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VIABILITY OF RAZORBACK-FLANNELMOUTH SUCKER HYBRIDS

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Abstract—Razorback sucker (*Xyrauchen texanus*) and flannelmouth sucker (*Catostomus latipinnis*) live in sympatry in the Colorado River basin. Although morphological intermediates have been described since 1889, hybrids were seemingly rare. Rarity of hybrids was likely attributed to razorback suckers' ability to find conspecific mates throughout the basin. Dams have segmented the Colorado River, altering habitat and isolating native fish populations. As a result, razorback suckers became endangered. Razorback suckers are uncommon in the Colorado River and hybridization could increase because of limited conspecific mates. To understand the impacts of hybridization on recovery of the razorback sucker, information on hybrid viability is needed. We compared hatch success and larval survival of artificially spawned razorback sucker, flannelmouth sucker, and their hybrids. We were able to successfully spawn and rear all combinations, implying that there are limited pre- and postzygotic isolation mechanisms, and hybrids are likely to survive in the wild.

Resumen—El matalote jorobado (*Xyrauchen texanus*) y el matalote boca de franela (*Catostomus latipinnis*) son simpátricos en la cuenca del río Colorado. Aunque intermedios morfológicos se han descrito desde 1889, los híbridos aparentemente fueron raros. La rareza de híbridos fue probablemente atribuible a la capacidad de los matalotes jorobados para encontrar a sus conespecíficos a lo largo de la cuenca. Presas han segmentado la cuenca del río Colorado, alterando el hábitat y aislando las poblaciones de peces nativos. Por ello el matalote jorobado llegó a ser una especie en peligro de extinción. Por ser *X. texanus* poco común en el río Colorado la hibridación pudo incrementar por la escasez de parejas conespecíficas. Para comprender los impactos de la hibridación sobre la recuperación del matalote jorobado se necesita información sobre la viabilidad de los híbridos. Comparamos las tasas de eclosión y supervivencia larval del matalote jorobado, matalote boca franela y sus híbridos artificialmente engendrados. Logramos el desove y producción de crías para todas las combinaciones, lo que sugiere que hay mecanismos reducidos de aislación pre- y post-cigóticos y que los híbridos pueden sobrevivir en la naturaleza.

Hybridization occurs when two different species successfully reproduce. Reproductive isolation mechanisms restrict gene flow between species, generally making hybrids rare (Kocher, 2004). Common reproductive isolation mechanisms include temporal and spatial separation of mating, zygotic failure, reduced fitness of hybrid offspring, and differences in breeding requirements (Freeman and Herron, 2007). Fishes in general have weakened reproductive isolating mechanisms because of similar spawning behaviors and external fertilization. Morphological intermediates between two catostomid species of the Colorado River, razorback sucker (*Xyrauchen texanus*) and flannelmouth sucker (*Catostomus latipinnis*), have been described as early as 1889 (Jordan, 1889). However, hybrids were rarely reported, possibly because there might have been fewer hybrids for the reason that razorback sucker undertook long spawning migrations that allowed for large congregations of conspecific mates or even spatial and temporal isolation from flannelmouth sucker (Wick et al., 1982; Minckley, 1983).

Currently, dams segment the Colorado River, which have altered historic river conditions from warm and turbid to relatively cold and clear, and have negatively affected razorback and flannelmouth suckers (Holden and Stalnaker, 1975; Minckley, 1983; Douglas and Marsh, 1998; Marsh et al., 2003; Albrecht et al., 2010, 2017). Compared with historic ranges, razorback sucker are confined to relatively small sections of the Colorado River, and while flannelmouth sucker are still widely distributed throughout the Colorado River basin, dams have fragmented their movement. The razorback sucker was designated as endangered in 1991 (United States Fish and Wildlife Service, 1991) largely because of habitat alteration and predation by nonnative species (Wick et al., 1982; Gutermuth et al., 1994; Weiss et al., 1998; Bestgen, 2008). Throughout the species' present-day ranges, the flannelmouth sucker remains relatively common despite habitat alterations (Mueller and Wydoski, 2004), while the razorback sucker remains relatively rare. The rarity of the razorback sucker increases its risk of hybridization with flannelmouth sucker because of limited numbers of conspecific mates (Hubbs, 1955; Tyus and Karp, 1990). Adult hybrids have been documented in the wild (Hubbs, 1955; Wick et al., 1982; Gutermuth et al., 1994; Douglas and Marsh, 1998); however, the viability of hybrids at early life stages have not been evaluated (Tyus and Karp, 1990).

Although genetic studies have shown that razorback sucker × flannelmouth sucker hybrids do produce reproductively viable offspring (Buth et al., 1987; Douglas and Marsh, 1998; Dowling et al., 2012), our study is the first to compare hatch success and larval survivability of razorback sucker × flannelmouth sucker hybrids to that of the parent species. Information is needed on hybrid viability to understand how hybridization may affect recovery and conservation of existing razorback sucker populations. We hypothesize that if hybrids have inferior hatch success and larval survival compared with the parental species, then hybridization with the flannelmouth sucker should not be a risk to the recovery and conservation of razorback sucker. However, if hybrids exhibit similar hatch success and larval survival to the parental species, then hybridization may likely impact razorback sucker genetics through introgressive hybridization (Buth et al., 1987). Our objective was to determine hybrid viability verses parental stocks in early life stages by quantifying hatch success and larval survival of artificially spawned razorback sucker, flannelmouth sucker, and their hybrids under controlled laboratory conditions.

We spawned hatchery-reared razorback sucker obtained from the Lake Mead Fish Hatchery, operated by the state of Nevada, and wild flannelmouth sucker collected from the Paria River, a tributary of the Colorado River, Arizona. We administered two doses of Ovaprim (Western Chemical, Ferndale, Washington) to each fish to ripen gametes; first we injected 0.5 mL/kg of body weight of Ovaprim, followed by an additional 0.25 mL/kg of body weight of Ovaprim 24 h after the original injection. We collected gametes in 50-mL polypropylene centrifuge tubes 48 h after the initial injection. We divided eggs from each female approximately equally among multiple tubes, dependent on number of eggs obtained from each individual female. We fertilized eggs in individual tubes with the milt of a single male of the appropriate species to complete four different progenies: razorback sucker female × razorback sucker male, flannelmouth sucker female × flannelmouth sucker male, razorback sucker female × flannelmouth sucker male, and flannelmouth sucker female × razorback sucker male. We labeled tubes with unique codes to track parent combinations to determine whether a parent's gametes were viable.

We were unable to achieve all four progeny combinations within the same year because eggs did not ripen in some females. We used three female razorback suckers, six male razorback suckers, and two male flannelmouth suckers to produce pure razorback sucker and razorback sucker female × flannelmouth sucker male progenies in 2016. In 2017 we used four female flannelmouth suckers, four male flannelmouth suckers, and three male razorback suckers to produce pure flannelmouth sucker and flannelmouth sucker female × razorback sucker male progenies. In 2016, we placed four replicates of fertilized eggs from each unique parent combination into individual containers constructed of 15.24-cm-diameter polyvinyl chloride pipes cut 25.4 cm in length. Containers contained four mesh-covered openings on the sides, each 7.6 cm in diameter, which allowed for water circulation. We separated hatching containers and placed them into four (2016) and two (2017) 83-L round rearing tanks filled with dechlorinated city water (Flagstaff, Arizona). In 2017, we replicated parent combinations only twice because fewer eggs were produced. In 2016 and 2017, an average of 774 ($SD = 620$) and 475 ($SD = 160$) eggs were in each container, respectively.

Egg-rearing tanks were equipped with one 7.6 × 3.8-cm air stone, a 380-L sump, and a biofilter containing 0.057 m^3 of Sweetwater® SWX Bio-Media (Pentair Aquatic Eco-Systems, Inc., Cary, North Carolina). The outflow from the filter was designed to circulate through and under the containers, providing oxygen to the eggs. We reared eggs at 20°C, maintained by ambient room air temperature. At 24 h postfertilization, we treated all eggs for fungus in a dip solution of 0.5 mL of methylene blue/3.78 L of water for 5 min each day for 2–3 days. Fungus treatments ceased when we observed movement within the eggs.

We photographed eggs 24 h postfertilization and

Table 1—Mean egg hatch success and lower and upper bounds of 95% confidence intervals (*CI*) for razorback sucker, flannelmouth sucker, and hybrids, with *n* representing the number of containers of eggs.

Year	Progeny	*n*	Mean hatch success, % (95% *CI*)
2016	Razorback sucker	20	4.24 (2.14–6.68)
	Razorback sucker ♀ × flannelmouth sucker ♂	8	10.67 (0.73–11.80)
2017	Flannelmouth sucker ♀ × razorback sucker ♂	8	7.88 (1.91–15.00)
	Flannelmouth sucker	9	2.62 (0.40–6.00)

posthatch larvae to facilitate accurate counts. Hatching occurred between 3 and 8 days postfertilization. We used a Canon Powershot SD750 camera (Canon Inc., Ōta, Tokyo, Japan) with a macro setting for all photographs. We counted eggs and larvae from the images using the multipoint tool in ImageJ (https://imagej.nih.gov/ij/). We calculated hatch success by dividing the number of larvae hatched by the number of eggs in each container. Bestgen (2008) determined that 25 mm total length was an important threshold for razorback sucker survival, and at a temperature of 20°C it took 36 days to reach this threshold. Thus, based on Bestgen (2008), we compared survival at 36 days posthatching by daily manual counts, image counts, and mortality collection. We calculated percent survival by dividing the number of surviving larvae after 36 days by the number of larvae hatched.

We used a negative binomial regression model to compare hatch and survival among treatments (e.g., hybrids vs. pure), comparing counts of eggs that hatched and fish that survived with an offset of the number of eggs or the number of hatched fish at the start. Our unit of measure for statistical analyses was individual egg- or larval-rearing containers. We have information on parental combinations, but we were unable to use that information in statistical analyses because of insufficient degrees of freedom. We conducted all statistical analyses using Program R (version 3.4.3; https://cran.r-project.org/).

In 2016, we removed 18 containers of eggs (9 pure razorback sucker, 9 hybrid), produced from one female razorback sucker from analyses because the eggs produced from this female were not viable and none hatched. Of the 28 remaining containers of eggs analyzed in 2016, there were only 2 that did not produce larvae. We excluded those containers in the analysis of larval survival. In 2017, one of the two rearing tanks leaked 5 days posthatching; therefore, we had to combine replicates of larval fish into one tank. There was not sufficient space in one tank to hold all of the containers of larvae separately, so we combined container replicates with low numbers of larvae, which resulted in a reduction in sample size from our hatch success trials to the survival trials. We also cultivated five containers of eggs in 2017 that did not produce larvae; consequently, we did not include them in the analysis of larval survival.

There was no significant difference in hatch success between hybrids and pure strains for 2016 ($P = 0.124$) or 2017 ($P = 0.212$; Table 1). For survival of larval fish there was no significant difference between hybrids or pure strains for 2016 ($P = 0.992$) or 2017 ($P = 0.464$; Table 2). A post hoc analysis of power using a power calculation for two proportions (different sample sizes; Cohen, 1988) revealed that power for hatch success was 0.097 for 2016 and 0.079 for 2017, and for larval survival it was 0.053 for 2016 and 0.33 for 2017. At the outset we knew that we would probably not have great statistical power as a result of logistics constraining our sample size, but believed that that there would be a greater effect size, because hybrids are relatively uncommon in the wild.

Razorback sucker female × flannelmouth sucker male and flannelmouth sucker female × razorback sucker male hybrids are capable of hatching and surviving. However, hatch success was generally low for all progeny combinations (3–10%), suggesting that our laboratory conditions were not ideal for egg rearing. It appears that fungus treatments with methylene blue were not effective because fungal infections plagued all containers of eggs both years, which likely contributed to the low hatch rates. We attempted to keep laboratory conditions the same from 2016 to 2017, but rearing temperatures were on average approximately 0.6°C warmer in 2017 (20.9°C, $SD = 0.40$) than in 2016 (20.3°C, $SD = 0.34$). There was a noticeable difference in larval survival from 2016 to 2017, which may be attributed to the difference in rearing temperatures, or there could be a difference in larval survival between razorback suckers and flannelmouth suckers when artificially spawned.

Table 2—Mean 36-day larval survival and lower and upper bounds of 95% confidence intervals (*CI*) for razorback sucker, flannelmouth sucker, and their hybrids, with *n* representing the number of containers of hatched larvae.

Year	Progeny	*n*	Mean larval survival, % (95% *CI*)
2016	Razorback sucker	18	94.3 (87.9–98.7)
	Razorback sucker ♀ × flannelmouth sucker ♂	8	94.8 (90.8–98.2)
2017	Flannelmouth sucker ♀ × razorback sucker ♂	6	64.5 (42.6–85.4)
	Flannelmouth sucker	4	40.0 (14.1–64.8)

Considering the rarity of razorback sucker and the high abundance of flannelmouth sucker in the Colorado River, hybridization is likely to increase (Hubbs, 1955; Tyus and Karp, 1990). Our results indicate that hybrids may hatch and survive at similar rates to that of the parent species. Given this possibility, hybridization could pose a serious threat to razorback sucker genetic integrity through introgressive hybridization (Buth et al., 1987). Understanding the mechanism of natural hybridization, be it random mixing of gametes or interspecific mate selection, could inform managers on the degree of severity to which natural hybridization takes place. If natural hybridization stems from random mixing of gametes, then hybridization will continue to occur at low levels. If natural hybridization stems from interspecific mate selection, then there is a high likelihood that in the future sections of the Colorado River may become hybrid zones where there are no longer genetically pure razorback suckers. More research needs to be conducted on the mechanisms of natural hybridization of razorback suckers to inform the development of management options to reduce the potential for hybridization and introgression in the future.

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