Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation

Razorback Sucker and Bonytail Chub Predator Recognition

U.S. DEPARTMENT OF THE INTERIOR
BUREAU OF RECLAMATION

December 2014

Lower Colorado River Multi-Species Conservation Program Steering Committee Members

Federal Participant Group

Bureau of Reclamation
U.S. Fish and Wildlife Service
National Park Service
Bureau of Land Management
Bureau of Indian Affairs
Western Area Power Administration

Arizona Participant Group

Arizona Department of Water Resources
Arizona Electric Power Cooperative, Inc.
Arizona Game and Fish Department
Arizona Power Authority
Central Arizona Water Conservation District
Cibola Valley Irrigation and Drainage District
City of Bullhead City
City of Lake Havasu City
City of Mesa
City of Somerton
City of Yuma
Electrical District No. 3, Pinal County, Arizona
Golden Shores Water Conservation District
Mohave County Water Authority
Mohave Valley Irrigation and Drainage District
Mohave Water Conservation District
North Gila Valley Irrigation and Drainage District
Town of Fredonia
Town of Thatcher
Town of Wickenburg
Salt River Project Agricultural Improvement and Power District
Unit "B" Irrigation and Drainage District
Wellton-Mohawk Irrigation and Drainage District
Yuma County Water Users' Association
Yuma Irrigation District
Yuma Mesa Irrigation and Drainage District

Other Interested Parties Participant Group

QuadState Local Governments Authority
Desert Wildlife Unlimited

California Participant Group

California Department of Fish and Wildlife
City of Needles
Coachella Valley Water District
Colorado River Board of California
Bard Water District
Imperial Irrigation District
Los Angeles Department of Water and Power
Palo Verde Irrigation District
San Diego County Water Authority
Southern California Edison Company
Southern California Public Power Authority
The Metropolitan Water District of Southern California

Nevada Participant Group

Colorado River Commission of Nevada
Nevada Department of Wildlife
Southern Nevada Water Authority
Colorado River Commission Power Users
Basic Water Company

Native American Participant Group

Hualapai Tribe
Colorado River Indian Tribes
Chemehuevi Indian Tribe

Conservation Participant Group

Ducks Unlimited
Lower Colorado River RC&D Area, Inc.
The Nature Conservancy

Lower Colorado River Multi-Species Conservation Program

Razorback Sucker and Bonytail Chub Predator Recognition

Prepared by:

Matthew W. O'Neill and William T. Stewart

Arizona Game and Fish Department

Lower Colorado River
Multi-Species Conservation Program
Bureau of Reclamation
Lower Colorado Region
Boulder City, Nevada
http://www.lcrmscp.gov

December 2014

O'Neill, M.W. and W.T. Stewart. 2014. Razorback Sucker and Bonytail Chub Predator Recognition. Submitted to the Bureau of Reclamation, Boulder City, Nevada, by the Arizona Game and Fish Department, Phoenix, Arizona. 16 p.

ACRONYMS AND ABBREVIATIONS

BONY	bonytail chub (*Gila elegans*)
BPNFCF	Bubbling Ponds Native Fish Conservation Facility
CFR	Code of Federal Regulations
cm^2	square centimeter(s)
ft	foot/feet
gal	gallon(s)
L	liter(s)
LCR	lower Colorado River
m	meter(s)
RASU	razorback sucker (*Xyrauchen texanus*)
Reclamation	Bureau of Reclamation

Symbols

>	greater than
≥	greater than or equal to

CONTENTS

Page

Summary S-1

Introduction 1
Methods 2
Study Area 2
Infrastructure Development 3
Conditioning Tanks and Shade Structure 3
Holding Tanks 3
Survival Trial Ponds 4
Experimental Design and Analysis 5
Hindering Predators 5
Conditioning Prey 7
Survival Trials 7
Results 8
Predator and Prey Size 8
Hindering Predators 8
Survival Trials 9
Conclusions 10
Management Implications 12
Future Work 13

Literature Cited 15

Tables

Table Page

1 The number of fish and number of trials conducted for each predator/prey combination 6
2 Length measurements and comparisons (Student's t-test) of predator and prey species for each predator/prey combination 8
3 Predator avoidance conditioning improves the survival of naïve hatchery-reared fish in 24-hour survival challenges 10
4 Predator avoidance conditioning did not change the size of prey fish that survived a 24-hour survival challenge 10

Figures

Figure Page

1 Map of BPNFCF showing the location of new infrastructure, including shade structures, holding tanks, and trial ponds. 2
2 Shade structure used to limit increasing water temperatures in conditioning tanks. 3
3 Eleven holding tanks (center and left side of photo) installed to hold predator and prey fish for the duration of this study..... 4
4 Galvanized steel tank with liner for holding our largest predators. 4
5 Concrete pond used for survival trials. 5
6 Optimal injection sites for preventing largemouth bass from capturing prey fish. 6
7 A greater percentage of conditioned (diamonds) than unconditioned (circles) RASU and BONY survive in 24-hour survival trials. 9

SUMMARY

The Lower Colorado River Multi-Species Conservation Program's Fish Augmentation Plan has a target goal to augment 620,000 bonytail chubs (*Gila elegans*) (BONY) and 660,000 razorback suckers (*Xyrauchen texanus*) (RASU) into the Lower Colorado River (LCR) (Bureau of Reclamation 2004). Existing populations of BONY and RASU are small to nonexistent in the LCR. Predation by nonnative fish may be the main source of mortality for stocked BONY and RASU in the LCR (Marsh and Pacey 2005; Kesner et al. 2010). Hatchery-reared fish are naïve to this risk and may not survive their first encounter with a predator. We developed a protocol to condition large groups of hatchery-reared BONY and RASU to recognize predators in an effort to improve post-stocking survival.

We used the cypriniform alarm pheromone, a chemical found in the skin of many fish species that alerts conspecifics to danger, to condition groups of predator-naïve BONY and RASU (prey fish) to recognize a predator. The alarm pheromone is obtained by sacrificing several individuals of a prey species and blending whole bodies into water to create a solution containing the pheromone. This solution is introduced to BONY and RASU in the presence of a predator with an incapacitated jaw. The jaw muscle of a predator fish is incapacitated via the injection of botulinum toxin into critical jaw-opening muscles, preventing it from capturing prey fish during conditioning.

Fish are conditioned to recognize a predator by placing a hindered predator into their tank and simultaneously adding the alarm pheromone. Following a 5-minute exposure to the alarm pheromone and hindered predator, the predator is removed, and the prey fish are moved into a concrete pond for a survival trial. Each survival trial combines prey fish (either all conditioned or all unconditioned) and actively feeding predators in a concrete pond for 24 hours. The percent survival of conditioned and unconditioned groups is then compared.

We found that predator recognition conditioning significantly improved the survival of BONY and RASU in 24-hour survival trials with largemouth bass (*Micropterus salmoides*), channel catfish (*Ictalurus punctatus*), and mixed bass and catfish. Survival of conditioned fish averaged 20-percent higher than unconditioned fish. There was no change in mean prey fish size, suggesting that all prey fish were susceptible to predation, and conditioning benefitted all prey fish sizes. Our novel conditioning methodology was successful in improving survival without prey fish seeing or experiencing predation during the conditioning process, suggesting this methodology may be successful at conditioning large groups of BONY and RASU in hatchery production ponds.

INTRODUCTION

An objective of the Lower Colorado River Multi-Species Conservation Program is to work toward the conservation of native species, including bonytail chubs (*Gila elegans*) (BONY) and razorback suckers (*Xyrauchen texanus*) (RASU), and their habitat. Populations of endangered BONY and RASU continue to remain very small despite intensive conservation and repatriation efforts. There are two primary hypotheses for why the abundance of native fish in the lower Colorado River (LCR) is low: (1) altered habitat conditions contribute to poor recruitment and survival and (2) nonnative fish in the LCR compete with and prey upon native fish, reducing growth and survival. Striped bass (*Morone saxitilis*) and members of the sunfish (Centrarchidae) and catfish (Ictaluridae) families are the most abundant nonnative piscivorous fish species in the LCR (Mueller 2005). Diet studies have indicated that these apex predators ingest stocked native fishes, thus contributing to low native fish abundance (Marsh and Brooks 1989; Karam and Marsh 2010). Efforts under the Lower Colorado River Multi-Species Conservation Program focus on habitat creation (the Habitat Conservation Plan) (Bureau of Reclamation [Reclamation] 2004) and the Fish Augmentation Plan (Reclamation 2006), which aims to increase native and endangered fish abundance.

Most stocked BONY and RASU are not exposed to fish predators before stocking (F. Agyogos 2014, personal communication). If predation is the primary factor of post-stocking mortality, it is plausible to attribute predator naïvety to high initial stocking mortality. Predator recognition conditioning has been identified as a potential method for increasing survival of hatchery-reared fish stocked into the wild (Suboski and Templeton 1989; Griffin et al. 2000).

An alarm substance known as "Schrekstoff," which is embedded in the skin of ostariophysian fish, is released when an individual's skin has been damaged (Mathuru et al. 2012). This alarm substance has been shown to elicit a fright reaction to conspecific skin extract (Brown et al. 2000), and this fright reaction has been used to condition many species of fish to avoid predation (Suboski and Templeton 1989). Prior to release, exposure of BONY and RASU to a predator, coupled with the addition of an alarm substance, may condition these fishes to view predators as a danger. If predator-naïve hatchery-reared fish can be conditioned to recognize and avoid predators, post-stocking mortalities attributed to predation may decrease. Previous work has suggested that BONY and RASU may be successfully conditioned to recognize predators and show altered behavior (Ward and Figiel 2013) and improved survival (Mueller et al. 2007), though differences in survival were not significant, and conditioning techniques would be difficult to use in large-scale hatchery settings.

Here, we developed a protocol for conditioning BONY and RASU to avoid largemouth bass (*Micropterus salmoides*) and channel catfish (*Ictalurus punctatus*) as proxies for striped bass and flathead catfish (*Pylodictis olivaris*).

We selected largemouth bass and channel catfish because of their similar hunting behaviors to striped bass and flathead catfish. Furthermore, both of these predator species may be present in areas where BONY and RASU are stocked. We used classical conditioning techniques by exposing these predator-naïve fish to a hindered predator in the presence of each prey species' alarm pheromone. The effectiveness of conditioning is measured by comparing the survival of conditioned and unconditioned fish after exposure to an actively feeding predator.

METHODS

Study Area

Trials were conducted at the Bubbling Ponds Native Fish Conservation Facility (BPNFCF) in Cornville, Arizona. Infrastructure needed to be developed onsite to accommodate this research and included installation of shade structures, holding tanks for predator and prey fish, and tanks for predator avoidance conditioning. Survival trials were conducted in existing concrete ponds (figure 1).

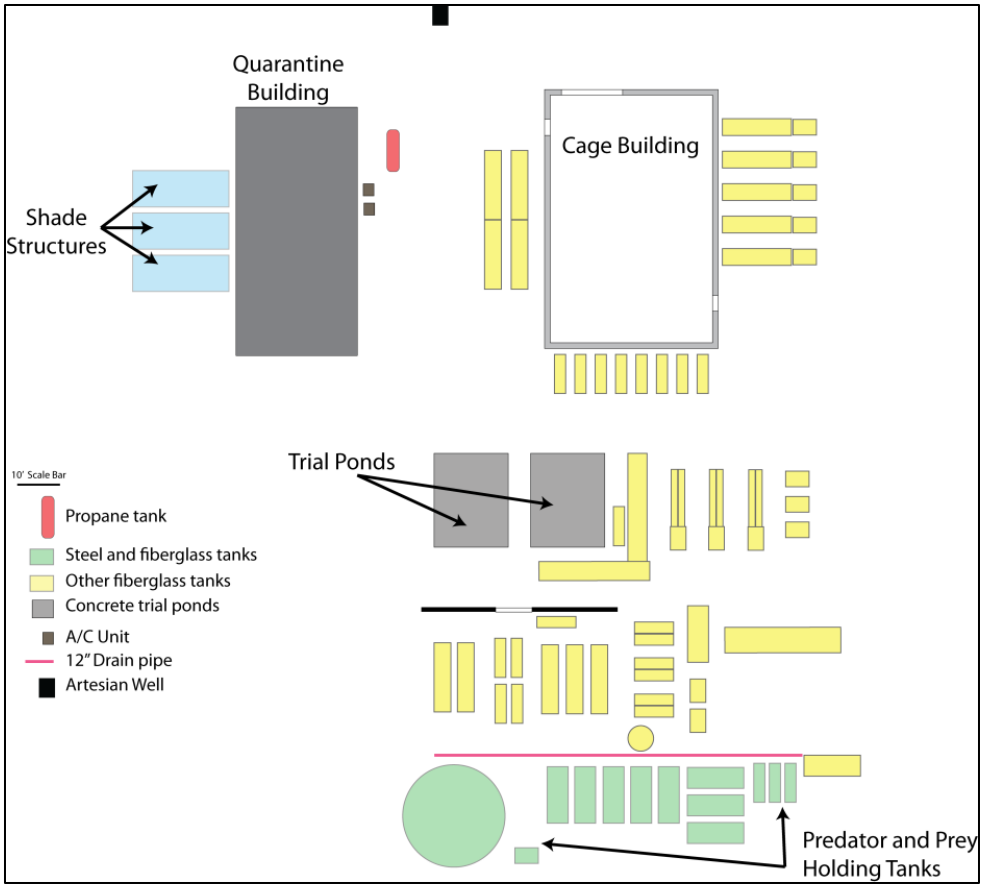

Figure 1.—Map of BPNFCF showing the location of new infrastructure, including shade structures, holding tanks, and trial ponds.

Infrastructure Development

Conditioning Tanks and Shade Structure

Fish were conditioned in 8-foot (ft) (2.4-meter [m])-diameter, 2-ft (0.6-m)-deep, 380-gallon (gal) (1,438.5-liter [L]) plastic stock tanks with recirculating water filters. Three shade structures were installed to maintain constant water temperatures during conditioning throughout the experiment (figure 2). This system can shade ten 8-ft (2.4-m)-diameter tanks. Due to difficulties with high winds, the shade structures were only used during low-wind periods and weighted with dirt-filled buckets and cinderblocks.

Figure 2.—Shade structure used to limit increasing water temperatures in conditioning tanks.

Holding Tanks

Eight 750-gal (2,839.1-L), three 150-gal (587.8-L), and one 15,000-gal (56,781.2-L) tank were installed to hold predator and prey fish (figures 1, 3, and 4). These tanks combined held over 21,000 gal (79,493.6 L) of water and provided adequate holding space for the prey fish used in this study. A 21-ft (6.4-m)-diameter, 8-ft (2.4-m)-deep galvanized steel tank with a polyvinyl chloride liner was installed for the predation trials, but we instead used this tank for holding large predators following mortality in smaller holding tanks (figures 2 and 4). A pump and blower on an independent, alarmed, electrical circuit recirculated water and provided aeration to all tanks.

Figure 3.—Eleven holding tanks (center and left side of photo) installed to hold predator and prey fish for the duration of this study.

Figure 4.—Galvanized steel tank with liner for holding our largest predators.

Survival Trial Ponds

The survival trials were conducted in two existing 20 x 18 x 4 ft (6.1 x 5.5 x 1.2 m) deep concrete ponds (figure 5). These ponds held approximately 4,000 gal (15,141.7 L) of water during the survival trials, and water temperatures were 66.2 degrees Fahrenheit (19 degrees Celsius) during the conditioning and survival trials. Both survival trial ponds had two groups of two cinderblocks that provided approximately 3,700 square centimeters (cm^2) of overhead cover. The cover was 8 inches (20.32 centimeters) or more off the bottom of the pond; therefore, it was accessible to both predator and prey species.

Figure 5.—Concrete pond used for survival trials.

Experimental Design and Analysis

We compared the survival rate of conditioned groups of fish to unconditioned groups of fish in the survival trials. We used two prey species (BONY and RASU) and three predator groups (largemouth bass, channel catfish, and both predator species together) for a total of six predator/prey combinations. Eight survival trials were completed for each predator group for both conditioned and unconditioned prey species (16 trials per predator/prey combination). This factorial design yielded a total of 96 survival trials (table 1). The variable number of prey in each predator/prey combination (due to number of fish available) resulted in percent survival values that cannot easily be compared between predator/prey combinations. We thus could not to use an analysis of variance to compare the overall survival of conditioned and unconditioned fish regardless of predator or prey species. We therefore used Student's t-tests to compare the survival rate (percent survival of a single trial) of conditioned trials to unconditioned trials within each predator/prey combination. For the same reasons, we also used Student's t-tests to identify differences in predator and prey fish size. These size comparisons were used to: (1) confirm that predator and prey size did not differ between conditioned and unconditioned groups and (2) identify differences in prey size pre- and post-survival trials.

Hindering Predators

We used botulinum toxin to paralyze jaw-opening muscles in the predators, allowing the predator fish to swim freely among the group of prey fish being conditioned. This technique allowed the predator to behave normally, providing physical, visual, and chemical cues without the ability to actually consume the prey fish. Following the methods of O'Neill and Gibb (2007), we injected

Table 1.—The number of fish and number of trials conducted for each predator/prey combination

Predator species	Prey species	# prey per trial	# predators per trial	# of trials
Largemouth bass	RASU	12	2	16
Channel catfish	RASU	8	2	16
Mix	RASU	8	1 each	16
Largemouth bass	BONY	20	4	16
Channel catfish	BONY	12	2	16
Mix	BONY	12	1 each	16

botulinum toxin into the hyoid musculature to reduce the opening velocity of the lower jaw. Retraction of the hyoid apparatus assists with rapid jaw depression and opercular expansion. We used six 0.2-microliter injections of botulinum toxin Type A complex (Metabiologics, Inc., http://www.metabiologics.com) into the hyoid region to paralyze these muscles (figure 6). The injected fish were allowed to recover for approximately 1 week and then were given small prey fish to confirm that they could not capture fish. The Type A complex can be purchased commercially with minimal Federal permitting requirements (42 CFR 73), and it is safe to work with once diluted.

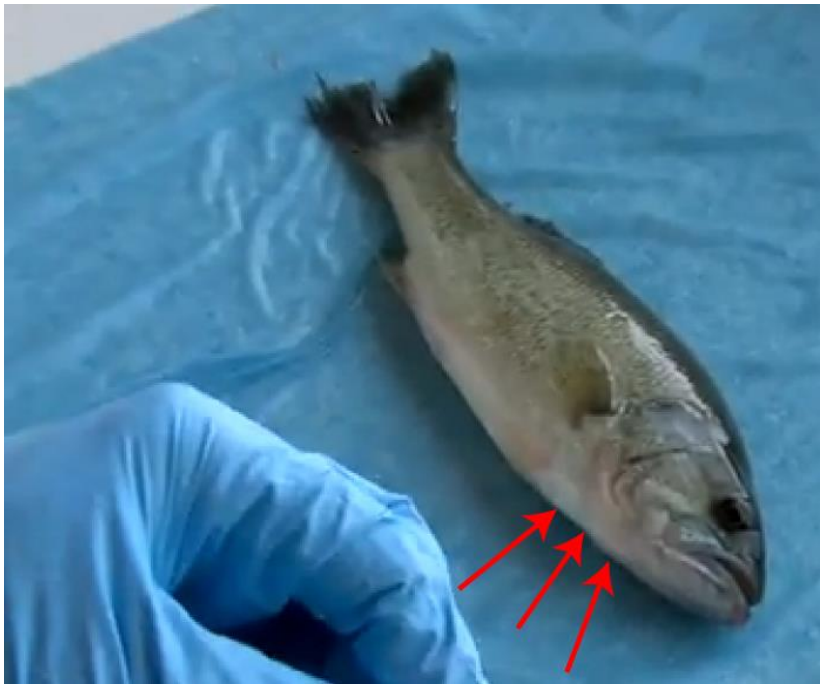

Figure 6.—Optimal injection sites for preventing largemouth bass from capturing prey fish.
We used three bilaterally paired injections; injection sites are marked with arrows.

Conditioning Prey

Prey fish were held in sixteen 150- or 700-gal fiberglass tanks on flow-through artesian well water at the BPNFCF (see figure 1). Prior to conditioning, we randomly selected and measured 8 to 20 fish from the holding tanks and placed them into 8-ft-round (300-gal) plastic tanks. The size range and number of fish per tank differed between predator/prey combinations depending on the size and number of prey fish available (see table 1). However, the number and size of prey fish per tank was constant within a predator/prey combination (see tables 1 and 2). Fish were allowed to acclimate to these tanks for at least 1 week, and then two tanks were randomly selected for survival trials (one conditioned and one unconditioned).

To condition prey, we first removed the net covers from the two tanks selected for the survival trials. We then prepared the alarm pheromone (made fresh a few minutes before each conditioning event) by randomly selecting three conspecific prey fish from the holding tanks. These fish were between 90 and 100 millimeters standard length for BONY and 75 and 85 millimeters standard length for RASU (approximate skin surface area of 26–34 cm^2 per fish for BONY and 14–20 cm^2 for RASU). Fish were euthanized with a swift blow to the head, placed into a small blender, and homogenized in 500 milliliters of water for 3–4 minutes until no large pieces remained. A hindered predator (one bass, one catfish, or one of each depending on the predator/prey combination being tested) was captured from a 150-gal holding tank, and the pheromone solution was placed into a bucket with the predator. We randomly selected from a total of five hindered bass and two hindered catfish as the conditioning predator. The predator and alarm pheromone solution were then poured into the conditioning tank of prey fish. Conditioning lasted 5 minutes, during which no observers walked within sight of the tank. After the 5-minute conditioning, the predator was removed from the conditioning tank, and the prey fish were quickly netted into separate buckets from both the conditioned group and the unconditioned group. These fish were then added to one of the two survival trial ponds.

Survival Trials

Immediately following conditioning, fish were moved to the survival trial ponds. Conditioned and unconditioned prey fish were placed in separate, randomly selected ponds. After allowing the prey fish to acclimate to the pond for 30 minutes, predators were added to the pond, and the 24-hour survival trial began (see table 1 for counts of predator and prey fish in each trial). Predator fish were randomly assigned to survival trials; most predators were used for several trials, and all predators were starved for at least 7 days prior to being used in a trial. After allowing predators to feed on prey fish for 24 hours, the water was drained from both ponds, the predators were removed, and the surviving prey fish were counted and measured. At the conclusion of all 16 trials for a prey/predator combination, the proportion of living fish for each conditioned and unconditioned group were compared using Student's t-tests as described above.

RESULTS

Predator and Prey Size

A difference in predator and prey sizes between conditioned and unconditioned fish in a predator/prey combination would complicate interpretation of our results by potentially altering the prey fish susceptibility to being eaten. We used Student's t-tests to confirm that predator and prey sizes within a predator/prey combination did not vary between conditioned versus unconditioned groups (table 2). No tests were significant (table 2), showing that there will not be an effect of size that complicates our results.

Table 2.—Length measurements and comparisons (Student's t-test) of predator and prey species for each predator/prey combination

		Prey size					Predator size				
Predator species	Prey species	Mean Prey Standard Length ± Std Error		Student's t-test parameters			Mean Predator Standard Length ± Std Erro		Student's t-test parameters		
		Conditioned	Unconditioned	t value	df	p-value	Conditioned	Unconditioned	t value	df	p-value
Bass	Razorback	49.44 ± 2.31	49.67 ± 2.37	-0.07	10.85	0.95	279.13 ± 6.19	281.07 ± 3.31	-0.28	10.57	0.79
Catfish	Razorback	54.40 ± 0.84	54.06 ± 0.99	0.26	13.62	0.80	366.56 ± 13.37	369.06 ± 8.22	-0.16	11.63	0.88
Mix	Razorback	51.89 ± 0.63	52.50 ± 0.80	-0.60	13.31	0.56	347.13 ± 9.43	317.63 ± 6.00	2.27	7.00	0.06
Bass	Bonytail	74.23 ± 4.38	69.43 ± 4.23	0.79	13.98	0.44	267.47 ± 10.70	263.97 ± 9.92	0.24	13.92	0.81
Catfish	Bonytail	67.68 ± 1.32	66.63 ± 0.85	0.67	11.97	0.51	360.56 ± 9.43	363.88 ± 12.75	-0.21	12.90	0.84
Mix	Bonytail	65.21 ± 1.13	66.71 ± 0.80	-1.09	12.65	0.30	327.81 ± 10.88	321.06 ± 10.55	0.45	13.99	0.66

Hindering Predators

Our use of botulinum toxin to paralyze jaw-opening muscles was successful in preventing prey capture for up to 5 months. Both largemouth bass and channel catfish, while unable to capture fish, were still able to open their jaws for respiration and, in fact, were still able to open their mouths to capture floating or sinking pellet food. This technique allowed us to use the same set of bass for conditioning prey fish for nearly all of the trials. During the course of the experiment, we injected a total of eight bass with botulinum toxin. Three of these bass perished soon after injections due either to toxin overdose or poor handling. Two other bass were unable to feed at all and perished within 1 month of injections, though these bass were usable for conditioning until they perished. The three remaining bass were able to feed on pellet feed but unable to catch fish prey for at least 6 months. These three bass did the majority of our conditioning. We also injected four channel catfish. Two of these fish perished fairly soon after injection likely due to infections following handling. The remaining fish survived for the duration of the experiment and were able to eat pellet food. These fish did not capture any prey fish, though we did not attempt to starve them to encourage them to capture fish.

Survival Trials

Predator avoidance conditioning caused a significant increase ($p > 0.001$ to 0.02) in survival of BONY and RASU in all predator combinations in 24-hour survival challenges (figure 7 and table 3). The improvement in survival BONY averaged 26 percent for all predator/prey combinations, and RASU survival improved 27 percent. Prey fish became extremely agitated during the conditioning process, showing rapid swimming and use of any available cover (including nets trying to catch them). Prey fish might still use the cinderblock structure in the tank for cover, but they would avoid the part of a block that a predator would hide in during conditioning. Unconditioned fish were much less agitated when they were removed before their survival trial and were more interested in escaping net capture.

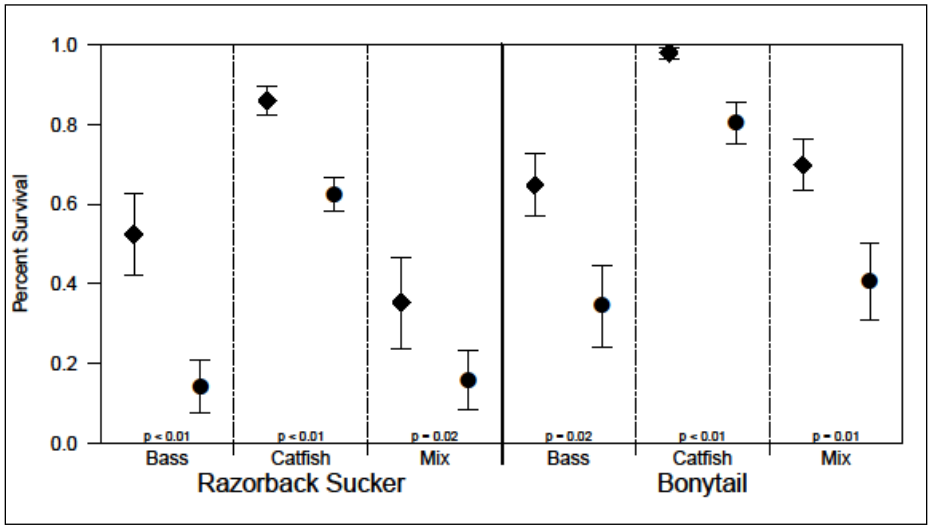

Figure 7.—A greater percentage of conditioned (diamonds) than unconditioned (circles) RASU and BONY survive in 24-hour survival trials.
Error bars denote standard error, and p values from Student's t-tests are reported beneath each trial combination.

When prey fish were added to the survival trial ponds, no differences in behavior (swimming speed or use of cover) between conditioned and unconditioned fish were observed. However, when predators were added to the survival trial ponds, the conditioned fish would either school up and remain several feet from any predators or they would disappear into or under cinderblocks. Unconditioned fish typically did not show any changes in behavior when predators were added and were documented using bass as cover.

Table 3.—Predator avoidance conditioning improves the survival of naïve hatchery-reared fish in 24-hour survival challenges

Predator species	Prey species	# fish per trial	Mean # of Conditioned Fish Consumed	Mean # of Unconditioned Fish Consumed	Conditioning Mean Percent Survival ± Std Error		Student's t-test parameters		
					Conditioned	Unconditioned	t value	df	p-value
Bass	Razorback	12	5.72	10.29	0.523 ± 0.102	0.143 ± 0.068	3.11	11.89	0.005
Catfish	Razorback	8	1.13	3.00	0.859 ± 0.037	0.625 ± 0.041	4.25	13.85	0.000
Mix	Razorback	8	5.18	6.73	0.353 ± 0.115	0.158 ± 0.074	2.40	7.00	0.024
Bass	Bonytail	20	7.04	13.11	0.648 ± 0.078	0.344 ± 0.103	2.36	13.04	0.017
Catfish	Bonytail	12	0.25	2.38	0.979 ± 0.014	0.802 ± 0.052	3.29	7.96	0.006
Mix	Bonytail	12	3.62	7.11	0.698 ± 0.065	0.407 ± 0.096	2.51	12.29	0.013

Finally, we did not find a difference in the size of prey fish between pre-survival length measurements and measurements of survivors after survival trials (table 4). The fact that smaller and larger fish were eaten at equal rates shows that all prey fish were capable of avoiding or escaping the predator – the major factor determining survival appears to be behavior for avoiding predators rather than a physical limitation of burst or sustained swimming speeds needed to avoid predation.

Table 4.—Predator avoidance conditioning did not change the size of prey fish that survived a 24-hour survival challenge

Predator species	Prey species	Prey size difference (post-trial SL - initial SL) Mean size difference (mm) ± Std Error		Student's t-test parameters		
		Conditioned	Unconditioned	t value	df	p-value
Bass	Razorback	2.39 ± 3.52	-3.54 ± 1.62	1.528	4.216	0.198
Catfish	Razorback	0.22 ± 0.62	0.96 ± 1.86	-0.379	7.293	0.716
Mix	Razorback	-0.20 ± 1.91	-1.45 ± 3.22	0.335	6.755	0.748
Bass	Bonytail	0.53 ± 0.42	1.19 ± 1.46	-0.437	5.823	0.678
Catfish	Bonytail	-0.31 ± 0.33	-0.07 ± 0.39	-0.462	11.657	0.653
Mix	Bonytail	-0.62 ± 0.49	-2.70 ± 2.21	0.919	5.484	0.397

CONCLUSIONS

Our data suggest that BONY and RASU can be conditioned to recognize predators and the conditioning can improve short-term survival. Furthermore, our conditioning technique did not require prey fish to witness a predation event, a detail critical for conditioning fish at a hatchery scale. Conditioning was effective in preparing fish to survive encounters with predators utilizing two very different feeding strategies: the visual-based ambush/pursuit strategy of the largemouth bass and the chemosensory-based searching strategy of the channel catfish. Our novel conditioning methodology enabled fish to experience all available cues

from predatory fish, prevented predation upon valuable prey species, and conditioned fish in the absence of predation, thus providing an optimal technique for conditioning large numbers of naïve fish.

Our experimental design was conservative in several ways to maximize the impact of significant results. The high predator density and minimal substrate availability during trials was intentionally unrealistic in order to provide a greater predator threat and survival challenge than these fish may encounter in the wild, where predators would likely be less frequently encountered and the habitat structure would be more complex. The 5-minute conditioning time was extremely short in duration to provide the absolute minimal learning opportunity as observed in literature (Griffin et al. 2000). This suggests that other conditioning techniques (longer conditioning durations, higher alarm pheromone concentrations, and multiple conditioning events) may yield even greater improvements in survival of conditioned fish.

While previous work has suggested BONY survival may be improved with predator conditioning (Mueller et al. 2007; Ward and Figiel 2013), the improved survival of conditioned RASU with both largemouth bass and channel catfish is particularly significant. Ward and Figiel (2013) found that predator conditioning altered the behavior of roundtail chub (*Gila robusta*), RASU, and Sonora sucker (*Catostomus insignis*). In their experiment, conditioned roundtail chub swam away from the predator, whereas conditioned individuals of both sucker species sat still on the bottom of the tank, showing almost no movement when a flathead catfish was present. Stationary hiding behavior might be effective against visual predators such as bass, but sitting still on the bottom could easily make suckers more susceptible to catfish predators (Rehage et al. 2009). While we were not able to closely observe fish behavior (to avoid spooking predators), the differential survival of conditioned and unconditioned RASU against channel catfish suggests that they have modified their behavior in some way that improves survival.

One concern we noted was that during the conditioning process, it was fairly easy to mistakenly cue the fish to fear the biologist instead of the fish predator. This occurred several times – once when a BONY was crushed while moving a filter or brick in preparation for conditioning and another time when we think the biologist moved too quickly when adding largemouth bass. BONY that cue in on something other than the bass as the reason for alarm pheromone release is clear when BONY use the bass as cover; these misconditioned animals were not used in survival trials. We therefore found it important to minimize the visibility of the biologist adding the predators during the conditioning process.

Finally, our novel conditioning technique that utilized hindered prey has shown to be an effective methodology. We therefore feel there is a good chance we can condition larger numbers of fish at production pond scales in a relatively short amount of time. Though working with powerful neurotoxins such as botulinum

requires extreme caution, we feel this method will be very effective in future conditioning studies and may be helpful in preventing much larger predators (such as striped bass and flathead catfish) from capturing fish in a production pond setting while still allowing the predators to behave normally.

BONY and RASU are among the most endangered fishes, and like many endangered fish, they now deal with severely modified habitat and nonnative fish predators. Hatchery stocking has been able to maintain an augmented population of several thousand RASU and smaller numbers of BONY in the Lower Colorado River Basin, but this has taken huge numbers of stocked fish to maintain these small populations. The extremely low post-stocking survival of hatchery-reared fish poses a major problem for BONY and RASU conservation planning. Improving the survival of stocked fish will certainly help increase the size of augmented populations, and larger numbers of reproducing adults may be able to overcome the challenges that currently prevent recruitment in the wild. In addition to helping enlarge augmented populations, increased survival rates will also make the investment in the stocking program more efficient and effective. We anticipate that our conditioning technique can be very helpful in improving the survival of stocked fish when applied to hatchery rearing ponds.

Management Implications

Though much work remains to better understand and refine predator avoidance conditioning for BONY and RASU, our conclusion is that predator avoidance conditioning can be started immediately. At this point, we can predict that conditioning fish will reduce their vulnerability to predation during and immediately after stocking, which is reputed to be a time of high vulnerability. The conditioning process for large ponds would be fairly straightforward: capture and euthanize a subset of prey fish, skin them and homogenize the skin to release the alarm pheromone, and then release predators in the pond while spreading the alarm pheromone. The simplest methodology would be to do this a day or two before a pond is harvested for stocking, so even if the learned predator recognition is not retained for a long period of time, the fish would be prepared to survive the first few days post-stocking.

The fact that there was no size difference of prey fish after each trial (predators were not more effective at capturing smaller fish) suggests that conditioning will improve survival of all sizes of hatchery-reared fish. While the current field data make clear that the larger the size at stocking means better chances of survival (Kesner et al. 2010), predator avoidance conditioning may be able to improve the survival rate of smaller fish. It is conceivable that we could condition and stock tens or hundreds of thousands of smaller fish and see similar or better survival as when we stock thousands of ≥ 300-millimeter fish.

Future Work

We see three major knowledge gaps to address to improve our implementation of predator avoidance conditioning. First, basic conditioning techniques need refinement. For this study, we used the minimum conditions found in literature (a 5-minute exposure to the alarm pheromone in the presence of a predator) and found improved survival. It is well documented that techniques such as longer conditioning durations, higher alarm pheromone concentrations, and multiple conditioning events elicit a longer predator recognition response (literature reviewed by Griffen et al., 2000). Second, how long the learned fear is retained is not known. Recent work with the June sucker (*Chasmistes liorus*) (Archer and Crowl 2014) suggests retention of learned predator recognition lasts less than 10 days, and Ward and Figiel (2013) also found reduced anti-predator behavior several weeks after conditioning. Finally, the effectiveness of conditioning in real life conditions is unknown. Habitat availability and other environmental conditions certainly play a role in determining how susceptible fish are to predation. We are currently developing a mesocosm study to: (1) better understand the retention of this learned behavior, (2) better replicate natural habitat and environmental conditions, and (3) refine our conditioning technique by examining multiple conditioning events and condition durations to maximize behavior retention.

LITERATURE CITED

Agyogos, Frank. 2014. Bubbling Ponds Fish Hatchery, Cornville, Arizona, personal communication.

Archer S.K. and T.A. Crowl. 2014. Retention of learned predator recognition in an endangered sucker *Chasmistes liorus liorus*. Aquatic Biology 20:195–202.

Brown G.E., J.C. Adrian Jr, E. Smyth, H. Leet, and S. Brennan. 2000. Ostariophysan alarm pheromones: laboratory and field tests of the functional significance of nitrogen oxides. Journal of Chemical Ecology 26:139–154.

Bureau of Reclamation (Reclamation). 2004. Lower Colorado River Multi-Species Conservation Program, Volume II: Habitat Conservation Plan. Final.

_____. 2006. Final Fish Augmentation Plan. Bureau of Reclamation, Boulder City, NV.

Griffin A.S., D.T. Blumstein, and C.S. Evans. 2000. Training captive-bred or translocated animals to avoid predators. Conservation Biology 14:1317–1326.

Karam A.P. and P.C. Marsh. 2010. Predation of adult razorback sucker and bonytail by striped bass in Lake Mohave, Arizona-Nevada. Western North American Naturalist 70:117–120.

Kesner B.R., A.P. Karam, C.A. Pacey, and P.C. Marsh. 2010. Demographics and Poststocking Survival of Repatriated Razorback Sucker in Lake Mohave, Annual Report. Bureau of Reclamation Agreement R09AP30002.

Marsh P.C. and J.E. Brooks. 1989. Predation by ictalurid catfishes as a deterrent to re-establishment of hatchery-reared razorback suckers. Southwest Naturalist 188–195.

Marsh P.C. and C.A. Pacey. 2005. Immiscibility of native and non-native fishes. Pages 59–63 *in* Restoring Native Fish to the lower Colorado River: interactions of native and nonnative fishes. U.S. Fish and Wildlife Service, Albuquerque, NM, and Bureau of Reclamation, Boulder City, NV.

Mathuru A.S., C. Kibat, W.F. Cheong, G. Shui, M.R. Wenk, R.W. Friedrich, and S. Jesuthasan. 2012. Chondroitin fragments are odorants that trigger fear behavior in fish. Current Biology 22:538–544.

Mueller G.A. 2005. Predatory fish removal and native fish recovery in the Colorado River mainstem: what have we learned? Fisheries 30:10–19.

Mueller G., J. Carpenter, R. Krapfel, and C. Figiel. 2007. Preliminary Testing of the Role of Exercise and Predator Recognition for Bonytail and Razorback Sucker. U.S. Geological Survey, Sacramento, CA.

O'Neill M.W. and A.C. Gibb. 2007. Botulinum toxin injections as a method for chemically denervating skeletal muscle to test functional hypotheses: a pilot study in Lepomis cyanellus. Physiological and Biochemical Zoology 80:241–249.

Rehage J.S., K.L. Dunlop, and W.F. Loftus. 2009. Antipredator responses by native mosquitofish to non-native cichlids: an examination of the role of prey naïveté. Ethology 115:1046–1056.

Suboski M.D. and J.J. Templeton. 1989. Life skills training for hatchery fish: social learning and survival. Fisheries Research 7:343–352.

Ward D.L. and C.R. Figiel. 2013. Behaviors of southwestern native fishes in response to introduced catfish predators. Journal of Fish and Wildlife Management 4:307–315.