**Prepared in Cooperation with the U.S. Fish and Wildlife Service, Willow Beach National Fish Hatchery**

# Preliminary Testing of the Role of Exercise and Predator Recognition for Bonytail and Razorback Sucker

By Gordon A. Mueller and Jeanette Carpenter, U.S. Geological Survey, and Robert Krapfel and Chester Figiel, U.S. Fish and Wildlife Service

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# Preliminary Testing of the Role of Exercise and Predator Recognition for Bonytail and Razorback Sucker

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## Summary

Hatchery-reared juvenile, <25-cm TL (total length), razorback suckers appeared curious and showed no sign of predator avoidance when initially placed with large (>45-cm TL) flathead catfish. Predator-naïve juveniles (20- to 25-cm TL) exhibited no discernable preference when provided areas with and without (52 percent and 48 percent, $n$ = 16 observations; 46 percent and 54 percent, $n$ = 20 observations) large flathead catfish. However, once predation occurred, use of predator-free areas nearly doubled in two trials (36 percent and 64 percent, $n$ = 50 observations; 33 percent and 67 percent, $n$ = 12 observations). A more stringent test examining available area indicated predator-savvy razorback suckers used predator-free areas (88 percent, $n$ = 21) illustrating predator avoidance was a learned behavior.

Razorback suckers exercised (treatment) in water current (<0.3 m/s) for 10 weeks exhibited greater swimming stamina than unexercised, control fish. When exercised and unexercised razorback suckers were placed together with large predators in 2006, treatment fish had significantly fewer ($n$ = 9, $z$ = 1.69, $p$ = 0.046) mortalities than control fish, suggesting increased stamina improved predator escape skills. Predator/prey tests comparing razorback suckers that had been previously exposed to a predation event with control fish, found treatment fish also had significantly fewer losses than predator-naïve fish ($p$ = 0.017). Similar tests exposing predator-savvy and predator-naïve bonytail with largemouth bass showed a similar trend; predator-savvy bonytail suffered 38 percent fewer losses than control fish. However, there was not a statistically significant difference between the test groups ($p$ = 0.143) due to small sample size. All exercise and predator exposure trials increased the survival rate of razorback sucker and bonytail compared to untreated counterparts.

## Introduction

Historically, fish culturists have based production on the most economical method of raising large numbers of fish. In other words, the most fish for the fewest dollars. Traditionally, survival has not been an issue with recreational species, which are typically stocked to augment depleted communities. However, in endangered repatriation programs, fish are not only being stocked into altered habitats, but ones that are already at full carrying capacity. Natives not only have to avoid predation, but also have to out-compete resident fishes for food and space. The practice of mass stockings to augment imperiled fishes has proved problematic worldwide (Philippart, 1995).

Since 1980, more than two-million bonytail (*Gila elegans*) and 15-million razorback sucker (*Xyrauchen texanus*) have been reintroduced into the Colorado River basin (unpublished data, U.S. Fish and Wildlife Service, Dexter National Fish Hatchery). Unfortunately, survival has been extremely low (<10,000), if not totally absent (Marsh and others, 2005). Marsh and Brooks (1989) reported that entire truck loads of hatchery razorback suckers were eaten by resident catfish within hours of release. Recent studies have shown a strong correlation between survival and size of fish when released. First-year survivorship for fish 30 cm long was 10 percent, compared to 26 percent for fish released at 35 cm (Marsh and others, 2005). The Lake Mohave Native Fish Work Group (NFWG) is an interagency program that is reintroducing razorback suckers in Lake Mohave. They recently recommended increasing the targeted stocking size from 35 to 50 cm for Lake Mohave. Even fish at this size remain vulnerable to large (>20 kg) striped bass (*Morone saxatilis*) and flathead catfish (*Pylodictis olivaris*) that are found in the system. There are also other issues in producing larger fish: it increases production costs (per fish) and decreases production numbers. It is unknown whether this change in stocking strategy will help increase populations.

Terrestrial researchers have recognized for several decades the importance of survival skills in the animals and birds used in repatriation programs. Current programs not only train, but test the performance of repatriated animals and birds in natural food recognition, foraging ability, and avoidance behavior to predators and humans prior to release. These survival approaches have proven critical in the reintroduction of the gray wolf (*Canis lupus*), California condor (*Gymnogyps california*), masked bobwhite quail (*Colinus virginianus ridgewayi*), and blackfooted ferret (*Mustela nigripes*) (Ellis and others, 1978; Fritts and others, 1997; Biggins and others, 1998; Bangs and others, 1998). Similar approaches have been adopted by Pacific Northwest salmon augmentation programs. Maynard and Flagg (2001) developed the Natural Rearing System (NATURES) program that incorporates natural feeding, physical conditioning, and predator recognition to improve post-stocking survival. However, similar research or culturing approaches are rare for warm-water species.

Mueller and others (2003) found that exercised razorback sucker were less prone to downstream dispersal compared to pond reared fish and Ward and Hilwig (2004) reported bonytail and razorback sucker exercised for two weeks exhibited greater swimming stamina than non-exercised fish. We proposed that if swimming performance was improved, it may also improve predator avoidance skills. The goal of this study was to test whether physical and/or behavioral conditioning would improve predator avoidance skills for bonytail and razorback sucker.

## Methods

The study was conducted at Achii Hanyo, a satellite hatchery facility of Willow Beach National Fish Hatchery (Willow BNFH). The facility is located near Parker, Ariz., and is operated by the U.S. Fish and Wildlife Service (FWS). Bonytail and razorback suckers were supplied by Willow BNFH. We used largemouth bass (*Micropterus salmoides*) as the predator for bonytail and flathead catfish (*Pylodictis olivaris*) as the predator in razorback sucker experiments. Study predators were collected from the Colorado River and returned unharmed following the study. Surviving native fish were included in native fish stockings elsewhere.

The study involved treatment, testing, and observation elements. Treatment activities involved exercising and exposing trial groups to predation prior to the actual predator/prey trials. The swimming performance of exercised and unexercised fish was measured using a flow chamber. Survival performance was determined by placing treated and control groups in large tanks with predators to actually measure predation rates. Working at the fish facility gave us a rare opportunity to spend considerable time observing these fish and conducting impromptu side experiments. Those observations and informal experiments are included in this report.

## Treatment

### Exercise Tanks

Four rectangular (1.2 m wide, by 7 m long, by 1 m deep) fiberglass tanks were modified and used as experimental rearing tanks. All contained a center divider and a screened enclosure at one end (fig. 1) that allowed water to flow around the tank. Two tanks were equipped with electric trolling motors (23 kg thrust), battery banks, and a charger (fig. 2), which provided the elements to create a flowing environment within the tank. The tanks were set up as pairs; water circulated in the treatment tank, whereas the control tank had no flow. This approach provided two complete systems for treatment and control groups and allowed us to work with two species at the same time.

Each tank had a net cover, a thermometer, and aeration stone. Generally, a small volume of fresh water was allowed to flow through to an overflow drain.

In February 2006, approximately 600 razorback suckers were transported from Willow BNFH and divided among the four test tanks. Their length averaged 198-mm TL (total length) (range 127 to 245 mm, $n = 44$). In February 2007, 600 razorback suckers were divided between two tanks (flow and control) and 600 bonytail were divided between the other set of tanks. Razorback suckers averaged 222-mm TL (range 190 to 260 mm, $n = 88$) and bonytail averaged 196-mm TL (range 150 to 260 mm, $n = 87$).

**Figure 1.** A pair of experimental rearing tanks modified with center dividers and caged ends. The right tank had a trolling motor (far end) that produced velocities up to 30 cm/s. Fish were held in the left tank without flow (control).

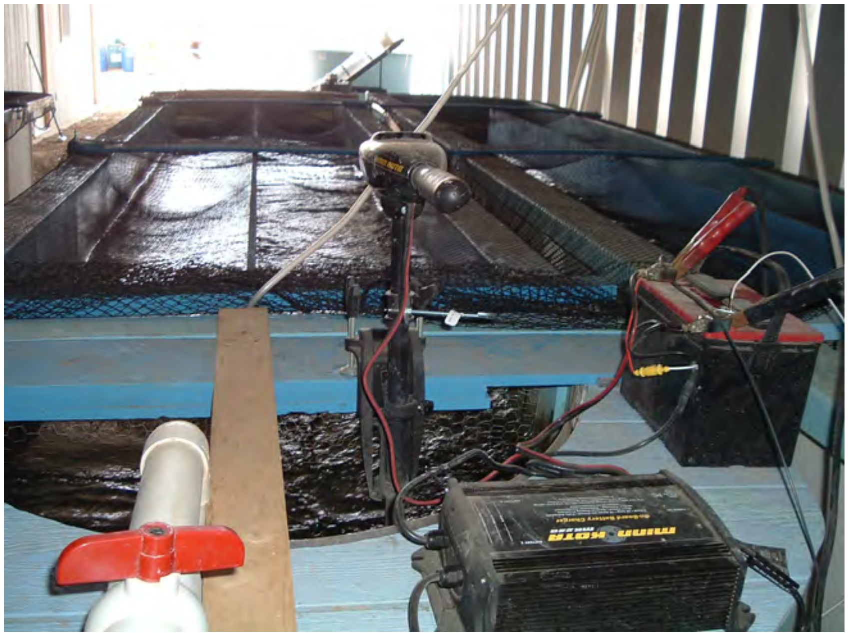

**Figure 2**. Photograph showing placement of the electric trolling motor that directed current through the flow exercise rearing tank.

The exercise regimen for 2006 and 2007 started in March and February respectively with an initial velocity of 7.5 cm/s. This increased in 2.5-cm/s intervals each week (7.5, 10.0, 12.5, 15.0, 17.5, 20.0, 22.5, 25.0, 27.5, and 30.0 cm/s) for 10 weeks and then maintained at 30 cm/s for the duration of the tests. The electric motors were turned on at 8 a.m.; flows were measured with a velocity meter and motor speeds adjusted to the scheduled velocities. Fish were subjected to current for eight hours from Monday through Friday and allowed to rest at night and during the weekends. Fish were fed a commercial pellet chow at dusk; tanks were cleaned weekly and fish received preventative chemical treatments for parasites and diseases or when they showed symptoms.

## Predator Exposure

Large predators were captured from the wild and brought into the facility at least one month prior to use. They were placed in large tanks and fed live nongame fish salvaged from local ponds. The 2006 plan was to net and release a large flathead catfish in each of the exercise tanks, allow the flathead catfish to feed for 24 hours, and then remove them. Surviving fish would be used to start the predator/prey trials one week later. However, this approach failed (see Observations section) causing us to abort that test in the 2006 trials and modify our predator exposure design in 2007. Instead of moving the predators, we moved the prey. Treatment groups consisted of <12 prey with the anticipation that one or two fish would be lost during the exposure process, leaving ≥10 prey for the predation experiment. Following a predation event, surviving prey were removed, measured, and combined with other treatment or control fish for the actual predator/prey experiment.

## Testing

### Swimming Performance Tests

A velocity chamber was used to measure swimming stamina. The chamber consisted of a DC-powered motor with a calibrated rheostat, enclosed propeller, and a flow chamber that was constructed of a small diameter 15-cm Plexiglas tube mounted inside a larger one. Water was circulated through the smaller tube and returned to the propeller from the interspace between the tubes. A terminal port allowed access to place and retrieve test fish. Fresh water was circulated through the apparatus to provide adequate dissolved oxygen (fig. 3).

**Figure 3.** Flow chamber used to measure swimming stamina.

Fish were subjected to velocity increments based on their body length (body length units/second [BL/s]). The test started with an acclimation period of 30 minutes at a flow rate of 0.5 BL/s. For example, a 20-cm-TL fish would be acclimated to a 10 cm/s velocity for 30 minutes. Following acclimation, velocities increased by 0.5 BL/s increments for 30-minute periods until the fish collapsed from exhaustion. At that point the experiment ended and critical swimming performance was calculated using the formula:

$U_{crit} = V_p + ((t_f/t_i)V_i))$, where,

$V_i$ is the velocity increment (cm/s).

$V_p$ is the penultimate velocity where the fish swam in the entire interval (cm/s).

$t_f$ is the elapsed time from the velocity increase to fatigue.

$t_i$ is the time interval.

The goal was to test 30 fish of similar size from both exercise and control groups.

## Predator/Prey Trials

### Razorback Suckers Versus Flathead Catfish

The predator/prey trials were designed to test whether exercise and predator exposure reduced short-term predation of razorback suckers compared to control fish. The predator/prey trials for razorback sucker and flathead catfish were conducted in a large 7-m-diameter fiberglass tank to allow fish adequate room to maneuver (fig. 4). The tank was partially buried into the ground and was shaded by a sun screen. Catfish shelters provided the predators a dark cavity in which to hide. Six shelters were fabricated by cutting plastic garbage cans (120 L) in half and attaching steel rebar along the cut edges to weigh the shelters down. Shelters were always used by the flathead catfish and were easily removed to capture the surviving prey. Water depth was maintained at 1 m, and water temperatures were recorded.

**Figure 4.** Photograph showing the 7-m-diameter tank used to conduct predator/prey experiments.

Flathead catfish were captured from the lower Colorado River. Five were used in the 2006 trials and six to seven were used in the 2007 experiments. Their length averaged 680-mm TL (range: 572- to 838-mm TL) and their weight ranged between 2.2 to 14.8 kg (Appendix A). Catfish were nocturnal, hiding in the shelters during daylight hours and roaming freely at night.

#### *2006 Trials*

Failure to precondition razorback suckers to predation left two test groups in 2006: razorback suckers that had been exercised and those that had not. Trials began with the selection of 10 fish from either group; they were measured and marked with a small pectoral-fin clip. Then ten similarly sized ($\pm$5-mm TL) fish were selected from the other test group and marked on the opposite pectoral fin (Appendix B). All 20 fish were introduced into the predation tank at the same time.

#### *2007 Trials*

These experiments were repeated in 2007; however, the comparison involved two treatment groups and a control; one new group had been exposed to predation. These trials started with 12 razorback suckers being selected from the exercise group; they were measured, marked, and placed in a tank with a flathead catfish to experience their first predation event. Then 12 similarly sized ($\pm$5-mm TL) fish were selected from the exercise and control groups. They were also marked and placed in a holding tank (Appendix C), so that all three groups experienced similar handling stress. The following morning, survivors from the predation tank were collected, measured, and matched with fish from the other two groups. The three groups were made distinguishable, by marking two groups differently and leaving the third unmarked. Marking consisted of clipping a small terminal divot from either left or right pectoral clip. The marking sequence was rotated among test groups. All prey were introduced into the predation tank at the same time.

Predator/prey experiments usually started in the morning, which allowed suckers time to acclimate before flathead catfish began feeding after dark. Experiments ran for one to six days, until roughly half the prey had been consumed. The tank was then partially drained, surviving suckers were dip netted, and the tank was refilled to start another trial. Survivors were measured and fins checked to determine which treatment group they originated from.

#### Bonytail Versus Largemouth Bass

We attempted to exercise bonytail in 2007; however, during the eighth week of the exercise regimen, bonytail from both exercise and control groups showed symptoms of ich (a parasitic—*Ichthyophthirius multifilis*—infection) and were treated with formalin. Control fish responded to treatment but the exercised fish died. We started another exercise group, but these fish also succumbed to another infection (3rd week) and died. No further attempts were made to physically condition bonytail.

We initiated the trials comparing predator-naïve with predator-savvy bonytail. These trials started with 12 bonytail selected from the control group; they were measured, marked, and placed in a tank with largemouth bass to experience their first predation event. Then 12 similarly sized (±5 mm) fish were marked and placed in a holding tank. Once predation was observed, survivors were removed and matched in number and size with predator-naïve fish. Fish were marked and transferred to a different predator tank to start the predation experiment. Three circular (2–4 m diameter) tanks were used, which contained varying numbers (2 to 15) of largemouth bass. Once predation occurred, surviving prey were removed, measured, and their origin of treatment determined. This typically took one hour to one day to occur.

## Observations

### Predation/Predator Avoidance?

The predator exposure attempt in 2006 left many unanswered questions that we fortunately were able to more closely examine. The flathead catfish were actively feeding prior to their transfer, but once moved they remained inactive for 48 hours. They were typically surrounded by curious razorback suckers, and there was no evidence by either predator or prey that predation occurred. Flathead catfish were then removed and placed in a 2-m-diameter tank. We quickly designed a set of impromptu tests to determine if the flathead catfish were feeding and if so, if we could detect predator avoidance behavior using a smaller number of prey.

#### Predator Avoidance Trials

Three predator avoidance experiments were conducted. Trial 1 consisted of a wood and wire mesh divider (8 by 10 cm) that was placed down the middle of the tank to provide a predator and a predator-free area. Ten razorback suckers were added to the tank that could swim through the large-mesh screen, allowing them access to either side. Periodically (2–5 times/d) we recorded prey distribution (predator side/sanctuary side) and if predation had occurred. Fish co-existed for seven days, suggesting the flathead catfish were still suffering from either handling or holding stress. For trial 2, the flathead catfish were moved to a larger (4 m) tank that contained two shelters that the catfish used during the daytime. A similar large-meshed divider was placed down the center of this tank, 10 new razorback suckers were added, and observation resumed. This experiment ran for 13 days. The third trial was similar to the previous test; the same two flathead catfish were used and 10 new razorback suckers were added. However, the sanctuary zone was reduced to one-fourth of the tank's area (fig. 5). For four days, we periodically recorded where fish were observed in the tank.

**Figure 5.** Tank configuration (one-fourth sanctuary) used for trial 3. The divider was placed along the center of the tank, dividing it into equal halves for trials 1 and 2.

## Statistical Analysis

### Razorback Sucker Predation Trials

Fish treatment varied between years: in 2006, treated fish were exercised only; in 2007, treated fish were exercised and exposed to a predation event. We were interested if treated fish were preyed upon less than control fish ($H_O$, predation was random and mortality of treatment and control fish was equal; $H_A$, predation was not random and mortality of treatment fish was less than mortality of control fish).

The number of predation trials in both years had relatively small sample sizes and values were often small and discrete (for example, number of fish eaten was <5 in many trials). Therefore, we used nonparametric tests to compare mortality of razorback suckers: either the Wilcoxon Matched Pairs Ranks test or Multi-Response Permutation Procedure (MRPP) Matched Pairs test. Significance was determined at the 0.05 level for all tests on predation.

### Bonytail Predation Trials

We examined predation of bonytail in small arena experiments using groups of two to three bonytail per treatment. A trial ended as soon as a predation event was observed, and we determined if the consumed fish was a treatment or control. We examined the binomial proportion of trial success using a Goodness of Fit test (HO, predation was random and ratio of trials was 1:1; HA, predation was not random and treatment fish succeeded in more trials than control fish).

### Flow Chamber Trials

We set up the experiment so that we evenly paired treatments; for example, we would test a 22-cm control fish and then a 22-cm exercised fish. We compared the cumulative distribution function of critical flow velocities ($U_{crit}$) between exercised and unexercised razorback suckers using a Kolmogorov-Smirnov two-sample test that provides information on maximum differences within the distributions, and the MRPP two-group comparison that focuses on average deviations. These nonparametric methods were used to test the hypothesis that exercised fish would handle critical flow velocities better than unexercised fish.

# Results

## Exercise Treatment

### Razorback Sucker

Exercised groups exhibited no sign of fatigue or stress. They roamed freely during exercise periods, easily navigating the velocity. They generally formed a large school that preferred the most upstream site near the motor and the downstream bend. Suckers in all four tanks typically schooled along the bottom of the tank during daylight hours, but they would disperse throughout the water column when the current was turned off at night.

### Bonytail

The two die-offs of bonytail were disappointments but not a surprise. We had been warned by hatchery personal that these fish are extremely susceptible to stress-related disease. However, we do not believe the exercise regimen in itself caused the mortality, but undoubtedly contributed to it. Three healthy bonytail were discovered among the exercised razorback suckers that had endured 20 weeks of flow; in addition, flow chamber results suggest bonytail are strong swimmers. Hatchery personnel reported the exercised bonytail quit feeding once the exercise regimen started. It has been reported (Mueller, 2006) that bonytail are nocturnal, and possibly the fish needed to be exercised at night rather than during the day. That combines with other existing stressors (for example, crowding and spawning season) that might have triggered chronic fatigue, leaving exercised fish more susceptible to the outbreak of *ich*. A similar outbreak of *ich* occurred at the end of our 2007 field season, which claimed all the remaining (>150) control bonytail.

## Predator Exposure

### Razorback Suckers—2006 Efforts

#### Impromptu Tank Experiments

In trial 1, the two flathead catfish and 10 razorback suckers co-existed in the tank experiment for seven days without predation occurring. Based on daytime observations, razorback sucker used both sides of the tank; 46 percent of the prey counted were found with the flathead catfish ($n$ = 20). There was no evidence of predator avoidance by razorback suckers (fig.6; Appendix B).

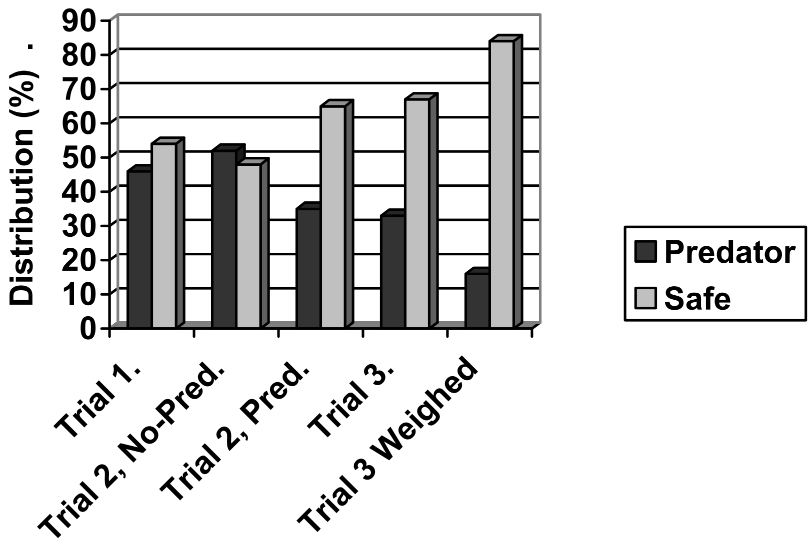


**Figure 6.** Distribution of razorback suckers observed in predator vs. safe (sanctuary) zone in a circular tank. Trials 1 and 2 (pre-predation) represent prey distribution prior to a predation event. Trial 2 (post-predation) and trial 3 illustrate prey distribution following a predation event. The size of the sanctuary in trial 3 was reduced from one-half to one-fourth of the tank's area. Observations were made periodically throughout daylight hours. Trial 1 occurred May 14–18, 2006 ($n$ = 20); trial 2 occurred May 21–27, 2006 ($n$ = 16); trial 2 post-predation occurred May 28–June 13 ($n$ = 50); and trial 3 occurred June 14–16 ($n$ = 12).

No predation occurred the first seven days of trial 2. Distribution of razorback suckers was similar to observations from trial 1: fish frequented both sizes of the tank equally (52 percent, $n$ = 16). However, following the predation event, prey distribution shifted immediately toward the sanctuary side (trial 2, post-predation; fig. 6) with twice as many razorback suckers frequenting the sanctuary zone compared to the area occupied by the predators. Predation occurred the first night in trial 3 and as with trial 2, prey were found twice as often (63 percent versus 37 percent, $n$ = 12) in the sanctuary zone. When considered in terms of available area, razorback suckers used the sanctuary zone six times more often than the predator zone.

### Razorback Suckers—2007 Efforts

Moving the prey into the predator tanks worked well. We established a 4-m tank with one flathead catfish we affectionately named "Oscar." Oscar had a feeding rate of less than one razorback per night. He generally ate one or two of the treatment fish then fasted for a night. However, on several occasions more were eaten and/or mortally wounded, which caused quite a bit of variability (2–10) in the size of some test groups.

### Bonytail

Largemouth bass generally feed during the day, but feeding rates varied from prey taken every few minutes to one in two days. Survivors of both species were removed as soon as predation occurred.

## Flow Chamber Experiments

### Razorback Suckers

Razorback suckers display the ability to "draft," maintaining their position for prolonged periods of time without having to swim. This made the test more of a comparison of this behavioral skill rather than physiological stamina. The chamber has a laminar flow filter constructed of 16-cm drinking straws, which redirects turbulence into parallel flow down the flow chamber. During our initial trials, some fish found what we called the "sweet spot," which was a location near the bottom of the laminar flow filter. Here fish could literally glide through the experiment.

On close examination we found that their head was against the chamber's floor, their pectoral fins were arched outward, flat against the sides of the chamber, and the posterior portion of their body angled upward. Apparently there was enough downward force to hold the fish in place. One fish used this technique and endured 4.5 hours and velocities that reached 1.0 m/s.

To reduce access to the "sweet spot," we built and installed a coarse screen that was positioned about 35 cm downstream of the filter. This reduced the length of the chamber to about 80 cm or about two-thirds of its original length, but prevented the prolonged drafting that we had witnessed before. While this helped, it did not eliminate the problem (behavior) entirely. Fish were still able to maintain themselves for periods of time without actively swimming. Taking advantage of this ability, they were able to hold themselves for a few seconds at velocities of 3.0 BL/s. Many would gradually slide backward, and upon reaching the screen, they would swim with a burst of energy to reestablish their position at the upper end of the flow chamber. There they would once again start their slow "slide" downstream. These respites undoubtedly helped fish conserve energy.

We determined critical flow velocities ($U_{crit}$) for 60 razorback suckers: 29 unexercised fish and 31 exercised fish. Summary data (table 1) indicated exercised fish had somewhat higher critical flow velocities and endurance than unexercised fish, but the difference was not significantly different.

**Table 1.** Comparison of distribution of body measurements, endurance (length of time fish endured increasing flows before exhaustion), and critical flow velocities ($U_{crit}$) tolerated by unexercised and exercised razorback suckers used in flow chamber experiments.

| Treatment | Statistical measurements | Total length Mm | Weight g | Endurance min | $U_{crit}$ cm/s | $U_{crit}$ body length/s |
|---|---|---|---|---|---|---|
| Exercised (n = 31) | Mean ± SE | 221 ± 2.5 | 98.4 ± 3.5 | 174.3 ± 6.9 | 53.1 ± 2.5 | 2.40 ± 0.11 |
| | Range | (200–245) | (58–137) | (64–236) | (11.9–75.5) | (0.56–3.43) |
| | Variance | 194.7 | 381.1 | 1465.3 | 192.3 | 0.41 |
| | Skew. coeff.[1] | 0.41 | 0.26 | –2.67 | 3.26 | 2.81 |
| | SW p-value[2] | 0.16 | 0.84 | 0.01 | 0.00 | 0.01 |
| Unexercised (n = 29) | Mean ± SE | 224 ± 2.6 | 100.0 ± 3.5 | 166.3 ± 7.4 | 50.4 ± 2.7 | 2.25 ± 0.12 |
| | Range | (198–245) | (62–130) | (80–241) | (16.7–74.4) | (0.82–3.43) |
| | Variance | 193.2 | 357.9 | 1594.4 | 211.9 | 0.43 |
| | Skew. coeff.[1] | –0.44 | 0.67 | 1.02 | 1.33 | 1.09 |
| | SW p-value[2] | 0.20 | 0.49 | 0.48 | 0.43 | 0.44 |

[1]Skewness coefficient = skewness/SE of skewness is considered significant if the absolute value is greater than 2.

[2]SW p-value: a significant p-value indicates data are not normal based on Shapiro-Wilk normality test.

Notably, the three control fish in the 21-cm-size class and the two control fish in the 25-cm-size classes had higher $U_{crit}$ (cm/s) than their exercised counterparts (fig. 7), which appeared to be the opposite trend compared to the other size classes (20-, 22-, 23-, and 24-cm TL). Therefore we examined the density distribution of $U_{crit}$ (BL/s) to see if these values may have altered the overall trend of $U_{crit}$ (BL/s). The five $U_{crit}$ (BL/s) values were not out of the ordinary compared to the rest of the distribution. Observed $U_{crit}$ (BL/s) for 21-cm control fish were 3.35, 2.75, 2.67; and 2.45 and 2.48 for the 25-cm control fish. The single 25-cm treatment fish had a $U_{crit}$ (BL/s) value of 1.52.

Although the size classes were slightly imbalanced, there were no significant differences in lengths between exercised and unexercised fish used in the flow chamber experiments (table 2; two-sample t-tests: $p = 0.5$). Therefore we used all 60 fish to examine the distributions of critical flow velocity ($U_{crit}$) between treatments. The $U_{crit}$ data were skewed to the left, especially for exercised fish (fig. 7; table 2). Therefore we ran nonparametric tests to compare distributions between treatments. The average deviations in cumulative distributions of $U_{crit}$ (BL/s) for exercised fish were not significantly different from unexercised fish (one-tailed MRPP two-group comparison; standardized test statistic = –0.15; $p = 0.15$). However, the maximal differences in cumulative distribution of $U_{crit}$ (BL/s) indicate exercised fish deviated significantly from control fish (fig. 7; one-tailed Kolmogorov-Smirnov two-sample test, D = 0.33; $p = 0.035$). Based on the latter statistical test and the distribution patterns, 50 percent more exercised fish than control fish (21 vs. 14) attained critical flow velocities of 2.5 body lengths/s or higher (fig. 8).

## Bonytail

Similar tests were conducted for bonytail without the screen modification. Even though we did not have an exercise group, we tested 30 control fish to develop base-line data for the species. Approximately 10 percent of the bonytail also exhibited the “drafting” behavior that was previously described. Trials were ended at the 4.5-BL/s level for convenience of time. Our primary concern was to test whether poor stamina contributed to the death of the exercised bonytail. Ten (33 percent) of our trial fish exceeded the 4.5-BL/s level of endurance, far surpassing the average endurance shown by razorback suckers (3.36 versus 2.24 (BL/s) (table 3). Average, median, and maximum swimming performances reported in table 3 are conservative values (Appendix B) since some fish exceeded our 4.5 BL/s test limit.

**Table 2.** Frequencies of razorback sucker by size class and treatment in flow chamber tests.

| Treatment | Razorback sucker size class (mm-TL) | | | | | | Totals |
|---|---|---|---|---|---|---|---|
| | 200 | 210 | 220 | 230 | 240 | 250 | |
| **Control** | 4 | 3 | 8 | 7 | 5 | 2 | 29 |
| **Exercised** | 4 | 7 | 8 | 5 | 6 | 1 | 31 |
| **Total** | 8 | 10 | 16 | 12 | 11 | 3 | 60 |

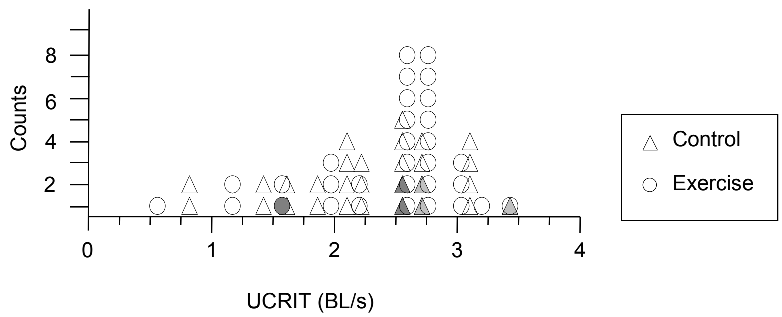


**Figure 7.** Density distribution of critical swimming speed ($U_{crit}$ in units of body length/s) of exercised and unexercised fish.

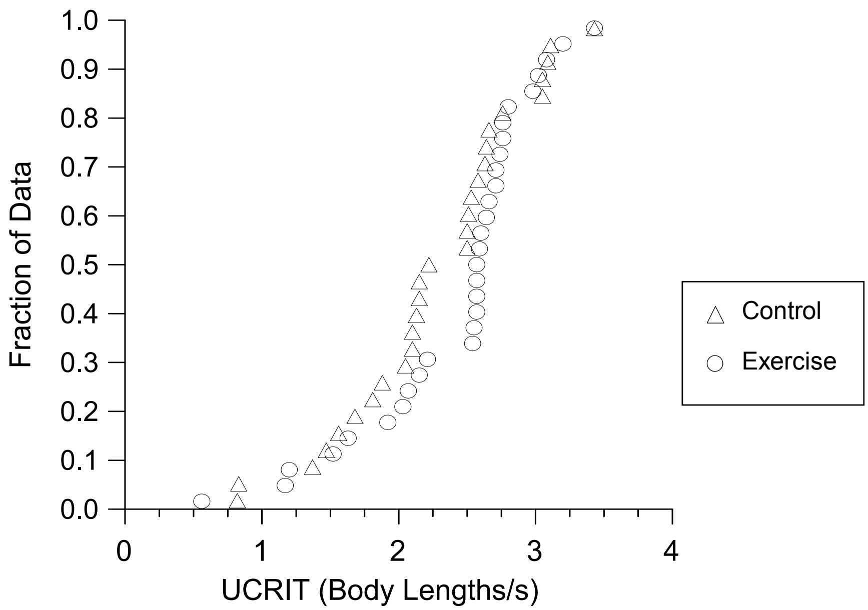


**Figure 8.** Cumulative distributions of critical flow velocity ($U_{crit}$ in body length/s) of exercised and unexercised (control) razorback suckers.

**Table 3.** Swimming performance[1] of bonytail.

| | Total length (mm) | Control (cm/s) | Control (BL/s) |
|---|---|---|---|
| **Average** | 181 | 59.98 | 3.36 |
| **Maximum** | 210 | 85.5 | 5.0 |
| **Minimum** | 150 | 17.27 | 0.91 |
| **Median** | 185 | 61.23 | 3.57 |

[1]Trials were limited to 4.5 BL/s; resulting averages are conservative.

## Predation Trials—2006

### Razorback Suckers

The 7-m tank gave prey ample room to escape. Nine trials using 20 razorback suckers (10 control and 10 treatment fish) were conducted (Appendix D). Initially, when razorback suckers were released into the tank, they swam frantically around and gradually would hide along or inside the shelters with the catfish (fig. 9). In time, they would gradually school and when disturbed, seek the protection of a shelter. All fish would disperse at night, including the flathead catfish.

Initially, each flathead catfish fed about once every four days. By the end of the trials, predators were feeding every other night. We recovered the prey fish by partially draining the tank. This didn't appear to disturb the flathead catfish, which became accustomed to this routine. When the depth had dropped to 10 cm, the shelters were placed outside the tank, and the razorbacks were dip-netted. The netting generally took less than two minutes. The shelters were immediately replaced, the drain closed, and the tank refilled; a process taking less than one hour. The flathead catfish appeared to become accustomed to this routine as it did not affect their feeding.

**Figure 9.** A school of razorback sucker shown exiting a flathead catfish shelter during a predation trial. Fish from both control and treatment groups were attracted to and typically associated with these structures during the initial phase of the test. These structures usually sheltered a large flathead catfish.

### Statistical Analysis

We conducted nine trials to compare mortality of exercised and control razorbacks. We compared the number of fish eaten of a total possible ten fish for each treatment (20 prey per trial). Mean mortality of exercised razorback sucker (3.33 ± 0.62 SE) was significantly lower than control fish (5.11 ± 0.74 SE) (fig. 10; one-tailed Wilcoxon Matched-Pairs Ranks test; n = 9, z = 1.69, p = 0.046).

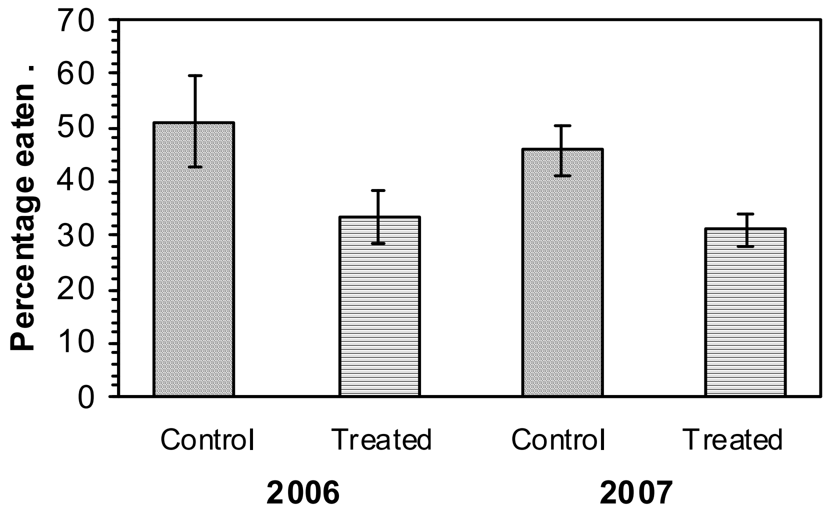


**Figure 10.** Percent mortality of razorback suckers (mean ± 95 percent Confidence limits) per treatment in 2006 and 2007. Treatment fish in 2006 were exercised only; treatment fish in 2007 were exercised and exposed to a predator event. In both years, treatment fish had significantly lower mortality than control fish (*p*<0.05).

## Predation Trials—2007

### Razorback Suckers

The first year we focused on testing whether flow conditioning increased a razorback sucker's escape performance. The second year our primary objective was to determine if an exposure to a predation event improved survival compared to predator-naïve fish. Twenty-three trials were conducted from April 10 to June 26, 2007. Initially, we mimicked the previous year's approach of using study groups of ten fish and allowing the predators whatever time necessary to reduce that number by half. However, the first two trials took nearly six nights to complete. During that period, we observed the razorback suckers schooling and realized that after the first evening, all these fish had some degree of predator experience. Avoidance behavior between the treated and control suckers would be most prevalent during the first encounter and decreased each consecutive night as fish were subjected to repeated predator aggression. We modified our study design to shorten the duration of the predation trial to one or two days by using smaller groups. As a result we increased our intended number of trials from 10 to 23.

## Statistical Analysis

We ran 29 predation trials originally with three treatments: unexercised, naïve razorback suckers (control fish); exercised, naïve razorback suckers; and exercised razorbacks that were exposed to a previous predation event. Numbers of fish per trial varied from two to ten per treatment, so prey numbers in a given trial ranged from six to 30 razorback suckers. Unlike the trials in 2006, in 2007 there was no difference in size of surviving fish between the three treatments (fig. 11; two-sample t-tests; $p = 0.65$).

The percentage of exercised, exposed razorback suckers consumed by predators (mean = 30.98 ± 4.41 SE) was significantly lower than for control fish (mean = 45.72 ± 4.88 SE) (fig. 10; one-tailed MRPP for Blocked Data; n = 42; standardized test statistic = –2.85; $p = 0.017$). These survival rates were very similar to what we observed in 2006 (fig. 10).

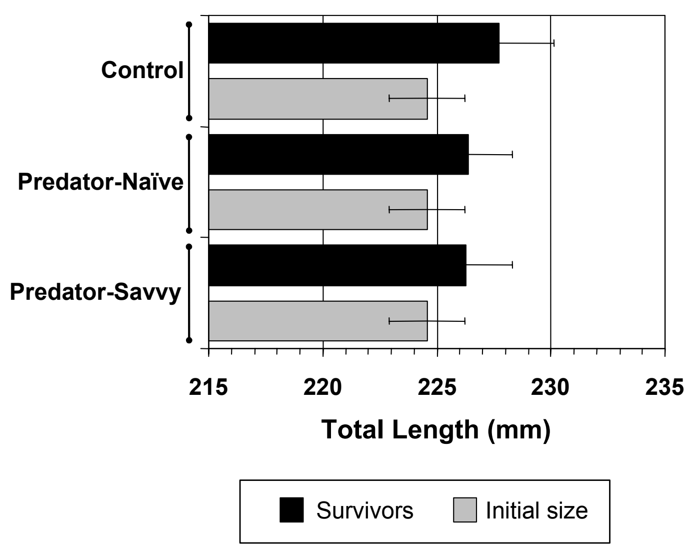


**Figure 11.** Total lengths (mean ± Standard Error) of initial and surviving razorback suckers used in predator experiments in 2007.

## Bonytail

In some trials, predation occurred within a few minutes after the interdiction of prey, and in one case, it took largemouth bass 48 h before they fed. Bonytail always schooled and generally gravitated toward the center of the tank or at the surface near the sides of the tank. We were able to complete 29 trials, which provided comparable data of largemouth bass predation on predator-savvy and predator-naïve bonytail. A total of 88 treatment and 88 control bonytail were used in these trials resulting in the loss of 18 (20 percent) treatment and 28 (32 percent) control fish (Appendix G).

### Statistical Analysis

We ran 29 trials to examine predation on exercised and unexercised (control) bonytail in small arena experiments. Numbers of bonytail varied from two to five pairs (control and treatment) of fish; thus number of prey in each trial ranged from four to ten bonytail. Of these 29 trials, 22 resulted in unequivocal success (no ties) by either control or exercised bonytail. Thus the expected ratio under the null hypothesis of random predation was 11:11. Exercised bonytail survived in 14 trials, whereas control fish survived in eight trials. However, we cannot reject the null hypothesis (one-tailed Goodness of Fit: $X^2 = 1.636$, df = 1; $p = 0.143$).

## Random Observations

Clear water allowed us to observe the relative number, location, and behavior of fish in all the tanks during the initial phases of all tests. Routine observations were conducted early in the morning, mid-day, in the afternoon, and occasionally after dark. We were interested in prey behavior (for example, schooling) and their distribution relative to the proximity of predators in the 2-m, 4-m, and 7-m tanks. Water temperatures were periodically checked, and the water depth was maintained at 1 m.

Behavior changes were quite dramatic for both species as were the mannerisms of the predators themselves. Largemouth bass generally fed during the day, which could be easily observed, but unfortunately, flathead catfish were entirely nocturnal. In those trials, we could only observe pre- and post-predation behavior for the razorback suckers.

### Bonytail

Largemouth bass generally fed during daylight. When placed with the bass, bonytail would school and initially intermixed with the largemouth bass. They exhibited no apparent avoidance behavior. The largemouth bass would eventually start crowding the bonytail causing them to more tightly school. This could take a few minutes and in some cases hours, depending upon the degree of aggression exhibited by the bass.

Prior to this series of experiments, predators were fed small nonnative fish salvaged from the rearing ponds. These included juvenile sunfish (*Lepomis* spp), threadfin shad (*Dorsoma petenence*), and common carp (*Cyprinis carpio*). When prey were placed in the circular tanks, they would immediately swim near the surface and against the tank's sides. This mimics the behavior of small fish that hug the shoreline when a predator was present. The potential angle (45°) of attack is greatly reduced at this position. The only exception is where the tank sides meet the bottom; there, a predator can drive its prey into the tank's side. On the surface with no backstop, the prey had a better opportunity to escape.

We witnessed the same behavior with bonytail that had been with predators for a length of time (fig. 12). On closer examination, these fish often showed signs of bruising and missing scales, which suggested they survived an unsuccessful attack. One of us (GAM) witnessed two such attacks; both times the bonytail had escaped. This behavior was only exhibited when bonytail were with predators; it was never observed in the control tanks where bonytail schooled at the bottom. All evidence suggests this is a predator-avoidance behavior, learned through predator aggression or actual predation of another individual.

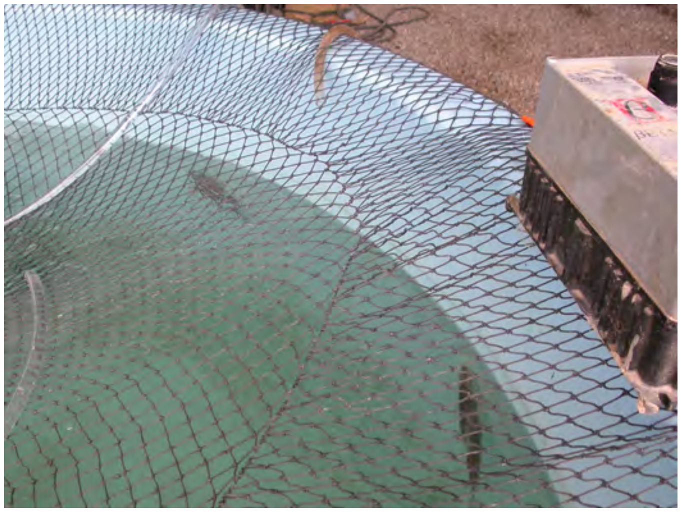

**Figure 12.** Common prey (feeder fish) and bonytail that had previously experienced predator aggression would often take a defensive stance of swimming at the upper sides of the tank. This position minimized the possible angle of attack and denied predators a backstop to trap prey.

## Razorback Sucker

During the flow chamber tests, we often heard fish splashing in tank no. four (control). This tank had a very active aerator that caused the water to up-well violently. On closer examination, we found that suckers were swimming vertically through the bubble plume and leaping out of the water. This behavior was not seen in the other three tanks but was a common occurrence in tank four. The nature or reason for this behavior is not known.

Initially we attempted to expose fish to predation by placing large flathead catfish in two tanks holding several hundred razorback suckers. Moving predators caused them to quit eating for several days. Both catfish went to the far end of each tank where they remained motionless for two days. The razorback suckers crowded around them in mass, possibly curious. Their attraction to the predators gradually decreased but the razorback suckers certainly did not exhibit predator avoidance. Their naïve curiosity would put them at greater risk in the wild.

We modified our study design to move the prey to the predator; this is also more representative of stocking practices. The handling ordeal caused the juvenile razorback suckers to flee, often seeking refuge in shelters whether they contained a predator or not. With time they calmed down, and often schooled in and around the shelters. Some fish remained inside the shelters.

Razorback suckers at night were found randomly distributed along the bottom and feeding on the tank's sides. We did not observe any type of schooling behavior after dark. Flathead catfish were generally active, slowly swimming around the tank. These observations were brief and limited since using an artificial light often startled the fish.

The following morning, razorback suckers were generally found inside the 10-cm-diameter inflow pipe or in shelters not occupied by a predator. They became highly skittish, avoided the catfish, and darted around the tank. In the pre-exposure tank, there was only one shelter that was always occupied by "Oscar," the pre-exposure predator. Survivors from that harrowing night always avoided going into that shelter.

### Changes in Pigmentation

Stress caused by handling or disease can cause blotching, discoloration, and behavioral changes in bonytail. Typically, healthy bonytail school near the bottom of the tank. When bonytail are stressed by some disease or parasite, the sickest fish are generally found by themselves near the surface in a lethargic state. Their bodies are either completely dark or blotched or banded and generally these fish die. The change in coloration can happen quite rapidly. For example, fish were observed hourly during the predator avoidance tank tests. One hour the bonytail appeared and behaved normally; the next hour, one could find a bonytail off by itself in a corner. It would be blotched or totally dark; within the next observation period it might be dead.

There were occasional (<1 percent) razorback suckers that were considerably darker than others (fig. 13). Unlike the bonytail, we did not experience any losses of these fish; however, their behavior was similar to the sick bonytail. They were often lethargic, avoided schooling, and were often found by themselves. We did observe razorback suckers changing color overnight, but the incidence was much lower than bonytail. The exact reason for this darker pigmentation or behavioral change is unknown, but we suspect it may be related to stress. We found no evidence it led to death.

**Figure 13.** Comparison of a normal colored razorback sucker (top) with one exhibiting darker pigmentation probably associated with stress.

## Discussion

Our observations supported previous reports that predator avoidance is not an inherited trait, but one that is learned (Fraser, 1974; Olla and Davis, 1989; Magurran, 1990). Our results also agreed with Brown and Warburton (1999), who reported that predator-naïve fish are often attracted to predators. Curiosity would be a lethal behavioral flaw in the wild. Razorback suckers were found to be naïve to the predators we tested. Our attempts to test bonytail failed due to a chronic outbreak of *ich.* However, once razorback suckers experienced predatory aggression or a predation event, they exhibited predator avoidance traits. We discovered through recent literature that there are apparent problems of mixing treated and control fish of which we were unaware. Regardless, we found that physical conditioning and prior predator exposure increased the survivability of treatment fish over control fish. All tests were within the probability range near $p = 0.10$, which for fish behavioral studies, is noteworthy.

## Experimental Challenges and Biases

Our results were conservative in terms of benefits derived by predator exposure. While the capability of handling stress for our study groups was identical, treatment fish received behavioral and physical trauma that control fish did not. Some treatment fish (both bonytail and razorback suckers) exhibited physical bruising (for example, discoloration, scale loss, and fin damage) from failed predator attacks, which undoubtedly placed them at a performance disadvantage (fig. 14). Unfortunately, the small number of fish we had to work with prevented us from removing all fish that showed signs of physical injury. Only those exhibiting difficulty in swimming or had major fin damage were removed. We suspect some of the bruising and possibly internal injuries went undetected and contributed toward poor escape performance leading to predation losses. This might be avoided in future studies by exposing large groups, for example, 100 prey and one predator.

In 2006, we used ten fish test groups where predation occurred over several days. In 2007, it became evident that after the first day, all the prey had been exposed to predator aggression and were exhibiting predator avoidance. We felt the benefit of pre-treatment decreased with each passing day. To address this, we shortened the duration and group size in the experiments. We opted to examine predation that occurred within a day or two, rather than rates acquired over several days.

Treatment and control fish schooled together, which was another factor we had not suspected would be a problem. Studies have shown that survivorship of some hatchery-reared fish increase if they are released among wild cohorts (Hvidsten and Johnsen, 1993). Berejikian and others (2000) suggested control fish may acquire anti-predatory behavior more quickly when released with trained fish through social learning processes. Brown and Laland (2001) recommended that treatment and control fish should be tested independently due to this problem; this is contrary to our approach. The schooling of control fish with the treatment groups may have inadvertently placed them in a better position than if they had been introduced to predators by themselves.

**Figure 14.** Bonytail exhibiting bruising, discoloration, and scale loss due to failed predation attempt during predator, pre-exposure treatments. Many trial fish suffered similar types of physical trauma during treatment sessions.

Lastly, by reducing the prey number and duration of the 2007 razorback sucker trials, we may have prematurely interrupted seeing the full benefits derived from treatment. We had four trials that exceeded four nights in duration in which we lost 8 predator-savvy, exercised fish; 13 predator-naïve, exercised fish; and 16 control fish. The predator-naïve, exercised fish showed a 19 percent advantage over the control fish, which was similar to our 2006 findings (22 percent). However, the full-treatment fish in those experiments out-performed the control fish by 50 percent. This is an extremely small number of observations and might simply be an anomaly; however, the trend certainly is intriguing and raises additional questions and possibilities.

## Management Significance?

These results suggest a glaring problem facing repatriation programs. Five decades ago, Miller (1954) suggested the discrepancy in survival rates between wild and hatchery-reared cutthroat trout was due to the absence of natural selection and that hatcheries might be better served (economically) to incorporate natural selection forces in the culturing process. To survive, stocked fish must not only avoid predators, they must also out-compete resident prey for food and space. This may be difficult for fish that are typically predator-naïve, fed commercial feeds, and have no experience with foraging for natural foods or utilizing complex environments. Poor stocking survival during the past two decades suggests something is missing in the culturing or reintroduction process. These problems have been reported worldwide for marine introductions and are now receiving more recognition for freshwater programs (Svsasand and others, 2000; Wisenden and others, 2004).

There are several excellent papers regarding the need and potential merit of incorporating life-skill training in aquaculture programs. Brown and Laland (2001) provided a review paper that focused on the issue of social learning skills of hatchery-reared fish. A second paper by Brown and Day (2002) stressed the need to "shift from husbandry to improving post-release behavioral performance." Both papers suggested that large increases in survival of hatchery-reared fish were possible. The main problem in testing these theories has been the large-scale treatment of fish and the practicality of conducting tests under field conditions. Mirza and Chivers (2000) conducted field tests for brook trout (*Salvelinus fontinalis*) and chain pickerel (*Esox niger*) and found measurable benefits for tests conducted in large stream enclosures. A similar approach testing survival in hatchery ponds containing a mixed predator/prey community provides an alternative approach.

## What is Happening in the Wild?

There is very little information available regarding what is happening to razorback suckers and bonytail after they are stocked. One fact most can agree with is that very few fish are contacted by field crews. Some biologists suggest we simply don't know where to look; however, the evidence to date is quite disturbing. Telemetry studies suggest stocked suckers disperse rapidly, seeking out someplace to hide (Mueller and Marsh, 1998). If that is typically the case, this dispersal may literally prevent them from experiencing a predation event until it is simply too late. It is quite possible stocked fish disperse and are simply picked off individually before having an opportunity to learn what their predators are. Newly stocked fish may literally swim into the "lion's den" as a result of current culturing practices. This may help explain observations by Marsh and Brooks (1989) of the rapid and complete loss of young razorback suckers during a stocking event in Arizona. They estimated it took catfish less than four days to decimate an entire stocking of hundreds of fish.

Hatchery fish appear to have the physiological tools needed for defense; they simply don't know how to use them. Being raised in a monoculture with the absence of other fish, including predators, and in an environment sterile of color, structure, or flow makes them naïve and highly vulnerable to their own curiosity and predators. Other researchers have demonstrated that the release of fright pheromones alone did not trigger predator avoidance; prey had to associate predator odors with the traumatic event (Brown and Smith, 1998; Brown and Godin, 1999). Predator avoidance is actually a learned response that is inhibited in present-day culturing programs. We believe flow conditioning and limited predator exposure could improve short-term survival rates of hatchery-reared bonytail and razorback sucker. Additional research is needed to help develop methods of mass exposure, determining the duration of the learned response, and the duration and velocity of flow regimens for optimal physical conditioning. These efforts should be followed by, or run concurrently with, the release of a large number of treated fish in the wild to determine actual predation rates.

## Conclusions

We provide conclusive evidence that behavioral and physical conditioning can improve the performance of bonytail and razorback suckers over their hatchery counterparts. The major conclusions drawn by this study are the following:

- Hatchery reared bonytail and razorback sucker are naïve to the predator threat by largemouth bass and flathead catfish.
- At initial contact, hatchery raised bonytail and razorback suckers freely approach predators.
- Predator avoidance is a learned behavior.
- Predation losses were reduced for flow-conditioned razorback sucker.
- Predation losses were reduced for razorback sucker having a previous predator experience.
- Predation losses were reduced for bonytail having a previous predator experience.
- All treatments (exercise and predator exposure) resulted in reduced predation losses.

A great deal was learned, but we also generated quite a few new questions that deserve further investigation. Unfortunately, we simply do not know if this type of conditioning would increase survival in the wild. Further research is justified to examine the predator/prey interactions in the field and to further refine predator avoidance and exercise techniques in hatchery settings. Some of those questions include the following:

- How long is predator avoidance retained?
- What is the most effective way of mass exposing treatment fish to predators?
- What predators should be used?
- What is the optimal duration and velocity for flow conditioning?
- What is the actual survival benefit in the wild?
- What is the economic savings?

## Acknowledgments

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# Appendix A. Data Pertaining to Large Predators Used in Predator/Prey Trials

| Flathead catfish (7) | Length (mm) | Weight (kg) |
|---|---|---|
| Average | 680 | 4.5 |
| Maximum | 838 | 7.1 |
| Minimum | 572 | 2.9 |

| Largemouth bass (18) | Weight (kg) |
|---|---|
| Average | 1.3 |
| Maximum | 2.5 |
| Minimum | 0.3 |

# Appendix B. Data Pertaining to Predators Avoidance Used in Predator/Prey Trials

| Trial 1. No predation | | | | Trial 2. No predation | | | |
|---|---|---|---|---|---|---|---|
| **Date** | **Time** | **Sanctuary zone** | **Predator zone** | **Date** | **Time** | **Sanctuary zone** | **Predator zone** |
| 14-May-2006 | 1300 | 9 | 1 | 21-May | 1630 | 8 | 2 |
| | 1600 | 9 | 1 | | 1800 | 9 | 1 |
| | 1830 | 1 | 9 | 22-May | 630 | 7 | 3 |
| 15-May | 630 | 8 | 2 | 23-May | 730 | 8 | 2 |
| | 1200 | 2 | 8 | 24-May | 800 | 9 | 1 |
| | 1330 | 10 | 0 | 25-May | 1600 | 10 | 0 |
| | 1630 | 2 | 8 | | 1900 | 6 | 4 |
| 16-May | 700 | 4 | 6 | 26-May | 700 | 7 | 3 |
| | 830 | 1 | 9 | | 1100 | 0 | 10 |
| | 1200 | 2 | 8 | | 1300 | 1 | 9 |
| | 1600 | 4 | 6 | | 1530 | 4 | 6 |
| | 1830 | 9 | 1 | | 1800 | 2 | 8 |
| 17-Jun | 600 | 5 | 5 | 27-May | 630 | 0 | 10 |
| | 900 | 1 | 9 | | 930 | 6 | 4 |
| | 1300 | 6 | 4 | | 1500 | 2 | 8 |
| | 1830 | 5 | 5 | | 1700 | 4 | 6 |
| 18-May | 630 | 8 | 2 | Total | | 83 | 77 |
| | 1200 | 9 | 1 | | | | |
| | 1630 | 9 | 1 | | | | |
| | 1900 | 4 | 6 | | | | |
| Total | | 108 | 92 | | | | |

Trial 1, *n* = 20; trial 2, *n* = 16

| Trial 2. Post-predation | | | | |
|---|---|---|---|---|
| Date | Time | Pred. zone | Sanct. zone | Survivor numbers |
| 28-May | 730 | 0 | 9 | 9 |
| | 1100 | 1 | 8 | 9 |
| | 1400 | 4 | 5 | 9 |
| | 1600 | 5 | 4 | 9 |
| | 1830 | 8 | 1 | 9 |
| | 2130 | 0 | 9 | 9 |
| 29-May | 730 | 3 | 6 | 9 |
| | 2100 | 0 | 9 | 9 |
| 30-May | 730 | 0 | 9 | 9 |
| | 1100 | 0 | 9 | 9 |
| | 1600 | 1 | 8 | 9 |
| | 2300 | 0 | 8 | 9 |
| 31-May | 800 | 1 | 7 | 8 |
| | 1300 | 1 | 7 | 8 |
| | 1700 | 0 | 8 | 8 |
| 1-Jun | 800 | 0 | 7 | 7 |
| | 1300 | 3 | 4 | 7 |
| | 2200 | 1 | 6 | 7 |
| 2-Jun | 730 | 1 | 6 | 7 |
| | 1400 | 4 | 3 | 7 |
| 3-Jun | 800 | 2 | 5 | 7 |
| | 1200 | 7 | 0 | 7 |
| | 2230 | 1 | 6 | 7 |
| 5-Jun | 1000 | 3 | 3 | 6 |
| | 1230 | 2 | 4 | 6 |
| | 1800 | 4 | 2 | 6 |
| 6-Jun | 545 | 4 | 1 | 6 |
| | 1400 | 3 | 1 | 6 |
| | 1845 | 4 | 2 | 6 |
| 7-Jun | 630 | 4 | 2 | 6 |
| | 924 | 3 | 2 | 5 |
| | 1311 | 1 | 4 | 5 |
| | 1905 | 2 | 2 | 5 |

| Trial 2. Post-predation—Continued | | | | |
|---|---|---|---|---|
| Date | Time | Pred. zone | Sanct. zone | Survivor numbers |
| 8-Jun | 545 | 4 | 0 | 5 |
| | 1300 | 4 | 0 | 5 |
| | 1900 | 1 | 4 | 5 |
| 9-Jun | 615 | 1 | 3 | 5 |
| | 1200 | 4 | 0 | 5 |
| | 1745 | 1 | 3 | 5 |
| 10-Jun | 1845 | 1 | 2 | 5 |
| | 1155 | 3 | 0 | 5 |
| | 1815 | 0 | 3 | 5 |
| 11-Jun | 700 | 1 | 1 | 5 |
| | 1300 | 3 | 0 | 5 |
| | 1815 | 2 | 1 | |
| 12-Jun | 600 | 2 | 0 | 5 |
| | 1300 | 2 | 1 | 5 |
| | 1730 | 1 | 2 | 5 |
| 13-Jun | 600 | 1 | 1 | 5 |
| | 1130 | 1 | 1 | 5 |
| Total | | 105 | 189 | |

| Trial 3. Predation | | | | |
|---|---|---|---|---|
| Date | Time | Pred. zone | Sanct. zone | Survivor numbers |
| 14-Jun | 650 | 8 | 1 | 9 |
| | 1330 | 8 | 1 | 9 |
| | 1800 | 1 | 7 | 9 |
| 15-May | 720 | 3 | 5 | 8 |
| | 1800 | 2 | 6 | 8 |
| | 2200 | 0 | 8 | 8 |
| 16-Jun | 500 | 1 | 7 | 7 |
| | 715 | 1 | 7 | 7 |
| | 1300 | 0 | 8 | 7 |
| | 1700 | 2 | 6 | 7 |
| | 2000 | 2 | 6 | 7 |
| | 2100 | 4 | 4 | 7 |
| Total | | 32 | 66 | |

Trial 2, $n = 50$; trial 3, $n = 12$

# Appendix C. Swimming Performance Data for Exercised and Unexercised Razorback Sucker and Unexercised Bonytail

| Trial | Razorback control | Razorback exercised | Bonytail control |
|---|---|---|---|
| 1 | 0.83 | 0.56 | 0.93 |
| 2 | 1.33 | 1.17 | 1.27 |
| 3 | 1.37 | 1.20 | 1.28 |
| 4 | 1.47 | 1.52 | 1.90 |
| 5 | 1.55 | 1.63 | 2.05 |
| 6 | 1.63 | 1.93 | 2.26 |
| 7 | 1.68 | 2.03 | 2.28 |
| 8 | 1.80 | 2.07 | 2.65 |
| 9 | 1.87 | 2.20 | 2.65 |
| 10 | 2.05 | 2.22 | 2.88 |
| 11 | 2.10 | 2.53 | 3.02 |
| 12 | 2.10 | 2.54 | 3.03 |
| 13 | 2.13 | 2.55 | 3.20 |
| 14 | 2.15 | 2.55 | 3.53 |
| 15 | 2.21 | 2.57 | 3.57 |
| 16 | 2.22 | 2.57 | 3.57 |
| 17 | 2.45 | 2.57 | 3.73 |
| 18 | 2.46 | 2.60 | 3.73 |
| 19 | 2.48 | 2.67 | 3.92 |
| 20 | 2.55 | 2.69 | 3.95 |
| 21 | 2.58 | 2.70 | 4.50+ |
| 22 | 2.63 | 2.72 | 4.50+ |
| 23 | 2.65 | 2.73 | 4.50+ |
| 24 | 2.67 | 2.80 | 4.50+ |
| 25 | 2.75 | 2.98 | 4.50+ |
| 26 | 3.04 | 2.99 | 4.50+ |
| 27 | 3.05 | 3.02 | 4.50+ |
| 28 | 3.08 | 3.08 | 4.50+ |
| 29 | 3.10 | 3.21 | 4.50+ |
| 30 | 3.35 | 3.43 | 5.00+ |
| Average | 2.21 | 2.40 | 3.36 |
| Maximum | 3.35 | 3.43 | 5.0+ |
| Minimum | 0.83 | 0.56 | 0.93 |

Values expressed as $U_{crit}$= BL/s (body length/second) values sorted in ascending order

# Appendix D. Total Length Data of Razorback Sucker Used and Survived Predator/Prey Experiments with Flathead Catfish

**Large (7 m) Tank Predation Tests**

Total length of individuals used in initial tests and those surviving.

| Trial 1 | | | | Trial 2 | | | |
|---|---|---|---|---|---|---|---|
| Test group | | Survivors | | Test group | | Survivors | |
| **Treated** | **Control** | **Treated** | **Control** | **Treated** | **Control** | **Treated** | **Control** |
| 240 | 206 | 212 | 200 | 215 | 205 | 218 | 205 |
| 220 | 195 | 230 | 230 | 215 | 225 | 225 | 225 |
| 220 | 200 | 200 | 212 | 223 | 197 | 223 | 197 |
| 212 | 245 | 215 | 242 | 225 | 220 | 225 | 220 |
| 208 | 218 | 218 | 220 | 204 | 212 | | 214 |
| 222 | 204 | 240 | 225 | 244 | 210 | | 222 |
| 230 | 230 | 207 | 203 | 195 | 215 | | 232 |
| 202 | 218 | 208 | 195 | 190 | 223 | | 240 |
| 210 | 230 | **216.3** | **215.9** | 205 | 225 | **222.8** | **219.4** |
| 205 | 218 | | | 200 | 240 | | |
| **216.9** | **216.4** | | | **211.6** | **217.2** | | |

| Trial 3 | | | | Trial 4 | | | |
|---|---|---|---|---|---|---|---|
| Test group | | Survivors | | Test group | | Survivors | |
| **Treated** | **Control** | **Treated** | **Control** | **Treated** | **Control** | **Treated** | **Control** |
| 194 | 190 | 215 | 225 | 242 | 206 | 230 | 215 |
| 209 | 206 | 212 | 215 | 215 | 228 | 215 | 215 |
| 211 | 210 | 222 | 225 | 212 | 188 | 242 | 200 |
| 212 | 215 | 220 | 194 | 206 | 200 | 212 | 187 |
| 215 | 207 | 195 | 225 | 222 | 214 | 185 | |
| 225 | 226 | 211 | 215 | 185 | 212 | 225 | |
| 221 | 222 | | 225 | 215 | 245 | 185 | |
| 210 | 226 | **212.5** | **217.7** | 206 | 218 | 205 | |
| 230 | 226 | | | 202 | 208 | 210 | |
| 224 | 222 | | | 212 | 215 | **212.1** | **204.3** |
| **215.1** | **215** | | | **211.7** | **213.4** | | |

| Trial 5 | | | | Trial 6 | | | |
|---|---|---|---|---|---|---|---|
| Test group | | Survivors | | Test group | | Survivors | |
| **Treated** | **Control** | **Treated** | **Control** | **Treated** | **Control** | **Treated** | **Control** |
| 235 | 235 | 239 | 225 | 215 | 220 | 245 | 225 |
| 220 | 220 | 218 | 210 | 212 | 211 | 235 | |
| 204 | 197 | 222 | 242 | 210 | 207 | 215 | |
| 215 | 216 | 205 | 219 | 233 | 235 | 219 | |
| 222 | 221 | 225 | | 220 | 220 | 195 | |
| 215 | 220 | 205 | | 188 | 192 | **221.8** | **225.0** |
| 200 | 195 | 219 | | 240 | 236 | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 225 | 227 | **219.0** | **224.0** | 192 | 192 | | |
| 215 | 215 | | | 191 | 191 | | |
| 210 | 210 | | | 222 | 225 | | |
| **216.1** | **215.6** | | | **212.3** | **212.9** | | |

| Trial 7 | | | | Trial 8 | | | |
|---|---|---|---|---|---|---|---|
| Test group | | Survivors | | Test group | | Survivors | |
| **Treated** | **Control** | **Treated** | **Control** | **Treated** | **Control** | **Treated** | **Control** |
| 186 | 184 | 195 | 200 | 240 | 170 | 240 | 190 |
| 210 | 202 | 210 | 240 | 180 | 245 | 200 | 240 |
| 236 | 240 | 230 | 205 | 200 | 195 | 242 | 245 |
| 208 | 206 | 205 | | 200 | 210 | | 215 |
| 185 | 188 | 195 | | 245 | 200 | | 252 |
| 195 | 191 | 210 | | 210 | 240 | **227.3** | **222.5** |
| 192 | 190 | 180 | | 240 | 245 | | |
| 215 | 211 | 180 | | 255 | 255 | | |
| 216 | 219 | **200.6** | **215.0** | 215 | 200 | | |
| 193 | 190 | | | 210 | 210 | | |
| **203.6** | **202.1** | | | **219.5** | **217** | | |

| Trial 9 | | | | Trial 10 | | | |
|---|---|---|---|---|---|---|---|
| Test group | | Survivors | | Test group | | Survivors | |
| **Treated** | **Control** | **Treated** | **Control** | **Treated** | **Control** | **Treated** | **Control** |
| 210 | 210 | 240 | 210 | 230 | 220 | 230 | 230 |
| 195 | 235 | 245 | 230 | 200 | 195 | 235 | 225 |
| 210 | 190 | 235 | 215 | 235 | 240 | 200 | 200 |
| 245 | 205 | 220 | 250 | 210 | 225 | 232 | 220 |
| 205 | 250 | 210 | 255 | 230 | 235 | 235 | 200 |
| 235 | 235 | 210 | 185 | 235 | 230 | 200 | |
| 200 | 210 | | 200 | 200 | 200 | 215 | |
| 225 | 200 | **226.7** | **224.2** | 235 | 225 | 235 | |
| 245 | 245 | | | 220 | 235 | **222.8** | **215.0** |
| 201 | 200 | | | 200 | 200 | | |
| **217.1** | **218** | | | **219.5** | **220.5** | | |

| Summary of survivor numbers | | |
|---|---|---|
| **Trial** | **Treatment** | **Control** |
| 1 | 8 | 8 |
| 2 | 4 | 8 |
| 3 | 6 | 7 |
| 4 | 9 | 4 |
| 5 | 7 | 4 |
| 6 | 5 | 1 |
| 7 | 8 | 3 |
| 8 | 3 | 5 |

| | | |
|---|---|---|
| 9 | 6 | 7 |
| 10 | 8 | 5 |
| **Total** | 64 | 52 |

## Appendix E. Razorback Sucker and Flathead Catfish Trials Showing the Duration of the Experiment, Number of Fish in Each Group, and Number of Survivors and Number of Prey Eaten from Each Group in 2007

| Date | Trial | Duration | Group no. | Treat | Naïve | Control | Treat | Naïve | Control |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Number of Survivors | | | Number Eaten | | |
| 10-April | 1 | 5 | 10 | 5 | 3 | 1 | 5 | 7 | 9 |
| 14-April | 2 | 2 | 2 | 1 | 0 | 0 | 1 | 2 | 2 |
| 16-April | 3 | 1 | 3 | 3 | 2 | 2 | 0 | 1 | 1 |
| 24-April | 4 | 2 | 3 | 1 | 2 | 2 | 2 | 1 | 1 |
| 28-April | 5 | 3 | 3 | 2 | 0 | 1 | 1 | 3 | 2 |
| 30-April | 6 | 2 | 4 | 2 | 4 | 2 | 2 | 0 | 2 |
| 2-May | 7 | 1 | 4 | 3 | 4 | 3 | 1 | 0 | 1 |
| 4-May | 8 | 1 | 3 | 2 | 0 | 2 | 1 | 3 | 1 |
| 7-May | 9 | 4 | 4 | 3 | 2 | 3 | 1 | 2 | 1 |
| 10-May | 10 | 2 | 4 | 2 | 3 | 3 | 2 | 1 | 1 |
| 12-May | 11 | 2 | 4 | 3 | 2 | 2 | 1 | 2 | 2 |
| 14-May | 12 | 1 | 4 | 2 | 2 | 2 | 2 | 2 | 2 |
| 15-May | 13 | 1 | 4 | 3 | 2 | 2 | 1 | 2 | 2 |
| 16-May | 14 | 1 | 3 | 1 | 1 | 3 | 2 | 2 | 0 |
| 18-May | 15 | 1 | 5 | 4 | 3 | 3 | 1 | 2 | 2 |
| 23-May | 16 | 1 | 5 | 4 | 5 | 2 | 1 | 0 | 3 |
| 24-May | 17 | 1 | 3 | 3 | 1 | 2 | 0 | 2 | 1 |
| 25-May | 18 | 1 | 5 | 2 | 5 | 4 | 3 | 0 | 1 |
| 26-May | 19 | 1 | 4 | 4 | 4 | 3 | 0 | 0 | 1 |
| 28-May | 20 | 2 | 4 | 3 | 4 | 2 | 1 | 0 | 2 |
| 31-May | 21 | 4 | 4 | 3 | 2 | 1 | 1 | 2 | 3 |
| 20-June | 22 | 1 | 3 | 3 | 1 | 1 | 0 | 2 | 2 |
| 26-June | 23 | 6 | 8 | 7 | 4 | 4 | 1 | 4 | 4 |
| Average | | 2 | 4.2 | | | | | | |
| Totals | | | 96 | 66 | 56 | 50 | 30 | 40 | 46 |

## Appendix F. Bonytail and Largemouth Bass Predator/Prey Trial Information Showing the Group Size and Numbers of Surviving and Lost Prey

| Date | Trial | Group no. | Treat | Control | Treat | Control | Treat | Control |
|---|---|---|---|---|---|---|---|---|
| | | | Survivors | | Eaten | | Least losses | |
| 19-April | 1 | 8 | 2 | 3 | 6 | 5 | | X |
| | 2 | 2 | 1 | 2 | 1 | 0 | | X |
| | 3 | 3 | 3 | 2 | 0 | 1 | X | |
| 20-April | 4 | 3 | 3 | 1 | 0 | 2 | X | |
| | 5 | 3 | 2 | 3 | 1 | 0 | | X |
| 21-April | 6 | 5 | 4 | 4 | 1 | 1 | | |
| 22-April | 7 | 3 | 3 | 1 | 0 | 2 | X | |
| | 8 | 3 | 2 | 2 | 1 | 1 | | |
| | 9 | 3 | 3 | 2 | 0 | 1 | X | |
| 23-April | 10 | 2 | 0 | 1 | 2 | 1 | | X |
| | 11 | 3 | 2 | 2 | 1 | 1 | | |
| 25-April | 12 | 3 | 3 | 1 | 0 | 2 | X | |
| | 13 | 2 | 2 | 0 | 0 | 2 | X | |
| | 14 | 2 | 2 | 1 | 0 | 1 | X | |
| 26-April | 15 | 2 | 1 | 1 | 1 | 1 | | |
| 27-April | 16 | 2 | 1 | 1 | 1 | 1 | | |
| 28-April | 17 | 2 | 1 | 2 | 1 | 0 | | |
| | 18 | 2 | 1 | 2 | 1 | 0 | | |
| | 19 | 2 | 0 | 2 | 2 | 0 | | X |
| 1-May | 20 | 3 | 3 | 2 | 0 | 1 | X | |
| | 21 | 3 | 2 | 3 | 1 | 0 | | X |
| 2-May | 22 | 3 | 2 | 3 | 1 | 0 | | X |
| | 23 | 3 | 3 | 2 | 0 | 1 | X | |
| | 24 | 3 | 2 | 3 | 1 | 0 | | X |
| 3-May | 25 | 2 | 2 | 1 | 0 | 1 | X | |
| | 26 | 3 | 3 | 2 | 0 | 1 | X | |
| | 27 | 3 | 3 | 2 | 0 | 1 | X | |
| 5-May | 28 | 3 | 3 | 1 | 0 | 2 | X | |
| | 29 | 5 | 4 | 2 | 1 | 3 | X | |
| | 30 | 2 | 2 | 1 | 0 | 1 | X | |
| Average | | 2.9 | | | | | | |
| Totals | | 88 | 65 | 55 | 23 | 33 | 15 | 8 |

## Appendix G. Total Lengths of Razorback Suckers Used in and Surviving Predator/Prey Experiments

| Trial | Length | Treatment | Naïve | Control | Trial | Length | Treatment | Naïve | Control |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 220 | 215 | 225 | 210 | 10 | 215 | 220 | 215 | 215 |
| 1 | 235 | 200 | 220 | | 10 | 220 | 225 | 220 | 225 |
| 1 | 220 | 205 | 215 | | 10 | 225 | | 230 | 230 |
| 1 | 210 | 220 | | | 10 | 230 | | | |
| 1 | 195 | 230 | | | 11 | 210 | 210 | 210 | |
| 1 | 205 | | | | 11 | 230 | 245 | 230 | 245 |
| 1 | 220 | | | | 11 | 245 | 235 | | 235 |
| 1 | 195 | | | | 11 | 235 | | | |
| 1 | 205 | | | | 12 | 225 | 225 | 235 | 235 |
| 1 | 230 | | | | 12 | 235 | 215 | 215 | 215 |
| 1 | 215 | | | | 12 | 205 | | | |
| 2 | 210 | 215 | | | 12 | 215 | | | |
| 2 | 215 | | | | 13 | 250 | 225 | 225 | 250 |
| 3 | 200 | 205 | 210 | 215 | 13 | 225 | 230 | 230 | 235 |
| 3 | 210 | 225 | 210 | 190 | 13 | 230 | 235 | 245 | 245 |
| 3 | 210 | 195 | | | 13 | 235 | 245 | | |
| 3 | 220 | | | | 13 | 245 | | | |
| 3 | 230 | | | | 14 | 250 | | | 250 |
| 4 | 210 | 240 | 220 | 220 | 14 | 220 | | | 220 |
| 4 | 225 | | 240 | 230 | 14 | 225 | 225 | 220 | 225 |
| 4 | 230 | | | | 15 | 215 | 215 | 215 | 230 |
| 4 | 210 | | | | 15 | 220 | 220 | 220 | 220 |
| 4 | 195 | | | | 15 | 230 | 230 | 230 | |
| 4 | 240 | | | | 15 | 220 | 220 | 220 | |
| 5 | 240 | 215 | | 215 | 15 | 215 | | 215 | |
| 5 | 215 | 210 | | | 16 | 215 | 215 | 225 | 225 |
| 5 | 210 | | | | 16 | 225 | 225 | | 215 |
| 6 | 210 | 210 | 210 | 235 | 16 | 220 | 220 | | |
| 6 | 230 | 235 | 230 | 195 | 17 | 260 | | 260 | |
| 6 | 235 | | 235 | | 17 | 210 | | 210 | 260 |
| 6 | 195 | | 195 | | 17 | 230 | 230 | 230 | 230 |
| 7 | 230 | 230 | 230 | 230 | 18 | 215 | 215 | 215 | 215 |
| 7 | 210 | 210 | 210 | 210 | 18 | 225 | 225 | 225 | 225 |
| 7 | 230 | 225 | 230 | 230 | 18 | 230 | 230 | 230 | |
| 7 | 225 | | 225 | | 18 | 245 | 245 | 245 | 245 |
| 8 | 230 | 230 | | 230 | 19 | 240 | 260 | 240 | 240 |
| 8 | 210 | 190 | | 190 | 19 | 260 | 230 | 260 | 230 |
| 8 | 190 | | | | 19 | 210 | 210 | 210 | |
| 9 | 200 | 210 | 210 | 210 | 19 | 230 | | 230 | |
| 9 | 210 | 210 | 230 | 210 | 20 | 210 | 240 | 230 | |
| 9 | 210 | 230 | | 230 | 20 | 240 | 230 | 220 | 230 |
| 9 | 230 | | | | 20 | 230 | 220 | | |
| | | | | | 20 | 220 | | | |

| | | | | | |
|---|---|---|---|---|---|
| | Total | 221.8 | 222.5 | 224.5 | 225.4 |
| | Maximum | 260 | 260 | 260 | 260 |
| | Minimum | 190 | 190 | 195 | 190 |

## Appendix H. Total Lengths of Bonytail Used in and Surviving Predator/Prey Experiments

| Trial | Length | Treatment | Control | Trial | Length | Treatment | Control |
|---|---|---|---|---|---|---|---|
| 1 | 165 | 165 | 180 | 15 | 190 | 190 | 190 |
| 1 | 185 | 185 | 185 | 15 | 175 | | |
| 1 | 180 | | 190 | 16 | 185 | | |
| 1 | 215 | | | 16 | 190 | 190 | 185 |
| 1 | 200 | | | 17 | 185 | | 185 |
| 1 | 150 | | | 17 | 205 | 205 | 205 |
| 1 | 185 | | | 18 | 185 | | 185 |
| 1 | 190 | | | 18 | 165 | 185 | 165 |
| 2 | 190 | | 190 | 19 | 165 | | 165 |
| 2 | 185 | 185 | 185 | 19 | 190 | | 190 |
| 3 | 175 | 175 | 175 | 20 | 230 | 230 | 230 |
| 3 | 160 | 160 | 190 | 20 | 240 | 240 | 240 |
| 3 | 190 | 190 | | 20 | 195 | 195 | |
| 4 | 175 | 175 | 190 | 21 | 255 | 255 | 255 |
| 4 | 175 | 175 | | 21 | 245 | | 245 |
| 4 | 190 | 190 | | 21 | 200 | 200 | 200 |
| 5 | 195 | 195 | 195 | 22 | 260 | 260 | 260 |
| 5 | 190 | 180 | 190 | 22 | 225 | | 225 |
| 5 | 180 | | 180 | 22 | 205 | 205 | 205 |
| 6 | 170 | 170 | | 23 | 205 | 205 | |
| 6 | 190 | 190 | 190 | 23 | 210 | 210 | 210 |
| 6 | 190 | 190 | 190 | 23 | 220 | 220 | 220 |
| 6 | 200 | | 200 | 24 | 210 | | 210 |
| 6 | 175 | 175 | 175 | 24 | 230 | 230 | 230 |
| 7 | 175 | 175 | | 24 | 230 | 230 | 230 |
| 7 | 175 | 175 | | 25 | 205 | 205 | |
| 7 | 180 | 180 | 180 | 25 | 220 | 220 | 220 |
| 8 | 180 | 185 | 180 | 26 | 220 | 220 | 220 |
| 8 | 185 | 190 | 190 | 26 | 215 | 215 | |
| 8 | 190 | | | 26 | 230 | 230 | 230 |
| 9 | 170 | 170 | 170 | 27 | 190 | 190 | |
| 9 | 160 | 160 | 160 | 27 | 235 | 235 | 235 |
| 9 | 175 | 175 | | 27 | 215 | 215 | 215 |
| 10 | 170 | | | 28 | 195 | 195 | |
| 10 | 180 | | 180 | 28 | 170 | 170 | |
| 11 | 190 | 190 | | 28 | 235 | 235 | 235 |
| 11 | 200 | 200 | | 29 | 250 | 250 | 250 |
| 11 | 185 | 185 | 185 | 29 | 235 | 235 | |
| 12 | 210 | 210 | 210 | 29 | 200 | | |
| 12 | 180 | 170 | 180 | 29 | 230 | 230 | |
| 12 | 170 | | | 30 | 205 | 205 | |
| 13 | 165 | 165 | | 30 | 220 | 220 | 220 |
| 13 | 170 | 170 | | | | | |
| 14 | 205 | 205 | 205 | Average | 196.0 | 198.4 | 201.8 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 14 | 175 | 175 | | Maximum | 260.0 | 260.0 | 260.0 |
| | | | | Minimum | 150.0 | 160.0 | 160.0 |