bonytails in the upper Colorado River basin. Adult Bonytails were stocked in the Green River and accessed Stewart Lake and Johnson Bottom (managed floodplain wetlands in the middle Green River, Utah) during high flows in May 2015 (Stewart Lake only) and 2016. Draining of Stewart Lake in September 2015 revealed 19 age-0 individuals of *Gila* sp. (37–64 mm TL) among over 405,000 fish. Four preserved specimens (41–48 mm TL) were verified as Bonytails by using morphological and molecular techniques. Otolith daily increment analysis confirmed reproduction by Bonytails in Stewart Lake. Bonytail reproduction was also noted during 2016 in Stewart Lake (probable) and Johnson Bottom. Young Bonytails survived despite the presence of abundant nonnative fish predators. Use of floodplain wetlands for reproduction may enhance the recovery of critically endangered Bonytail in the upper Colorado River basin.

The endangered Bonytail *Gila elegans* is the rarest native fish in the Colorado River basin, a system that is highly developed with water storage and diversion infrastructure and that contains many abundant invasive fishes. As one of four main-stem, large-bodied fish species that are federally listed as endangered in the basin (Minckley 1973; Carlson and Muth 1989), few wild Bonytails have been collected in the last 35 years, and they are now likely extirpated (Vanicek and Kramer 1969; Kaeding et al. 1986; USFWS 2002). General reasons for the demise of native fishes in the Colorado River basin—all of which likely also affected Bonytails—include habitat alteration and destruction; disruption of natural flow, temperature, and sediment regimes by main-stem dams and diversions; and the negative effects of nonnative fishes (Dill 1944; Olden et al. 2006; Bestgen et al. 2007). However, specific factors associated with reductions of formerly widespread and abundant Bonytails are poorly understood because few ecological studies were conducted prior to their precipitous decline (Vanicek et al. 1970; Holden and Stalnaker 1975a, 1975b; Behnke and Benson 1983; Minckley and Marsh 2009).

To assist with recovery efforts, hatchery propagation of Bonytails began in 1981 with 11 adults (six females and five

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Received September 1, 2016; accepted January 2, 2017

males) that were captured from Lake Mohave (Hamman 1982; Minckley et al. 1989; Johnson and Jensen 1992). Hatchery-produced Bonytails have been stocked in an attempt to enhance populations throughout the Colorado River basin (Minckley 1995; Mueller 2006; Bestgen et al. 2008). For example, over 500,000 Bonytails have been repatriated in the upper Colorado River basin from 2000 to 2016, with 63% stocked in the Green River subbasin and the balance stocked in the Colorado River subbasin. Stocking goals specified the use of Bonytails at least 200 mm TL (Nesler et al. 2003; Integrated Stocking Plan Revision Committee 2015), but since 2013, fish with mean TLs greater than 250 mm have been stocked in an effort to increase survival. It was further recommended that Bonytail stocking levels in the upper Colorado River basin be increased from about 16,000 to 35,000 fish annually. Bonytails are tagged with PIT tags prior to release, except that some fish stocked in the early 2000s were coded wire tagged.

Despite the large number of Bonytails released into streams of the upper Colorado River basin, few have been recaptured during extensive surveys, and survival is presumed to be low (e.g., Bestgen et al. 2007, 2008). Furthermore, there had been no documented evidence of Bonytail reproduction in the upper Colorado River basin, although localized reproduction was detected in the lower Colorado River basin (Mueller et al. 2005). Stocking in upper basin streams occurs in a variety of habitat types, including high-gradient, canyon-bound reaches as well as lower-gradient, alluvial sections, often at sites where last-known wild individuals were captured or where floodplain wetlands exist (Mueller 2006; Bestgen et al. 2008). Use of floodplain wetlands and selected riverine backwaters was in response to successful stocking of Bonytails in isolated off-channel ponds of the lower Colorado River (Marsh et al. 2013a, 2013b). In those ponds, when isolated from nonnative fish predators and piscivorous birds, adult Bonytail survival has been high and reproduction has been noted in a variety of lentic habitats ranging from hatchery ponds to restored wetlands that were created specifically for endangered fishes (Marsh 2004). Survival of and subsequent successful reproduction by adult Bonytails in natural settings are required as a first step toward the species' recovery. Here, we report the first documented reproduction by hatchery-produced Bonytails stocked in the upper Colorado River basin and the survival of young Bonytails through the first summer.

METHODS

Study area.—The middle Green River in east-central Utah flows through a relatively broad, alluvial valley reach, where the channel is low gradient and sand-bottomed (Figure 1). Off-channel wetlands are relatively abundant in the floodplain of this reach and connect with the Green River when spring snowmelt runoff is sufficient (Hedrick et al. 2010). One such wetland, Stewart Lake, is designated as waterfowl habitat but also has potential to assist with endangered fish recovery. Regulation of the Green River by Flaming Gorge Dam (a U.S. Bureau of Reclamation [USBR] facility), located 178 river kilometers (rkm) upstream of Stewart Lake and 238 rkm upstream of Johnson Bottom, has resulted in lower peak flows and seasonally higher base flows than had occurred naturally (Muth et al. 2000). These modified conditions are partially attenuated downstream by (1) flow from the mostly unregulated Yampa River, a large Green River tributary that is situated 73 rkm upstream of Stewart Lake; and (2) increased magnitude of spring peak releases from Flaming Gorge Dam, which were designed to benefit native fishes (Muth et al. 2000; Bestgen 2015).

Fish sampling in Stewart Lake and Johnson Bottom was part of a larger study that was conducted to evaluate the effectiveness of spring flows from Flaming Gorge Dam for inundating floodplain wetlands and simultaneously entraining the larvae of Razorback Suckers *Xyrauchen texanus* to enhance recovery of that species (Valdez and Nelson 2004; Bestgen et al. 2011). The warm, food-rich wetlands promote growth and survival of the vulnerable early life stages of native fishes (Bestgen 2008). Floodplain connections with the Green River and wetland filling depend on the joint effect of unregulated Yampa River flows and the timing, magnitude, and duration of Flaming Gorge Dam releases (Brunson and Christopherson 2005; Bestgen et al. 2011). Higher-magnitude spring flow releases from Flaming Gorge Dam are now timed to coincide with the first presence of Razorback Sucker larvae in the Green River (rather than the typically earlier peak of the Yampa River) so as to maximize the entrainment of larvae into floodplain wetlands (Bestgen et al. 2011; LaGory et al. 2012).

Wetland inflows are managed with upstream and downstream gates or have uncontrolled breaches that allow for wetland filling when Green River flows rise. When river flows begin to decline from peak, the gates are closed to retain water and fish, thereby benefiting fish survival and growth over the summer. Stewart Lake connects with the Green River through the outlet gate at flows of about 99 m^3/s (3,500 ft^3/s). Surface area of the wetland varies in size depending on the stage of the Green River; at average spring peak flows of about 525 m^3/s (18,524 ft^3/s), the wetland area is about 231 ha (Bestgen et al. 2011), and depth is 2 m or more. The substrate is mostly silt, and emergent macrophytes (mainly cattails *Typha* sp.) are abundant. Johnson Bottom is smaller, with a surface area of about 170 ha at average Green River peak flows. It was first modified in 2015 to include (1) a single gate-controlled canal (which functions as an inlet and an outlet) with a culvert connection and fish kettle (connection flow of about 241 m^3/s) and (2) a second uncontrolled breach downstream (connection at about 340 m^3/s), which allows for enhanced access by Green River flows.

Fish sampling and specimen analysis.—To prevent invasion by larger-bodied nonnative fishes during filling, the inlets to the wetlands are screened in early spring when flows are low. Stewart Lake is screened with 1-cm-wide picket

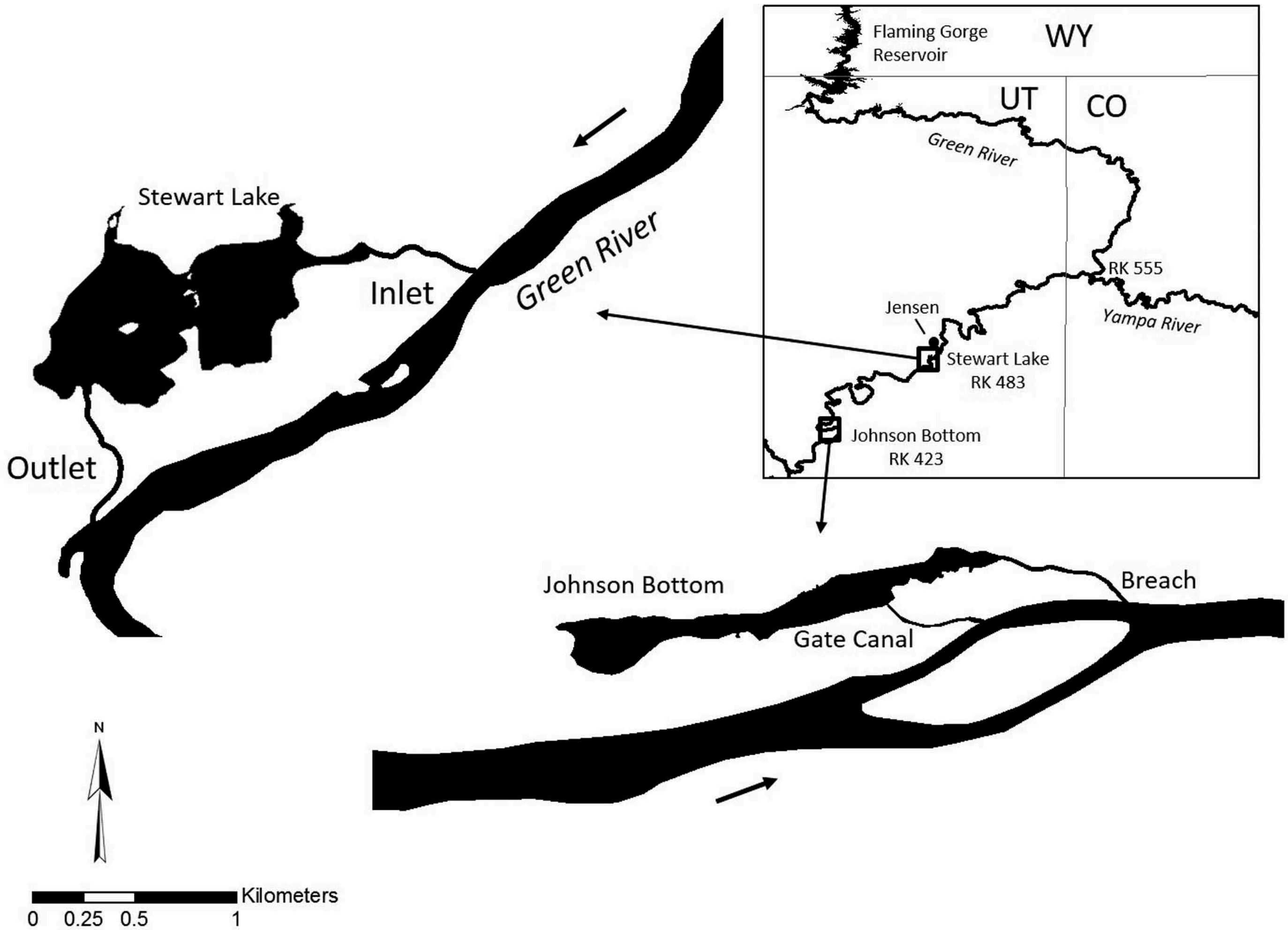

FIGURE 1. Map of the floodplain wetlands (Stewart Lake and Johnson Bottom) in the middle Green River basin (inset map), northeastern Utah, where sampling for Bonytails was conducted. Jensen, Utah, is the nearest town. Numbers on the inset map indicate river kilometer (RK) locations; arrows adjacent to the Green River channel indicate the flow direction. The wetland surface area at Green River base flow level is depicted, but the surface area expands as filling proceeds at higher river stages.

weirs, and Johnson Bottom is screened by a culvert perforated with 15- × 150-mm slots. Screens allow Razorback Sucker larvae and some other early life stages to pass, but some small-bodied, nonnative fishes (e.g., Fathead Minnow *Pimephales promelas* or Green Sunfish *Lepomis cyanellus*) also access the wetlands through screens, and larger-bodied fish sometimes jump over the weirs or enter the wetlands when screens or breaches are submerged by higher river flows. Although most water that enters the wetlands can be screened at lower flows, efficiency of screens to exclude nonnative fishes is not known. At higher stages, Green River flows into the wetlands are unabated. Stewart Lake has been drained annually since 2012 in late summer (early September to mid-October) to remove selenium burdens that leach from the soil (Naftz et al. 2005). During autumn wetland draining, the results of springtime entrainment are assessed by netting fish from enclosures with 0.25–1.00-cm-wide bars that screen outlet flows. Captured and preserved fish were identified and counted, and endangered fish were weighed (g), measured (mm TL), scanned for the presence of a PIT tag, and tagged if no tag was found. Additional 2015 and 2016 Stewart Lake sampling occurred in July with fyke nets (15.2-m wing; 0.64-cm mesh), trammel nets (2.5-cm inside mesh), and gill nets (experimental nets; mesh size range = 1.3–7.6 cm), mainly to remove Common Carp *Cyprinus carpio* that gained access to the wetland during the spring.

Two age-0 *Gila* specimens from Stewart Lake sampling in 2015 were aged by using otolith daily increments to establish hatching dates in relation to the timing of gate closure, which eliminated the connection with the Green River. Standard otolith microincrement techniques were used for analyses and were similar to those successfully employed for aging other western riverine cyprinid larvae (Bestgen and Bundy 1998; Haworth and Bestgen 2016).

Genetic analysis.—Genetic analysis was conducted by the Molecular Ecology Laboratory at the Southwestern Native Aquatic Resources and Recovery Center (U.S. Fish and Wildlife Service [USFWS], Dexter, New Mexico). Genotypes from 22 Bonytails, 198 Humpback Chub *G. cypha*, and 113 Roundtail Chub *G. robusta* (Table 1) were compared to 7 Bonytail samples collected from Stewart Lake in 2015. Genomic DNA was extracted using DNeasy Blood and Tissue Kits (Qiagen) in accordance with the manufacturer's instructions; the samples were then stored at –80°C. Fifteen microsatellite loci were amplified via PCR using forward primers (Applied Biosystems) labeled with one of four fluorescent dyes (Table 2). Amplification was carried out in 10-μL reactions consisting of 1 μL of template DNA, 0.8× Qiagen Multiplex PCR Kit, and up to 200 nM each of forward and reverse primers by using a GeneAmp 9700 or ProFlex PCR System (Applied Biosystems). Touchdown cycling (temperature decreased by 0.2°C per cycle) consisted of one cycle at 95°C for 15 min; 35 cycles of 95°C for 45 s, annealing at 56°C for 60 s, and extension at 72°C for 60 s; and a final extension at 72°C for 30 min. Amplified microsatellite loci were visualized on an automated 3130XL Genetic Analyzer (Applied Biosystems) with GeneScan LIZ 500 size standard, and loci were genotyped with GeneMapper 5 (Applied Biosystems).

The Bayesian clustering method of STRUCTURE version 2.3.2 (Pritchard et al. 2000) was used to estimate the proportion of ancestry in the Stewart Lake samples that was attributed to each of the three *Gila* species. The admixture model was applied, which assumes gene flow among populations and allows for correlated allele frequencies across populations. Twenty iterations were performed with *K* set at 3 clusters to represent the three *Gila* species present in the Green River. All runs had a burn-in of 250,000 iterations followed by 250,000 iterations of data collection. A discriminant analysis of principal components (DAPC; Jombart et al. 2010) was performed using the package "adegenet" (Jombart and Ahmed 2011) in R version 3.2.3 (R Core Team 2015) to visualize the genetic differences between locations and among years. The number of principal component axes included was determined through cross-validation, and all discriminant axes (DAs) were included in the analysis. The minimum number of breeders was calculated from the age-0 fish genotypes.

RESULTS AND DISCUSSION

Except for slight groundwater seepage, Stewart Lake and Johnson Bottom were essentially dry before connecting with the Green River each spring, so there was no resident fish community prior to wetland filling. In 2015, the Stewart Lake filling period (May 9–28) began relatively early in the reproductive season for Green River fishes, and the water was cold (<16°C) during that time; the filling period in 2016 was later (May 31–June 14), but water temperatures were usually less than 16°C. During 2016, Johnson Bottom was connected with the Green River from May 9 to June 29. Razorback Sucker larvae are typically the only early life stage of native fish present in early spring when Green River flows rise and first connect with floodplain wetlands (Bestgen et al. 2011).

Five Bonytails (262–329 mm TL; presumably adults) were among the large-bodied fish that were collected during Stewart Lake monitoring in July 2015 or during draining on September 1–14, 2015; those individuals likely jumped the inlet weir in May to access the wetland. Adult Common Carp and a single Northern Pike *Esox lucius* were also observed accessing the floodplain by jumping the picket weir when Stewart Lake was filling. The five Bonytails captured were originally stocked on May 6, 2015, in

TABLE 1. Summary of collection locations and years for tissue samples from *Gila* species used for genetic comparisons with the Stewart Lake samples (MEL = Molecular Ecology Laboratory; ARRC = Aquatic Resources and Recovery Center).

MEL ID number	*N*	Sampling location	Drainage	Year
		Humpback Chub (total *N* = 198)		
GcypW13LCR_001-050	50	Little Colorado River	Lower Colorado River	2013
GcypW12BR_001-051	50	Black Rocks	Upper Colorado River	2012
GcypW09GR_001-050	48	Desolation Canyon	Green River	2006
GcypW15DC_001-050	50	Desolation Canyon	Green River	2015
		Roundtail Chub (total *N* = 113)		
GrobW14CHEV_001-009	9	Chevelon Creek	Lower Colorado River	2014
GrobW12BR_001-050	50	Black Rocks	Upper Colorado River	2012
GrobW12WP_001-006	6	Whirlpool	Green River	2012
GrobW12YC_001-018	18	Yampa Canyon	Yampa River	2012
GrobW06MC_001-048	31	Muddy Creek	Little Snake River	2006
		Bonytail (total *N* = 22)		
GeleDX12_001-030	22	Southwestern Native ARRC	Lower Colorado River	2012

TABLE 2. Locus codes, primer sequences (F = forward; R = reverse), and repeat motifs for the 15 microsatellite loci that were used to screen tissue samples from *Gila* specimens captured in Stewart Lake (Green River subbasin), Utah, during 2015.

Locus	Primer sequences	Repeat motif	Reference
Gel226	F: VIC-TTGCACATGAACTTACATAGAGG R: ACCGTAGATAAAAACAATACAACG	$(TAGA)_3TT(GATA)_7GATA(GACA)_6GATA$	Keeler-Foster et al. (2004)
Ca12	F: VIC-GTGAAGCATGGCATAGCACA R: CAGGAAAGTGCCAGCATACAC	$(CA)_{16}$	Dimsoski et al. (2000)
Gbig87	F: NED-TGTGGCTTTAAGTAAATGATGACC R: TCGGGTGTTATAGAAAATGTTCC	$(GATA)_{10}$	Meredith and May (2002)
Gel227	F: 6FAM-TGTGAGATGGTTGTGCAAAG R: TTTTAATTGGCCACGACACA	$(CTAT)_6(CTGT)_{14}(CTTGT)_4$	Keeler-Foster et al. (2004)
Cypg3	F: 6FAM-AGTAGGTTTCCCAGCATCATTGT R: GACTGGACGCCTCTACTTTCATA	$(CAGA)_2(TAGA)_{11}$	Baerwald and May (2004)
Gbig294	F: PET-TGTTCCCCTCATCATCATAG R: AGAACAATAGAACAATACACAGA	$(GATA)_7$	Meredith and May (2002)
Pluc13	F: NED-GGGTGGGTGGCTAAGGTAGG R: TTCACATAAATGTGTAATCTAGAGAAGTGC	ATCT	Martin et al. (2015)
Pluc22	F: VIC-TCATGTGCTCCTATCTGAGTGC R: TCATTCTTGAAATCTCATTGGTCC	AAAG	Martin et al. (2015)
Pluc03	F: 6FAM-AGGTTCTTTCTTCTTCATCATGG R: TGAGTAGCATCATGTCTTGCG	AATAG	Martin et al. (2015)
Pluc11	F: PET-CTTTGCGACGTCGATTTCC R: AACAATGCGGTTCTTCATGC	ATCT	Martin et al. (2015)
Gel228	F: PET-CCAGAAAATAATGCAACTCTTG R: CTGTCACAGGATCTCCAGAAG	$(TCTA)_{21}$	Keeler-Foster et al. (2004)
Pluc19	F: 6FAM-GAGTTGTTTCATACAGGTATGCCC R: TTAGTTTCTTGCTGATGGATGG	ATCT	Martin et al. (2015)
Pluc30	F: PET-AAATGCTGAATGTTGTAAATGAGC R: TCTGCACGGAATCCATTAGC	AAAG	Martin et al. (2015)
Pluc41	F: 6FAM-GAATGCATTGATTCAGCTCACC R: TGCACATACTGTTGGTTTGGC	AAAG	Martin et al. (2015)
Pluc44	F: NED-AATACAATTACGCAACAGAAGCC R: CCATTTAAAGATAAACAGCAAGTGG	AAAG	Martin et al. (2015)

the Green River at the Split Mountain Boat Ramp, 31 km upstream of Stewart Lake. One of the five Bonytails (266 mm TL) was initially detected by a flat-plate PIT tag antenna positioned just outside of the fish screen in the inlet canal to Stewart Lake on May 13, 1 week after stocking and when the wetland was filling. The two Bonytails that were captured in early September were 325 and 329 mm TL and had grown 71 and 83 mm over the 4-month period since stocking. The 325-mm TL fish nearly doubled in mass from 125 to 240 g; recapture mass was not recorded for the other individual. The other three Bonytails, including the one detected in the inlet canal, were recaptured during July sampling in Stewart Lake and constituted three of the seven Bonytails that were included in genetic analyses; no information on length at recapture was available. Stewart Lake draining in 2016 resulted in the capture of 24 large-bodied, presumably adult Bonytails (252–458 mm TL); at least 19 of those fish were stocked in the outlet canal on June 10, 2016, outside of the Stewart Lake gate and fish screen (n = 451 total stocked), but the provenance of the remainder is yet unknown. Forty-three large-bodied Bonytails (mean TL = 275 mm) were captured during the autumn 2016 draining of Johnson Bottom; 41 of the 43 were fish that had been stocked into the downstream wetland breach on May 13 (n = 1,041 total fish stocked; mean TL = 223 mm) as the river and wetland were connecting. The other two captured Bonytails were stocked during 2015 in the Green River near the Johnson Bottom gate canal. The Bonytails stocked in 2016 gained an average of 52 mm and 43 g in mass from mid-May through late September; no growth information was available for the other recaptures.

During the 2015 draining of Stewart Lake, 19 small-bodied, age-0 *Gila* sp. were captured (37–64 mm TL). Four of those individuals (41–48 mm TL) died during handling and were preserved in 100% ethanol, while the remainder were released into the Green River. In 2016, nine presumptive age-0 Bonytails (45–63 mm TL; one mortality preserved) were captured in Stewart Lake, and five (53–72 mm TL; three mortalities preserved) were captured from Johnson Bottom. Age-0 *Gila* specimens were identified by using Snyder et al. (2016). Morphological characteristics indicated that the preserved young *Gila* specimens collected from Stewart Lake in 2015 and from Johnson Bottom in 2016 were Bonytails (Table 3). Determinations were based on the specimens' narrow least caudal peduncle depths; long dorsal and anal fin lengths; a dorsal fin ray count of 10; an anal fin ray count of 10 or 11; and moderately oblique, terminal mouths, with the terminus of the upper lip above the bottom of eye level (Holden and Stalnaker 1970; Douglas et al. 1989, 1998; Muth 1990; Snyder et al. 2016). Lateral pigmentation was also extensive and extended well below eye level, a pattern more likely for Bonytails and Roundtail Chub than for Humpback Chub of similar size. Most morphological comparisons of consequence were with Bonytails and Roundtail Chub, as Humpback Chub are rare in alluvial valley reaches such as the middle Green River, and upstream populations are now small or extirpated (Finney 2006; Bestgen et al. 2008). However, Humpback Chub were included in analyses for completeness.

The only 2016 *Gila* specimen preserved from Stewart Lake had morphological traits consistent with Bonytails except for anomalous dorsal and anal fin ray counts (nine rays each, but the fins were very long and oddly shaped, indicating possible developmental issues) and a slightly deeper caudal peduncle, and taxonomic confirmation awaits genetic analysis. However, based on photographs of two other 2016 Stewart Lake age-0 *Gila* specimens (released alive) that were typical of Bonytails and based on the absence of any other *Gila* spp. adults, we presume that Bonytails reproduced in Stewart Lake during 2016.

Based on genetic examination, all 2015 Stewart Lake samples clustered as Bonytails in the STRUCTURE analysis (coefficient $q > 0.97$); no genetic analyses were performed on other samples. The DAPC conducted in adegenet explained

TABLE 3. Selected morphological characters (typical ranges, not extremes, are reported) for juvenile Bonytails captured from two floodplain wetlands (Stewart Lake, 2015: n = 4; Johnson Bottom, 2016: n = 3) of the middle Green River, Utah, presented in comparison with the characteristics of congeners as reported by Snyder et al. (2016). Fin lengths and body depths are expressed as a percentage of SL; ranges are given in parentheses.

Species	SL range (mm)	Dorsal fin length (% of SL)	Anal fin length (% of SL)	Depth at caudal peduncle (% of SL)	Dorsal/anal fin ray counts	Mouth characteristics
Bonytail (wild) (n = 7)	31–48	23 (22–24)	20 (18–21)	5.5 (5–6)	10/10 (10/10–10/11)	Terminal, upper lip above bottom of eye
Bonytail	22–44	22 (20–24)	20 (18–22)	6 (5–7)	10/10 (10/10–10/11)	Terminal, upper lip above bottom of eye
Roundtail Chub	19–50	20 (18–23)	18 (15–21)	8 (7–10)	9/9 (9/9–9/10)	Terminal, upper lip above bottom of eye
Humpback Chub	18–45	24 (21–28)	20 (17–23)	7 (6–8)	9/10 (9/9–10/10)	Low to subterminal, upper lip at or below bottom of eye

40% of the variance in the data set (Figure 2). The first DA separated Bonytails from Roundtail Chub and Humpback Chub. The second DA separated Roundtail Chub from Humpback Chub. The adults and age-0 *Gila* specimens that were captured in Stewart Lake were found to group definitively with Bonytails. The age-0 fish genotypes indicated that at least two females and two males must have contributed to 2015 reproduction; this was somewhat surprising given the few age-0 fish examined and the few adults that were apparently present in Stewart Lake.

Although we report the first conclusively documented Bonytail reproduction in recent times in the upper Colorado River basin, reproduction was also suspected in two floodplain wetlands (Above Brennan and Leota-10) in the middle Green River during summer 2003 after the stocking of adults into the wetlands and the subsequent capture of age-0 *Gila* specimens (Modde and Haines 2005). The absence of other adult *Gila* sp. in the vicinity suggested that the young fish captured were likely Bonytails, but no specimens were preserved for taxonomic verification. Thus, reproduction by Bonytails in Green River floodplain wetlands cannot be verified with certainty based on 2003 records.

Because *Gila* spp. do not typically spawn in the Green River until late spring or summer on the descending limb of snowmelt flood flows and after floodplain wetlands are disconnected from the river (Muth et al. 2000; Bestgen et al. 2011), the entrainment of Bonytail larvae from the river into wetlands was improbable. Otolith daily increment analysis confirmed that age-0 Bonytails captured from Stewart Lake in 2015 were spawned well after gate closure and disconnection of the wetland from the Green River on May 28. The largest and smallest age-0 preserved specimens (48 and 41 mm TL; captured on September 3) had hatching dates of June 24 and 27, respectively, with spawning dates likely 4–7 d earlier based on egg incubation times at summer water temperatures (Hamman 1982).

Growth of otolith-aged specimens was rapid (0.63 and 0.52 mm/d, respectively, assuming 5.5 mm TL at hatching; Snyder et al. 2016) but similar to growth rates reported for the early life stages of other native Green River cypriniform fishes in warm, food-rich environments (Bestgen 1996, 2008; Bestgen et al. 2006). Based on spacing of otolith increments, growth was faster during approximately the first 30 d of life than later, perhaps indicating reductions in food supply or increased stress beginning in late July or early August.

Sampling of the Stewart Lake and Johnson Bottom fish communities during autumn draining in 2015 and 2016 yielded a total of 18 fish species—possibly 19 if the presence of Roundtail Chub is verified (Schelly and Breen 2015; R. C. Schelly, unpublished data; M. T. Jones, unpublished data; www.coloradoriverrecovery.

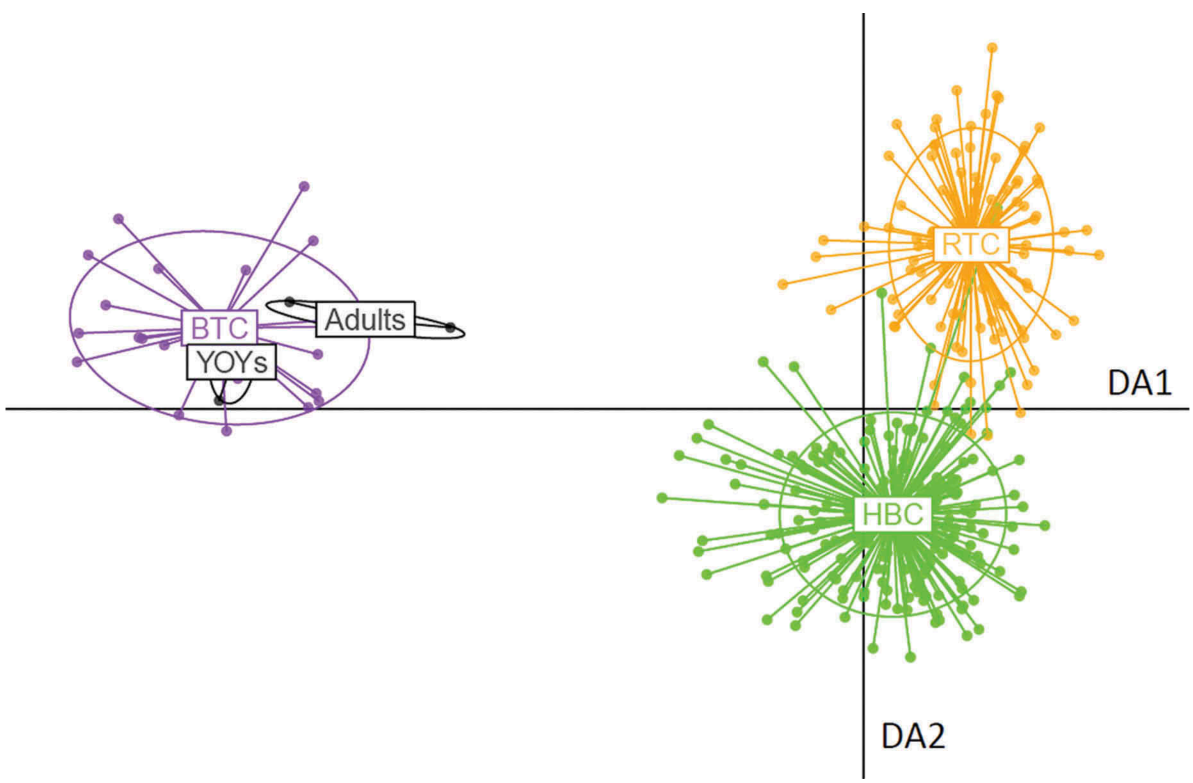

FIGURE 2. Discriminant analysis of principal components based on the genotypes of Stewart Lake samples and the three *Gila* spp. found in the Green River, Utah, near Stewart Lake (DA = discriminant axis; BTC = Bonytail; HBC = Humpback Chub; RTC = Roundtail Chub). Ellipses represent 95% confidence limits about the data for each taxon, and those for adults and age-0 fish (young of the year [YOY]) indicate genotypes that were sampled from Stewart Lake in 2015.

org/documents-publications/work-plan-documents/project-annual-reports.html). By late summer in 2015 and 2016, wetland fish communities were composed primarily (>98%) of nonnative species (Table 4); based on specimen size, most were age-0 fish that had been produced in the wetlands. For example, among 405,295 fish collected from Stewart Lake in 2015, the only native taxa were Bonytails (24 age-0 and adult fish) and Razorback Suckers (age-0 fish: $n = 87$, TL range = 75–152 mm; older fish: $n = 10$, TL range = 245–315 mm). The Green Sunfish, a potential predator of young *Gila* (Dudley and Matter 2000), was abundant in 2015 ($n = 131{,}377$, TL range = 17–151 mm) and much more so than in 2014 ($n = 329$) or 2016 ($n = 38{,}448$), when age-0 Razorback Suckers were more abundant. Common Carp ($n = 111{,}317$, TL range = 19–183 mm; excludes adults captured in July) and Fathead Minnow ($n = 154{,}224$, TL range = 18–85 mm) were also abundant in Stewart Lake during 2015 and in both wetlands during 2016. Presence of abundant and introduced predatory fishes, including the Black Bullhead, Green Sunfish, and crappies *Pomoxis* sp., likely precludes higher native fish survival in floodplain wetlands.

TABLE 4. Relative abundance of fishes captured from two floodplain wetlands (Stewart Lake and Johnson Bottom) of the middle Green River, Utah, during 2015 and 2016 (A = abundant, >1,000 individuals captured; C = common, 100–1,000 individuals captured; R = rare, <100 individuals captured; dash = the species was not found in that location and year). Relative abundance in Johnson Bottom is based on qualitative assessments, as not all fish were enumerated. The presence of Roundtail Chub is questionable because of unusual specimen morphology; specimen identification via genetic analysis is underway.

Species	Stewart Lake 2015	Stewart Lake 2016	Johnson Bottom 2016
Native			
Bonytail *Gila elegans*	R	R	R
Bonytail or Roundtail Chub *G. robusta*	R	R	
Colorado Pikeminnow *Ptychocheilus lucius*		R	
Razorback Sucker *Xyrauchen texanus*	C	A	
Speckled Dace *Rhinichthys osculus*		R	R
Introduced			
Black Bullhead *Ameiurus melas*	A	R	A
Black Crappie *Pomoxis nigromaculatus*[a]		R	C
Brook Stickleback *Culaea inconstans*	R	C	R
Channel Catfish *Ictalurus punctatus*	R	R	R
Common Carp *Cyprinus carpio*	A	A	A
Creek Chub *Semotilus atromaculatus*	R	R	
Fathead Minnow *Pimephales promelas*	A	A	A
Green Sunfish *Lepomis cyanellus*	A	A	A
Iowa Darter *Etheostoma exile*	R	R	R
Northern Pike *Esox lucius*	R		
Red Shiner *Cyprinella lutrensis*	A	C	C
Redside Shiner *Richardsonius balteatus*	R	R	
Sand Shiner *Notropis stramineus*	C	R	
White Sucker *Catostomus commersonii*	C	C	R

[a]Fish were presumed to be Black Crappies, but the presence of White Crappies *P. annularis* cannot be ruled out.

Efforts to increase the survival of stocked Bonytails have been initiated by the Upper Colorado River Endangered Fish Recovery Program. Green River flow pattern and temperature recommendations for Flaming Gorge Dam releases, which attempt to restore aspects of a more natural flow regime, are expected to improve habitat for Bonytails and other native fishes (Muth et al. 2000). Use of additional stocking areas, including alluvial valley river reaches and their associated floodplain wetlands, was successful in 2015 and 2016, and such efforts to increase the survival, reproduction, and recruitment of Bonytails should continue (Bestgen et al. 2008). Bonytails have exhibited successful reproduction and recruitment in the lower Colorado River when stocked in ponds isolated from predaceous nonnative fishes (Mueller 2006; Marsh et al. 2013a, 2013b). The effects of size at stocking into the upper Colorado River are the focus of ongoing analyses, as larger fish may survive at higher rates (Badame and Hudson 2003; Nesler et al. 2003; Zelasko et al. 2010). Additional techniques to improve poststocking survival may include novel acclimation techniques (Chart and Cranney 1993), pre-stocking exercise regimens, or exposure to predators prior to release to increase predator avoidance behaviors. An understanding of optimal river conditions and release techniques may enhance the survival of stocked Bonytails.

Since the decline of wild Bonytail populations several decades ago, many large- and small-bodied piscivores have proliferated, thus posing an increased threat to the recovery of all endangered fishes in the upper Colorado River (Tyus and Saunders 2000; Mueller 2005; Bestgen et al. 2006; Johnson et al. 2008). Although Channel Catfish were widespread and abundant prior to the decline of Bonytail populations, species such as the Northern Pike, Smallmouth Bass *Micropterus dolomieu*, Walleye *Sander vitreus*, and small-bodied Red Shiner have dramatically increased in distribution and abundance since the early 1970s and are now widespread and abundant in a wide variety of habitats throughout the upper Green River basin (Holden and Stalnaker 1975a, 1975b; Mueller 2005; Bestgen et al. 2006). Ongoing actions to reduce the abundance of nonnative predators—particularly large-bodied species—in the upper Colorado River basin may increase the short-term survival of stocked Bonytails. However, long-term solutions are required, including more effective removal techniques and the reduction or elimination of source populations (Zelasko et al. 2016).

A better understanding of how environmental conditions, habitat use and spawning success in floodplain wetlands, and interactions with nonnative fishes affect the health, physical condition, and survival of Bonytails could facilitate this species' recovery. Further understanding of the potential for interactions with extant populations of congeneric wild chubs is also a consideration and may assist with the conservation of other *Gila* populations in the upper Colorado River basin. Successful reproduction of Bonytails and survival of their young—despite the presence of abundant nonnative fishes—in two off-channel wetlands of the middle Green River indicate that the expanded use of such locations in the upper Colorado River basin may improve the conservation status and advance recovery efforts for this critically endangered species.

ACKNOWLEDGMENTS

This study was funded by the Recovery Program for Endangered Fish in the Upper Colorado River. The Recovery Program is a joint effort of the USFWS; USBR; the Western Area Power Administration; the states of Colorado, Utah, and Wyoming; upper basin water users; environmental organizations; and the Colorado River Energy Distributors Association. Funding was administered by the USBR under Cooperative Agreements with Colorado State University, the Larval Fish Laboratory, and the Utah Division of Wildlife Resources. Project administration was facilitated by D. Speas, D. Parcesepe, and C. Morales. Laboratory, field, and administrative assistance was provided by M. Fiorelli, J. Christopherson, K. Holder, L. Johnson, S. Harrison, J. Herdmann, S. Raber, M. Stanton, M. Tohl, B. Anderson, C. Brundage, A. Disch, T. Hedrick, E. McKall, J. Swenson, G. Tournear, and C. Smith. T. Czapla provided unpublished data; A. A. Hill constructed Figure 1; and M. Haworth assisted with otolith aging. We especially thank W. Wilson and the Molecular Ecology Laboratory at the USFWS Southwestern Native Aquatic Resources and Recovery Center for conducting molecular analyses and providing all related results. Comments from T. Chart, K. McAbee, T. Czapla, A. Hill, and anonymous reviewers improved the manuscript.

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