Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation

Salinity Tolerances for Egg and Larval Stages of Razorback Sucker 2007-2008

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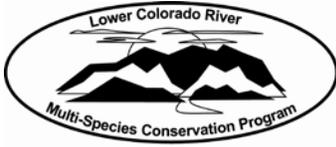
Lower Colorado River
Multi-Species Conservation Program

Salinity Tolerances for Egg and Larval Stages of Razorback Sucker 2007-2008

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Abstract

The success of the numerous habitats currently being used to rear razorback suckers *Xyrauchen texanus* along the lower Colorado River has been somewhat hit or miss in terms of number of fish produced and overall survivorship. One of the key problems has been determining what factors result in a successful habitat. High salinity and low dissolved oxygen are thought to be problems in several of the current habitats being used to rear these fish. To determine the effects of high salinity on early life stages of razorback sucker and on in-pond recruitment, razorback sucker eggs and larvae were exposed to a range of salinities to determine the critical lethal limits of hatching and survival. Egg and larval responses were measured as percent hatch and percent mortality at 72 hours, respectively. Larvae were also monitored for 45-60 days at all experimental salinities to determine long-term survivability. Successful hatching of razorback sucker eggs occurred at salinities up to 12,000 microsiemens/centimeters (µS/cm), while lethal salinity to 50% of larvae occurred near 27,000 µS/cm when larvae had been acclimated. Long-term survival of larval razorbacks was observed at 20,000-23,000 µS/cm.

Introduction

The Lower Colorado River Multi-Species Conservation Program (LCR MSCP) is developing 360 acres of backwater habitat for razorback sucker (*Xyrauchen texanus*) and bonytail (*Gila elegans*), two endangered native fishes of the Colorado River Basin (Figure 1). Strategies for establishing suitable habitat include making small or large changes to existing backwaters, or creating new backwater habitat through excavation of undeveloped land. Most of these created backwater habitats will be floodplain ponds and sloughs isolated from the main river channel. Once created these habitats will be managed and maintained as native fish refugia. Due to high air temperatures, low humidity, and limited hydrologic exchange with the adjacent river, salinity typically increases in isolated floodplain ponds along the lower Colorado River. Freshening of these ponds may need to occur periodically to reset water quality conditions. In this two-year study, we evaluated salinity tolerances for egg and larval stages of razorback sucker in an effort to help fishery managers develop freshening schedules for these backwater habitats as well as aid in future site selection.

Figure 1: Ponds developed for native fish at the Imperial National Wildlife Refuge (USBR photograph by Andy Pernick)

Study Area

Fieldwork associated with this study took place in the LCR MSCP's river reach 2, Lake Mohave, Arizona-Nevada. This study was designed to experimentally determine the critical lethal limits of salinity with respect to razorback sucker eggs and larvae. This was

accomplished by determining the maximum salinity at which eggs can successfully hatch, the maximum salinity at which larval fish can survive for 72 hours (h), and the long-term survivability of larval fish under different salinities for a period of 1-2 months. We observed long-term survival of larval fish until their transition to juveniles as described by Snyder et al. (2004). All laboratory work was conducted at the LCR MSCP fisheries office, Boulder City, Nevada.

Methods

Adult razorback suckers were collected by trammel net and electrofishing from shoreline areas of Lake Mohave in March 2007 and 2008 (Figure 2). The timing of fieldwork was scheduled to coincide with the Lake Mohave razorback sucker spawning season and to take advantage of ripe adult fish available from known spawning locations. Seven female and nine male razorbacks were captured in 2007, and 12 females and eight males were captured in 2008 for use as brood fish. Adult fish were separated by sex, and held in separate live wells for a period of 18-24 hours prior to being manually spawned. Eggs from females and sperm from males were captured simultaneously in the same 9.5 liter (L) container partially filled with one of the experimental salinities (2007: 1,000, 3,000, 6,000, 10,000, 15,000, and 20,000 μS/cm; 2008: 10,000, 12,000, 14,000, 16,000, and 18,000 μS/cm). Multiple females were used in each spawning when possible, and multiple males were always used. Temperatures of experimental salinities were near 20° Celsius (C) at time of fertilization.

Figure 2: Razorback sucker spawning group, Tequila Cove, Lake Mohave, Nevada (USBR photograph by Jon Nelson).

Experimental salinities were produced using deionized (DI) water and Instant Ocean® synthetic sea salt, and salinity values were selected based on the tolerances of associated fish species as well as to provide us with a wide range of salinities for study. Morgan et al. (1981) observed striped bass (*Morone saxatilis*) eggs and larvae to develop and survive at salinities up to 14,000 μS/cm, while the known lethal effects of salinity for Colorado pikeminnow (*Ptychocheilus lucius*) have been determined to be in the 18,000 μS/cm range (Nelson and Flickinger 1992). Flathead catfish (*Pylodictis olivaris*) tolerances have been shown to be even higher, with concentrations averaging over 20,000 μS/cm at 18°C (Bringolf et al. 2005). Other freshwater fish species that are found in isolated habitats also have considerable salt tolerances, often in excess of 20,000 μS/cm (Ostrand and Wilde 2001).

During fertilization, gametes were gently mixed together, and calcium bentonite was added to prevent fertilized eggs from clumping together or from adhering to the side of the container. Fertilized eggs were then transferred to floating Nitex® cloth hatching trays. Prior to transfer, hatching trays were placed in large containers of corresponding salinities in preparation for the fertilized eggs. Eggs were allowed to water harden overnight and were then removed from hatching trays via small dip net. Eggs were placed into 3.8-L aquaria bags, along with sufficient amounts of corresponding saline water, and arranged in a small cooler for transport to our laboratory.

The laboratory portion of this study was conducted from March to early May of both years. The laboratory was outfitted with twenty 38-L aquaria prior to spawning adult fish. Egg tanks were set up in triplicate, with three tanks for each of the experimental salinities, and tanks were filled with approximately 8 L of water at the required salinities. A single 25 cm × 40 cm floating hatching tray was placed in each tank and tanks were numbered for individual identification. Egg densities for all spawning salinities were estimated volumetrically based on measurements of eggs/mL. Eggs from individual spawning salinities were divided equally between hatching trays in the three tanks. With the exception of the 15,000 μS/cm spawning in 2007, multiple females were used in each spawning. Eggs from each spawning were mixed together for transport, and assuming each adult fish supplied viable gametes, all tanks received fertilized eggs of mixed parentage. Total egg volumes varied between salinities as a result of the individual fecundity of the female or females used.

For the duration of this experiment a 12-hour light, 12-hour dark photoperiod was maintained to mimic vernal conditions. Daytime hours were sustained using both natural and overhead artificial light. Water temperatures for egg tanks were maintained between 18 and 20°C, and water exchanges were performed daily to prevent fouling during incubation. In doing this, researchers took great care to disturb eggs as little as possible. Fungal growth was also a concern at this stage, so each hatching tray was dipped in a 1:150 formalin solution. Egg tanks were examined for fungus routinely and dead (white/opaque) eggs were removed.

Once hatch larvae were swimming, hatching trays were removed and tanks were thoroughly cleaned. At this time all fish were counted and combined into single tanks of their respective salinities. One hundred and fifty larvae from each of the combined tanks were separated and

placed in individual tanks, one tank for each cohort of 150 at the salinity they were spawned in. These larvae acted as the control group for the duration of the experiment.

Throughout the larval portion of the experiment, we performed water changes every one to two days on all tanks while maintaining temperatures between 19 and 21°C. As larval yolk sacs were absorbed, we began feeding twice daily using brine shrimp. Tanks were cleaned prior to each feeding and brine shrimp were siphoned into small dip nets and rinsed with DI water before being introduced into tanks. Salinity and general water quality readings were taken routinely using a Hydrolab Quanta® meter.

2007 Larvae Trials — Salinity toxicity tests began by observing larvae in the salinity they were spawned in for 168 hours. During this period, salinity and temperature measurements were taken and mortalities were recorded as they occurred. Also during this period, an additional six aquaria were set up for use as long-term holding tanks. These tanks were used to determine long-term survival as well as provide space for larvae not being immediately utilized during the following experimental trials. After seven days, limited mortality was observed in spawning salinities that successfully produced larvae. Limited mortality was defined as less than 10%.

Seventy-two hour trials began with larvae from all salinities being exposed to each of the higher experimental salinities (Figure 3). As was done with the control group, cohorts of 150 larvae were again used in this trial. Tanks were observed routinely each day and mortalities were counted and removed as they occurred. Percent mortality was recorded at 72 hours.

Figure 3: First trial tank setup. Refer to key in the lower right corner to identify both the source salinity and the salinity larvae were exposed to during this period.
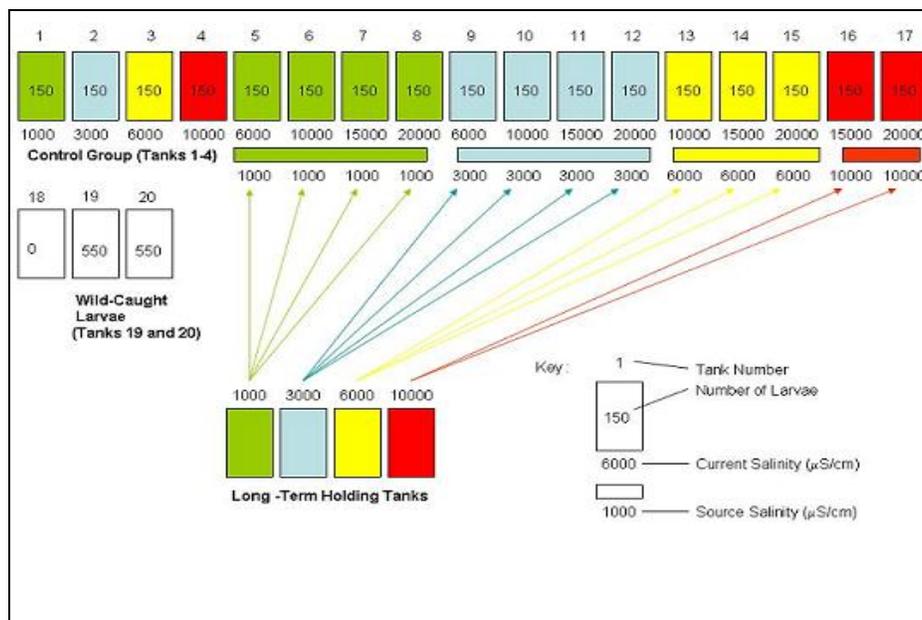
Information gathered from the first trial indicated that additional experimental salinities would be required to determine the 72-hour salinity tolerance of razorback larvae. Salinities of 23,000, 26,000, and 29,000 µS/cm were prepared for our second 72-hour trial, and larvae from 1,000, 6,000, 10,000, and 20,000 µS/cm were exposed in the same manner as previously outlined. Fewer than 450 larvae were available from the 20,000 µS/cm source following the previous trial, so cohorts of 104, 141, and 126 larvae were used in this instance. Percent mortality was again recorded at 72 hours.

2008 Larvae Trials — For the 2008 study year, two changes were made with respect to our larval trial. First, cohort sizes were doubled from the previous year to 300 larvae per tank. This was done to provide more flexibility with larvae from all salinities if the need to examine other effects, such as relative growth or condition factor, arose. Second, larvae were exposed to higher salinities incrementally, as opposed to moving them directly from low to high salinities. Findings from the first study year indicated that survival of larvae may be improved when they are tempered/acclimated from lower to higher salinities. This period of acclimation more closely mimics natural salinity increases and gives us a better idea of how this species may react in ponds along the LCR. In addition, tempering razorback larvae may reduce stress response associated with moving them from relatively low salinities directly into higher salinities.

Control tanks for larvae spawned in 10,000 and 12,000 µS/cm were set up in triplicate, and an additional four tanks housed 14,000 µS/cm larvae for long-term observation. For tempering trials, six 38-L aquaria were used. Three of these aquaria contained 10,000 µS/cm source larvae and three had larvae from the 12,000 µS/cm spawn. Tempering was accomplished by increasing salinities in each tank at a rate of 500µS/cm per day. Each increase was followed by a 24-hour acclimation period before salinities were increased again. Tempering continued until an increase in mortality was observed, at which time salinities were held at their current values and larvae were monitored for 72 hours.

Results

2007 Hatching Success — Four to 10 days were required for complete hatch of eggs at all salinities. Eggs fertilized in 1,000, 3,000, 6,000, and 10,000 µS/cm developed normally and larvae began swimming by 24 hours post-hatch. Eggs in 15,000 µS/cm tanks were observed to be of comparatively reduced size. This was likely due to the osmotic effects of this higher salinity. Larvae from these tanks hatched early, were small, and were few in number. None survived past 16 hours. No development was observed in 20,000 µS/cm tanks.

Hatch success was variable among salinities with a successful brood (Table 1). Results were similar to those of Haines (1995) and Marsh (1985), who observed 67% and 35% hatch, respectively, for eggs incubated at 20°C. Results also indicate that successful hatching is unlikely for salinities of 15,000 µS/cm or greater. Experimental salinities for the second study

year were based on these findings and chosen to more accurately define the upper salinity tolerance for successful egg development.

Table 1: 2007 Percent Hatch - Mean (±SD) percent hatch of razorback sucker eggs subjected to experimental salinities. Number of eggs and larvae from eggs are combined totals for three replicate treatments.

Salinity (μS/cm)	Number of Eggs *	Larvae from Eggs	% Hatch
1000	7500	4115	54.8 ± 4.7
3000	7800	4421	56.6 ± 1.5
6000	3750	1125	30.0 ± 2.3
10000	7200	1579	21.9 ± 9.4
15000	2400	0	0
20000	8750	0	0

* - number of eggs estimated based on 50 eggs/mL measurement.

2007 Larvae Trials — The first larval trial conducted during the 2007 study year resulted in limited mortality (0-26%). Following the initial 72 hours, larvae were observed for an additional 240 hours to evaluate any delayed mortality. This additional period resulted in minimal change to mortality percentages (Table 2).

Table 2: 2007 Larvae Trials - First larval trial percent mortality at 72 h and 240 h.

			72 h		240 h	
Tank #	Source (μS/cm)	Current (μS/cm)	Mortalities	%	Mortalities	%
5	1000	6000	15	10.0	16	10.6
6	1000	10000	21	14.0	23	15.3
7	1000	15000	38	25.3	39	26.0
8	1000	20000	12	8.0	16	10.6
9	3000	6000	2	1.3	4	2.6
10	3000	10000	4	2.6	9	6.0
11	3000	15000	10	6.6	15	10.0
12	3000	20000	40	26.6	46	30.6
13	6000	10000	0	0	1	0.6
14	6000	15000	1	0.6	2	1.3
15	6000	20000	4	2.6	9	6.0
16	10000	15000	1	0.6	2	1.3
17	10000	20000	23	15.3	23	15.3

For our second trial, larvae were initially observed for the predetermined 72-hour period. Observations were, however, extended to 312 hours after improved survival was noted in the 20,000 μS/cm source tanks (Table 3). Larvae from 1,000, 6,000, 10,000, and 20,000 μS/cm exposed to 23,000 μS/cm during the second trial did well. Percentage of mortality was low and ranged from 0 to 18% over 72 hours. Greater than 50% of larvae from 1,000 and 10,000

μS/cm exposed to 26,000 μS/cm died within 72 hours, while larvae from 6,000 and 20,000 μS/cm died at 96 hours and 312 hours, respectively. This range, 72-312 hours, suggests that fish from the 20,000 μS/cm source may have survived longer because they had been acclimated to a significantly higher salinity for a longer period of time. Larval fish from 1,000 and 6,000 μS/cm exposed to 29,000 μS/cm had 100% mortality at 72 hours. Eighty-eight percent of the 10,000 μS/cm source larvae exposed to 29,000 μS/cm also died within 72 hours. Delayed mortality was once more observed with larvae from the 20,000 μS/cm source. At 72 hours, only 1.6% mortality had occurred. Mortality of greater than 50% required a total of 216 hours. Again, this suggests some degree of acclimation may have occurred. Parry (1966) observed the period of time required for acclimation to be species dependent. The acclimation period during this study year could still be considered relatively short when compared to the time it would take for these changes to occur naturally within an isolated body of water. With further evaluation of incremental exposure to higher salinities, the time required for acclimation and the upper salinity tolerance for this species may be more accurately determined.

Table 3: 2007-Second larval trial percent mortality at 72h, 312h, and time to ≥ 50% mortality.

Tank #	Source (μS/cm)	Current (μS/cm)	72 h		312 h		≥ 50% Mortality		
			Mort.	%	Mort.	%	Time(h)	Mort.	%
7	1000	23000	27	18.0	29	19.3	N/A	N/A	N/A
8	1000	26000	77	51.3	139	92.6	72	77	51.3
9	1000	29000	150	100.0	150	100.0	24	147	98.0
10	6000	23000	3	2.0	5	3.3	N/A	N/A	N/A
11	6000	26000	29	19.3	121	80.6	96	75	50.0
12	6000	29000	150	100.0	150	100.0	24	110	73.3
13	10000	23000	4	2.6	11	7.3	N/A	N/A	N/A
14	10000	26000	129	86.0	143	95.3	72	129	86.0
15	10000	29000	132	88.0	141	94.0	24	75	50.0
16	20000	23000	0	0	0	0	N/A	N/A	N/A
17	20000	26000	5	3.5	68	48.2	N/A	N/A	N/A
18	20000	29000	2	1.6	108	85.7	216	68	53.9

Mort. – total mortality for treatment period, N/A – indicates mortality of less than 50% for treatment period.

Mortality rates for control and long-term holding tanks were examined to determine the difference in long-term survival between salinity levels. Control and long-term holding tanks for 1,000, 3,000, 6,000, and 10,000 μS/cm were monitored over a 60-day period. Control tanks for 15,000 and 20,000 μS/cm were obtained by retaining 1,000 μS/cm source fish that had been exposed to these salinities during the first trial. These tanks were monitored for a total of 50 days, which includes their participation in the first trial.

Within the first five days, mortality for 1,000, 3,000, 6,000, 10,000, 15,000 and 20,000 μS/cm control tanks totaled 9.3, 8.6, 37, 23, 26, and 11%, respectively. Most of the mortality for the

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control group occurred within these first few days, after which rates slowed to approximately 0.34 mortalities per day. Over the 50-60 day period, control tanks for 1,000, 3,000, and 20,000 μS/cm showed minimal mortality, totaling 11.3, 8.6, and 12%, respectively. Control tanks for 10,000 and 15,000 μS/cm had slightly higher percentages of 24 and 26.7%, and the 6,000 μS/cm control had the highest mortality rate at 42.7%.

Mortality rates for long-term holding tanks ranged from 6.5 to 100%. Again, most mortality occurred in the first few days. The exception in this case was the 1,000 μS/cm tank, which experienced considerable mortality over the first two weeks. Dead larvae were comparatively smaller and showed high incidence of crooked backs. Crooked backed larvae were also observed swimming and often had small amounts of fungus growing on them. Larval densities and fungus or infection resulting from handling are likely factors contributing to this mortality. Densities present in 3,000 μS/cm holding tanks were similar, but mortality rates for those were lowest overall. This indicates a possible therapeutic effect of this salinity that may have prevented mortality due to fungus or infection. Piper et al. (1982) suggest a similar salt concentration for extended treatments of bacterial disease and external parasites on hatchery raised fish species. Congruent with our control group findings, 6,000 μS/cm larvae had the highest mortality during long-term observation. One hundred percent mortality occurred for this treatment; however, this is partly due to the low starting numbers.

2008 Hatch — As was the case during the 2007 study year, hatch rates for successful salinities varied (Table 4). Eggs fertilized in 10,000, 12,000, and 14,000 μS/cm developed successfully and hatched in five to nine days. Resultant larvae began swimming within 30 hours of hatching. Similar to our findings from comparative salinities during the first study year, eggs fertilized in 16,000 and 18,000 μS/cm salinities were of reduced size and unsuccessful. These eggs were examined after five days and discarded when no further development was observed.

Table 4: 2008-Mean (±SD) percent hatch of razorback sucker eggs subjected to experimental salinities. Number of eggs and larvae from eggs are combined totals for the three replicate treatments.

Spawning Salinity (μS/cm)	Number of Eggs *	Larvae from Eggs	% Hatch
10000	7350	3089	42.0 ±5.2
12000	7350	2533	34.5 ± 6.9
14000	7350	366	5.0 ± .01
16000	2300	0	0
18000	2300	0	0

* - number of eggs for 10, 12, and 14000 μS/cm estimated based on 49 eggs/mL measurement, 16 and 18000 μS/cm based on 92 eggs/mL measurement.

2008 Larval Trial — Due to an insufficient number of larvae available from the 14,000 μS/cm hatch, larvae for this trial came only from the 10,000 and 12,000 μS/cm spawning salinities. Larvae from the 14,000 μS/cm hatch were, however, kept in their respective tanks and

grouped with the control tanks for observation. Our results indicate that tempering these fish did have a positive effect on survival when compared to our findings from the previous year. Significant mortality did not occur until salinities approached 27,500 μS/cm. Four of the six trial tanks had greater than 50% mortality at 72 hours, with salinities ranging from 27,300 to 27,500 μS/cm. The remaining two tanks took 96 hours to achieve greater than 50% mortality and had salinities of 27,500 and 27,750 μS/cm (Table 5).

Table 5: 2008 - Salinity tempering trial. Concentration and time to ≥ 50% mortality.

Tank #	Source (μS/cm)	Current (μS/cm)	Time (h)	% Mortality
11	10000	27500	96	55.3
12	10000	27450	72	52.6
13	10000	27750	96	52.4
14	12000	27500	72	68.9
15	12000	27300	72	78.1
16	12000	27450	72	64.2

Long-term survival was monitored in control tanks over a 45-day period. Mortality for 10,000 and 12,000 μS/cm control tanks remained low overall and ranged from 1.6 to 7%. Mortality for 14,000 μS/cm was considerably higher, ranging from 50 to 58% between the four tanks. The high mortality for these tanks is likely an effect of being spawned in this salinity as we observed larvae enduring much higher salinities with less mortality during both study years. The key factor separating these groups is that larvae able to survive at higher salinities were all spawned at salinities below 14,000 μS/cm.

Discussion

Razorback sucker eggs fertilized in experimental salinities between 1,000 and 12,000 μS/cm, and incubated between 18 and 20°C, developed normally and produced larvae within 10 days. For these salinities, 22 to 56% of the fertilized eggs were successful. Eggs were most successful at 1,000 and 3,000 μS/cm with hatch rates of 55 and 56%, respectively. These findings suggest that even though eggs are able to develop and hatch at higher salinity levels, moderate to low salinities promote greater egg success. Eggs fertilized in 14,000 μS/cm also produced larvae with a 5% hatch overall. This low rate of success indicates this value is very near the maximum that these eggs can tolerate.

Razorback sucker larvae were also exposed to a wide range of salinities (1,000-29,000 μS/cm) during this two-year study. Results from the first year showed that of the chosen experimental salinities, 26,000 μS/cm was the minimum value lethal to 50% of larvae at 72 hours. Observations also showed that survival at 23,000 μS/cm was possible as relatively low mortality rates of 0, 3, 7, and 19% were observed in these four trial tanks over 312 hours.

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Further observations made during the first study year led to the hypothesis that acclimating larvae to increasing levels of salinity would improve survival at higher salinities. During the second study year this hypothesis was tested, and the minimum salinity lethal to 50% of larvae at 72 hours increased to 27,300 µS/cm. Parity was observed in all tanks used in this trial with lethal salinity ranging from 27,300 to 27,750 µS/cm. Depending on the method by which larvae are exposed to extreme salinities, the maximum salinity tolerance may range from 23,000 to 27,750 µS/cm.

Long-term survival of larval razorbacks may be possible in salinities up to 20,000 µS/cm when other water quality parameters are favorable. Results from the 2007 study showed only 12% mortality for larvae exposed to 20,000 µS/cm over a 50-day period. Although it appears larvae are also able to survive at salinities as high as 23,000 µS/cm, our experiment did not allow for a long enough period of observation to evaluate long-term survival. With the success observed at these higher experimental salinities, it is not surprising that larvae handled the low and mid-range experimental salinities fairly well. This is of significant importance as low and mid-range salinities are found in the majority of habitats managed for this species.

The large reservoirs of the LCR, including lakes Mead, Mohave, and Havasu, all support adult populations of razorback sucker and have salinities that range between 800 and 1,100 µS/cm depending on flow. The exceptions to this range can be found in two of the more turbid Lake Mead inflow areas, which include the Las Vegas Wash inflow and the Muddy River/Virgin River inflow. Salinities reported in these areas of Lake Mead average closer to 2,300 µS/cm and range in excess 4,000 µS/cm (Bureau of Reclamation 2010). Despite these higher salinities, these are two of the major, known spawning locations for Lake Mead razorback sucker. The documentation of continued recent recruitment of razorback sucker in these areas of Lake Mead (Albrecht et al. 2010) indicates that as observed in the laboratory, these mid-range salinities do not prevent successful egg and larval development.

Backwater habitats on Lake Mohave currently used as grow-out ponds for razorback suckers also tend to have slightly higher salinities than the reservoir and range from 1,000 to 3,500 µS/cm. The Davis Cove native fish sanctuary pond, also found on Lake Mohave, has had even higher salinities recorded (in excess of 5,000 µS/cm) while supporting small populations of razorbacks (Mueller 2007). It should be noted that although this species shows the ability to tolerate relatively high salinities, preferred salinities may be found in the low to mid-range values. Meador and Kelso (1989) investigated behavioral responses of largemouth bass *Micropterus salmoides* to various salinities (0-17,000 µS/cm) finding that young largemouth preferred the lowest available salinity and adult largemouth preferred salinity in the 4,000 µS/cm range. Earlier research also showed that the maximum salinity at which largemouth bass eggs can successfully develop lies in the 5,000 to 7,500 µS/cm range (Tebo and McCoy 1964). These observations begin to demonstrate the importance of understanding how each life stage reacts to differences in salinity. In managing razorback sucker habitat, identifying tolerances as well as preferences is important for creating a successful environment for these fish.

Based on observations made during this two-year study, it appears that the salinity levels currently found in habitats managed for this species will not prevent the survival of early life stages. The effect of high salinity on later life stages of RASU (juveniles and adults) is, however, still unknown. An important point to consider when discussing lethal salinities for RASU is that eggs display much greater sensitivity to salinity than do larvae. Management decisions that alter the salinity concentration of native fish habitat should therefore focus on providing conditions that promote the greatest success for newly fertilized eggs.

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