LFL Contrib. 98

Effects of the Fish Anesthetic Tricaine on Larval and Early Juvenile Razorback Sucker, *Xyrauchen texanus*

Final Report

Submitted to

Edmund J. Wick
Cooperative Parks Study Unit
U.S. National Park Service
1201 Oakridge Drive, Suite 250, Rm 49
Fort Collins, Colorado 80525
Telephone: (970) 225-3540; Fax: 221-4222

5 September 1997

LFL Contribution 98

By

Darrel E. Snyder

LARVAL FISH LABORATORY
Department of Fishery & Wildlife Biology
Colorado State University
Fort Collins, Colorado 80523

Telephone: (970) 491-5295, Fax: 491-5091

Effects of the Fish Anesthetic Tricaine on Larval and Early Juvenile Razorback Sucker, *Xyrauchen texanus*

Final Report

Submitted to

Edmund J. Wick
Cooperative Parks Study Unit, National Park Service, USDI
1201 Oakridge Drive, Suite 250, Rm 49, Fort Collins, Colorado 80525

By

Darrel E. Snyder
Larval Fish Laboratory, Colorado State University
Fort Collins, Colorado 80523

5 September 1997

Abstract

Field identification and handling of live fish larvae often requires use of an anesthetic to induce temporary paralysis. To assess the effectiveness of aqueous solutions of the anesthetic tricaine (Finquel™) for rapid but safe immobilization of larval and early juvenile razorback sucker, I tested lab-reared protolarvae at 0 (controls), 50, 100, and 200 mg/l, mesolarvae at 0, 6.25, 12.5, 25, 50, 100, 200, 400 and 800 mg/l, and recently-transformed juveniles at 0, 50, 100, 200 and 400 mg/l. For each of three trials per treatment, I recorded times to loss of: equilibrium, reflex response (full immobilization except for breathing), and breathing motions. After full loss of equilibrium by all eight or ten fish in a trial (or 5 min if fish remained upright), I retained half in anesthetic for an additional 5 min and the other half for 15 min. I then transferred the fish to freshwater, recorded recovery times, and monitored survival for 4 d. For this investigation, I considered optimal immobilization and recovery times to be less than 1 and 10 min, respectively. Tricaine concentrations of 50 mg/l or less failed to immobilize completely all fish in any treatment and 100 mg/l was borderline for protolarvae. Only the 200 mg/l treatment for protolarvae met the optimal criteria. Concentrations of 100 and 200 mg/l best approached the criteria for mesolarvae; likewise for early juveniles except that 15-min exposures were not safe for juveniles at either concentration, or even for 5 min at 400 mg/l. Except for juvenile exposures much over 5 min, median concentrations of about 150 mg/l are most likely to approximate the goal for larvae and early juveniles and are therefore recommended. For early juveniles, concentrations somewhat less than 100 mg/l may immobilize the fish without loss of breathing and thereby allow exposures of 15 min or longer, but immobilization will likely require a few minutes.

For mesolarvae, and probably for protolarvae, concentrations of 400 and 800 mg/l also were safe, even for 15-min exposures, and resulted in almost instantaneous immobilization but much longer recovery times. As a corollary to the latter results and other observations, high doses of tricaine, at least up to 1,600 mg/l, are ineffective for euthanasia of razorback sucker and probably other fish larvae not yet relying heavily on gills for respiration.

Introduction

For some investigations, biologists need to examine closely and manipulate live fish larvae and juveniles under magnification. A need to do so in the field arose in 1994 when researchers in the Upper Colorado River Basin decided to try to identify and segregate living razorback sucker *Xyrauchen texanus* larvae (and possibly young-of-the-year juveniles) from other fish collected by light traps in Green River (Utah) backwaters. To facilitate such examination with as little stress as possible, the fish must be temporarily paralyzed with an anesthetic. Tricaine was selected for this purpose, but guidelines for its use with catostomid larvae have not been published and reported effective concentrations for larvae and juveniles of other species are highly variable. The objective of this investigation was to determine effective concentrations for rapid but safe immobilization of batches of razorback sucker larvae and early juveniles and their quick recovery after an exposure sufficiently long to allow for identification, separation, and possibly measurement. We assume that concentrations found safe and effective for various life stages of razorback sucker also will be safe and effective for comparable life stages of other catostomid fishes and at least safe for most other taxa. However, in many cases, survival of species other than catostomids would be immaterial since those specimens would likely be fixed and preserved for subsequent laboratory analysis.

The razorback sucker is an endangered species endemic to the larger rivers of the Colorado River Basin in southwestern United States. As part of the recovery effort in the Lower Colorado River Basin, researchers have been collecting wild razorback sucker larvae, rearing them in predator-excluded backwaters, and later releasing them at much larger sizes that have a greater probability of survival (Burke 1985). Some investigators thought the approach was worth trying in the Upper Colorado River Basin as well, especially after light traps set in riverine backwaters were found effective for capture of live razorback sucker larvae in good condition (Muth and Wick 1997). This ability to capture live razorback sucker larvae also expanded possibilities for other in-field investigations including enclosure studies.

However, unlike the lower basin where nearly all sucker larvae collected in mainstem reservoirs (e.g., Lake Mohave) are razorback sucker, upper basin collections usually include other species of very similar appearing suckers and numerous other fish from which the much less abundant razorback sucker larvae need to be segregated. To identify living fish under a microscope, count and measure them, and segregate them for rearing, experiments, or return, the fish larvae need to be immobilized and manipulated with as little stress or harm as possible. This is best accomplished with a general anesthetic.

Tricaine (ethyl m-aminobenzoate methanesulfonate or tricaine methanesulfonate), sold commercially as Finquel™, MS-222™, and Metacaine™, is water soluble and by far the most commonly used fish anesthetic and tranquilizer in the United States (Marking and Meyer 1985). As Finquel™, it is the only anesthetic registered with the FDA (U.S. Food and Drug Administration) for use with food fish, specifically those in the families Ictaluridae, Salmonidae, Esocidae, and Percidae (Schnick et al. 1989, Summerfelt and Smith 1990). This suggests that aside from carbon dioxide, which is categorized as GRAS (generally recognized as safe) by the FDA, tricaine is probably among the safer anesthetics available

for use with fish. Accordingly, tricaine was selected for paralytic anesthetization of specimens in field collections likely to include young razorback sucker.

For adult and older juvenile fish of six families, Argent Chemical Laboratories (1987, product package information brochure) summarized data from published literature and reported a range of recommended concentrations for rapid (2-5 min) anesthesia from 80 to 330 mg/l with maximum exposure times of 3 to 28 min and recovery times of 3 to 40 min, depending on the target species (Table 1). Lethal concentration data summarized from the same source suggests that exposure times must be carefully monitored. For salmonids and ictalurids the recommended effective concentrations are actually greater than corresponding 15-min LC_{50}s (lethal concentration, 50%; Table 1). Summerfelt and Smith (1990) provide a relatively comprehensive review of effective concentrations, induction and recovery times, toxicity, and related matters, as well as comparable information for alternative anesthetics.

A review of the published literature revealed no guidelines for use of tricaine with catostomid larvae and few and quite varied reports of effective concentrations for the larvae and early juveniles of other families. For goldfish *Carassius auratus* protolarvae (~5-6 mm TL, total length), and red drum *Sciaenops ocellatus* protolarvae (~2-3 mm TL), Massee et al. (1995) reported 75 and 55 mg/l, respectively, as the lowest effective concentrations of tricaine with 100% survival (see Snyder and Muth 1990 for definitions of developmental intervals of fish). For fountain darter *Etheostoma fonticola* juveniles (21-23 mm TL), Brandt et al. (1983) reported 60 mg/l as the lowest effective concentration of tricaine with 100% survival. At 50 mg/l Brandt et al. found that the darters were only partially immobilized but reported mortalities beginning at 80 mg/l with 0% to 30% survival for

TABLE 1.–Recommended concentrations of Finquel™ (tricaine) for rapid (2-5 min) anesthesia of large juvenile and adult fish with maximum exposure times, expected recovery times, and 15-min and 60-min exposure LC50s (lethal concentration for 50% of test organisms). Data abstracted from Argent (1987).

Family	Concentration (mg/l)	Max. Exposure (min)	Recovery Time (min)	15/60 min LC_{50} (mg/l)
Salmonidae	80-135	4-12	3-19	65/56
Esocidae	150	8-28	8-31	
Cyprinidae	150-200			
Ictaluridae	140-270	4-11	3-24	139/110
Centrarchidae	260-330	3-5	7-11	
Percidae	100-120	7-18	5-40	

specimens exposed to 100 mg/l for 15 to 18 min. For striped bass *Morone saxatilis*, Chapman et al. (1988a) safely used concentrations of 200 to 250 mg/l for 4 to 12-day-old larvae, Chapman et al. (1988b.) used 50 to 67 mg/l for 8 to 40-day-old larvae and juveniles, and Henderson-Arzapalo et al. (1992) used 110 to 123 mg/l for 19 to 71-mm-TL juveniles (~30 to 60-day-old). Summerfelt and Smith (1990) noted that 20 mg/l is commonly used to anesthetize fingerling Pacific salmon and that Barton and Peter (1982) had reported short exposure to 50 mg/l sufficient for immobilization of fingerling (early juvenile) rainbow trout.

With so little and varied data on effective concentrations of tricaine for early life stages of fish, experiments were needed to determine optimal concentrations for razorback sucker larvae and early juveniles. Accordingly I designed a series of tests to assess the observable effects of a wide range of tricaine concentrations and determine the best range of tricaine concentrations for rapid immobilization (less than a minute if possible), short recovery times (preferably less than 10 min–as suggested by Summerfelt and Smith 1990) for 5 and 15-min exposures beyond loss of equilibrium (estimated time range needed to examine fish), and good survival (100%) after exposure based on a monitoring period of 96 h.

Methods

Fertilized eggs of razorback sucker were obtained in March 1994 from Dexter National Fish Hatchery and Technology Center (New Mexico) and in early May from Ouray National Fish Hatchery (Utah). The eggs were further incubated and hatched and the larvae reared for testing in the indoor facilities of the Colorado State University Aquatic Research Laboratory. Early larvae were fed brine shrimp nauplii twice daily and maintained in culture trays in a trough with aerated flow-through well water. Later larvae and early juveniles were reared in troughs and fed dry food. Water temperature was about 18 to 19°C. Only fish that appeared healthy and active were used in the experiments. I tested 9 to 11-mm TL protolarvae shortly after swimup with moderate to little yolk, 12 to 14-mm flexion to postflexion mesolarvae, and approximately 28 to 35-mm early juveniles (Figure 1; see Snyder and Muth 1990 for definitions of these developmental intervals). Most razorback sucker larvae collected by light traps in the Green River had been 11 to 14-mm mesolarvae. Accordingly, I conducted my most comprehensive set of experiments with mesolarvae.

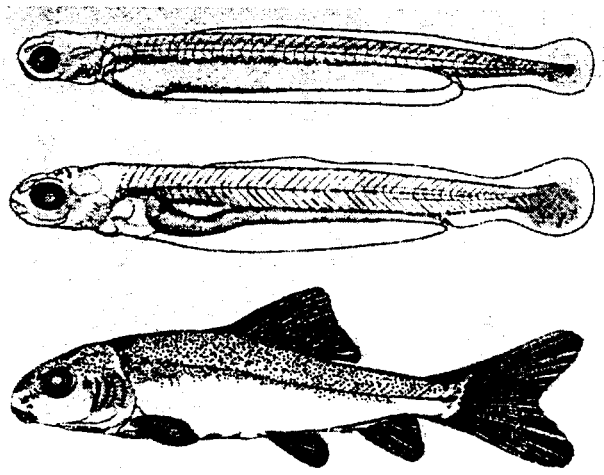

FIGURE 1.–Illustrations of razorback sucker representative of developmental intervals of tested: protolarvae with and without yolk, ~9-11 mm total length (top); flexion to postflexion mesolarvae, ~12-14 mm (middle); early juveniles, 28-35 mm (bottom). Drawings by C. Lynn Bjork, originally published in Snyder and Muth (1990).

Prior to each set of experiments, I prepared anesthetic solutions at twice the ultimate test concentrations with well water (same source as that used for rearing the fish). When diluted with an equal amount of well water, these stock solutions of tricaine (Finquel™) yielded a series of eight progressively halved test concentrations from 800 to 6.25 mg/l for trials with mesolarvae. Subsequent trials with protolarvae and early juveniles were conducted with a subset of these concentrations–50, 100, and 200 mg/l; also 400 mg/l for juveniles (I did not have enough protolarvae to test at this concentration). Control trials for each developmental interval were conducted at 0 mg/l tricaine.

The well water used for rearing and experiments had a specific conductivity of about 600 to 720 µS/cm, total hardness of 360 to 380 mg/l, total alkalinity of 250 to 260 mg/l and pH of 7.9 to 8.6 (based on samples allowed to set at least 1 d before analysis–D. W. Beyers, personal communication). Since use of this water for stock solutions of tricaine resulted in a pH of 8.0 to 8.1 for concentrations of 12.5 to 400 mg/l, 7.6 for 800 mg/l, and 5.1 for 1,600 mg/l, additional buffering to avoid the adverse effects of acidic tricaine solutions (Summerfelt and Smith 1990) was unnecessary. During the experiments, water temperature in test dishes often rose from 18 to 19°C (rearing-trough water) to 20 to 21°C (room temperature).

For each of three replicate trials per treatment, I transferred batches of 10 mesolarval, 8 protolarval, or 8 juvenile fish to measured amounts of rearing-trough (well) water in culture or petri dishes. I then added an equal volume of the appropriate anesthetic stock solution to yield the desired treatment concentration, swirled the dish to mix, and recorded times to specific responses by the first, one-half, and all fish in the batch. Targeted anesthetic responses were total loss of equilibrium (fish roll over and remain on their sides or backs) and, if occurring within subsequent exposure periods of 5 and 15 min after total loss of equilibrium by all fish in the batch (or failing that, an initial 5-min exposure), loss of reflex reactivity (fish fully immobilized and unresponsive to touch as they are gently moved, turned, and held by forceps for examination under a stereo-microscope) and loss of breathing motions (opercular, branchial, and jaw movements; apnea through medullar collapse). The 5 and 15-min exposure periods after loss of equilibrium approximate the time needed to examine and identify small samples or subsamples of captured fish.

Five min after loss of equilibrium by the entire batch of fish in a trial (or an initial 5-min exposure), I removed half of the fish (i.e., 5 mesolarvae, 4 protolarvae, or 4 juveniles) to a dish with fresh rearing-trough water, examined them for signs of life (e.g., a beating heart), and recorded times to recovery of breathing motions, equilibrium, and apparently normal swimming and response behaviors by one-half and all specimens. Fifteen min after loss of equilibrium, I did likewise with the remaining fish for each trial. For mesolarvae, I ran an additional set of 100 mg/l trials with extended 30 and 60-min exposures. Times were recorded to the nearest 1, 5, or 10-s interval up to 1.5 min, 0.5-min interval between 1.5 and 5 min, and 0.5 or 1-min interval thereafter (sometimes 5-min intervals for times of about an hour or longer). Controls were treated similar to test treatments but with less physical handling during examination (fish would not remain still and could not be easily positioned or held on their sides or backs).

After recovery, I maintained each exposure group of each batch of fish in appropriately

labeled holding containers (clear plastic cups) and monitored survival twice daily for 96 hours. The holding containers were arranged in a trough with a shallow bath of flowing well water to maintain temperature in the containers at about 18 to 19°C. Recovered fish were fed twice daily and most of the water in each holding container was renewed daily. Fish that failed to recover in one hour were examined for signs of life (e.g., heart beat) and, if present, were checked periodically during the next few hours for recovery; if still alive but not recovered after a few hours, these fish were transferred to an appropriately labeled holding container for continued monitored along with fish that had recovered.

At the conclusion of each set of experiments, all fish were euthanized, then fixed and preserved in 10% formalin. After several days they were transferred to 3% buffered formalin for continued preservation as voucher and future reference material. Fish were euthanized by placing them in high (presumably lethal) concentrations of tricaine (800 mg/l, 1,600 mg/l, or higher unmeasured concentrations) for 1 to 5 min before transferring them to 10% formalin; fish not killed as expected by overdose of tricaine were killed by the formalin.

Results

Results are summarized in Tables 2 and 3 with details for individual trials and observations in Appendices 1 to 4. Times recorded below are the maximum for individual trials, that is, the time by which all fish in a particular trial succumbed to a specific anesthetic response or state of recovery. In most cases, times for some individuals were very much shorter (Appendices 1-4).

Mesolarvae, 12 to 14 mm TL

For mesolarvae (Table 2, Appendices 1 and 2), tricaine was ineffective at concentrations below 50 mg/l. Ten-min exposures at 6.25 mg/l (5 min initial exposure plus 5 min additional exposure) had no notable effect, except on one specimen which appeared a bit lethargic and occasionally lay on its side. Similar 10-min exposures at 12.5 mg/l specimens seemed to irritate the larvae as well as make a few listless. At 25 mg/l, some specimens began to momentarily and sometimes repeatedly lose equilibrium; others just seemed sluggish. For partially anesthetized fish, recovery times were similar or actually greater for fish exposed 5 min beyond the initial 5-min exposure than for fish exposed for an additional 15 min. Recovery of equilibrium by all affected specimens per trial took 0.3 to 1.3 min (range among trials, mean given in Table 2) for fish exposed an additional 5 min and 0.5 to 0.8 min for those exposed an additional 15 min. Comparable recovery of normal swimming and behavioral responses took 1.5 to 3.5 min and 1.0 to 1.5 min respectively.

At 50 mg/l, it took 2.0 to 2.5 min for all mesolarvae to lose equilibrium (and stay that way), but 5 min later a few still reacted to touch with a spurt of upside down swimming. All fish continued normal breathing motions throughout exposure. The subsets of fish removed to fresh water 5 min after all had lost equilibrium recovered equilibrium in 2.5 to 4.0 min and normal swimming and behavior in about 4.5 to 7 min. Subsets removed to fresh water 15 min after loss of equilibrium recovered equilibrium in 3.5 to 5.0 min and normal swimming and behavior in 6 to 7 min.

At 100 mg/l, I ran two treatments, one with the usual 5 and 15 min exposure periods

TABLE 2.–Summary of results for experiments to determine safe and effective concentrations of tricaine for anesthetization of razorback sucker mesolarvae, about 12-14 mm total length. Treatment times are the means of times by which all specimens in individual trials fully reached the specified end points. Partial responses or full responses by fewer than all specimens are indicated by "p" and not applicable observations by "na." The second 100 mg/l treatment was run for extended-exposure periods of 30 and 60 min. See text and Appendices 1 and 2 for details.

	Tricaine Concentrations, mg/l, for Mesolarvae									
Experimental End-Points	0	6.25	12.5	25	50	100	200	400	800	100
Anesthetization										
Mean Minutes to Full Loss of:										
Equilibrium	-	-	-	p	2.3	1.0	0.3	0.0	0.0	1.0
Reflex Reactivity	-	-	-	p	p	2.2	0.4	0.0	0.0	2.8
Breathing Motions	-	-	-	-	-	3.8	0.4	0.0	0.0	3.8
Recovery of Fish Retained in Tricaine										
5 Min After Loss of Equilibrium										**(30 min)**
Mean Minutes to Recovery of:										
Breathing Motions	na	na	na	na	na	2.8	9.2	14.7	22.3	?
Equilibrium	na	na	na	0.7	3.3	5.7	12.0	16.7	23.7	28.8
Normal Swimming/Responses	na	na	na	2.2	5.5	8.2	14.3	20.3	26.0	36.3
15 Min After Loss of Equilibrium										**(60 Min)**
Mean Minutes to Recovery of:							[1]			
Breathing Motions	na			na	na	6.5	17.8	28.3	32.0	p
Equilibrium	na			0.6	4.3	10.0	21.0	31.0	36.2	p
Normal Swimming/Responses	na			1.3	6.3	13.3	26.0	36.3	48.0	p
Survival of Fish Retained in Tricaine										
5 Min After Loss of Equilibrium										**(30 Min)**
Mean Percent Survival to:										
0 h	100	100	100	100	100	100	100	100	100	100
1 h	100	100	100	100	100	100	100	100	100	100
24 h	100	100	100	100	100	100	100	100	100	100
48 h	100	100	100	93	100	100	100	100	100	100
72 h	100	100	100	93	100	100	100	100	100	100
96 h	100	100	100	93	100	100	100	100	100	100
15 Min After Loss of Equilibrium										**(60 Min)**
Mean Percent Survival to:										
0 h	100			100	100	100	100	100	100	100
1 h	100			100	100	100	100	100	100	100
24 h	100			100	100	100	93	100	100	73
48 h	100			100	100	100	93	100	100	58
72 h	100			100	100	100	87	100	100	58
96 h	100			100	100	100	87	100	100	58

[1] Mean times based on two of three trials; solitary specimen in third trial never recovered equilibrium.

TABLE 3.–Summary of results for experiments to determine safe and effective concentrations of tricaine for anesthetization of razorback sucker protolarvae, about 9-11 mm, and early juveniles, about 28-35 mm total length. Treatment times are the means of times by which all specimens in individual trials fully reached the specified end points. Partial responses or full responses by fewer than all specimens are indicated by "p" and not applicable observations by "na." See text and Appendices 3 and 4 for details.

	Tricaine Concentrations, mg/l									
	Protolarvae				Early Juveniles					
Experimental End-Points	0	50	100	200	0	50	100	200	400	
Anesthetization										
Mean Minutes to Full Loss of:										
Equilibrium	-	p	0.7	0.5	-	p	1.7	0.6	0.4	
Reflex Reactivity	-	p	p	0.6	-	p	1.7	0.6	0.4	
Breathing Motions	-	-	2.6	0.6	-	-	p	3.0	1.1	
Recovery of Fish Retained in Tricaine										
5 Min After Loss of Equilibrium										
Mean Minutes to Recovery of:										
Breathing Motions		na	1.7	3.5			1.8	11.0	p	
Equilibrium		2.7	3.7	6.3			3.7	11.5	p	
Normal Swimming/Responses		4.7	5.7	9.7			5.8	13.2	p	
15 Min After Loss of Equilibrium										
Mean Minutes to Recovery of:										
Breathing Motions	na	na	2.0	4.3	na	na	p	p	-	
Equilibrium	na	2.3	4.1	6.6	na	1.0	p	p	-	
Normal Swimming/Responses	na	4.0	5.5	8.7	na	2.5	p	p	-	
Survival of Fish Retained in Tricaine										
5 Min After Loss of Equilibrium										
Mean Percent Survival to:										
0 h		100	100	100			100	100	100	
1 h		100	100	100			100	100	8	
24 h		100	100	100			100	100	8	
48 h		100	100	100			100	100	8	
72 h		100	100	100			100	100	8	
96 h		100	100	92			100	100	8	
15 Min After Loss of Equilibrium										
Mean Percent Survival to:										
0 h	100	100	100	100	100	100	100	100	100	
1 h	100	100	100	100	100	100	75	100	0	
24 h	100	100	100	100	100	100	75	8	0	
48 h	100	100	100	100	100	100	75	8	0	
72 h	100	100	100	100	100	100	75	8	0	
96 h	100	100	100	100	100	100	75	8	0	

after loss of equilibrium, and the other with extended-exposure periods of 30 and 60 min after loss of equilibrium. Equilibrium was lost by all fish per trial in 0.6 to 1.5 min, reflex reactivity in 2.0 to 4.0 min, and breathing motions in 3.0 to 5.0 min (combined ranges for both treatments, six trials). Batches of fish that were removed to fresh water 5 min after loss of equilibrium recovered breathing motions in 2.0 to 4.0 min, equilibrium in about 4.5 to 7 min, and normal swimming and behavior in 7 to 10 min. Those removed to fresh water 15 min after loss of equilibrium recovered breathing motions in 5 to 8 min, equilibrium in 9 to 11 min, and normal swimming and behavior in 13 to 14 min.

Mesolarvae exposed for 30 min (beyond loss of equilibrium) in two of three 100-mg/l extended-exposure trials recovered breathing motions in less than 21.5 to 25 min, equilibrium in 21.5 to 25 min, and normal swimming and behavior in 25 to 29 min. Maximum recovery times were similar for fish exposed for 60 min in one of the same two trials; in the other, one specimen was accidentally damaged and killed and another specimen exhibited only infrequent movement of branchial apparatus and pectoral fins, sometimes with a brief burst of swimming (quivering) on its back, and never recovered. All fish in these two trials except the accidentally killed and latter specimen survived and appeared normal four days later. Recovery times for fish exposed for 30 min in the third trial were greater for some specimens and nearly twice as long for one (40 min for recovery of breathing and equilibrium and 55 min for recovery of normal swimming and responses), but all survived. In contrast, none of the third-trial fish exposed for 60 min recovered breathing or body movements, although their hearts were beating strongly upon transfer to fresh water; all died within 24 h except one which died the next day. I have no explanation for what happened to fish in this third trial, but suspect a mistaken test concentration greater than 100 mg/l.

At 200 mg/l, all fish lost equilibrium in 0.2 to 0.4 min, and both reflex reactivity and breathing motions almost simultaneously in 0.2 to 0.6 min. Fish removed to fresh water 5 min after loss of equilibrium recovered breathing motions in 7.5 to 10.5 min., equilibrium in 9 to 13.5 min., and normal swimming and behavior in 12 to 16 min. All but one fish removed to fresh water 15 min after loss of equilibrium recovered breathing motions in 9.5 to 26 min, equilibrium in 12 to 30 min, and normal swimming and behavior in 14 to 38 min. The single exception recovered breathing in about 2.5 h but it was abnormally slow and for at least the next couple hours the fish remained on its back but occasionally twitched or swam (on its back)–it died within 17 hours after exposure. A second 15-min-exposure specimen in the same trial, one which appeared to recover fully, died between 48 and 72 h after exposure.

At 400 and 800 mg/l, all fish lost equilibrium, reflex reactivity, and breathing motions almost immediately. Fish in the 400 mg/l treatment that were removed to fresh water after 5-min exposures recovered breathing motions in 13 to 17 min, equilibrium in 14 to 20 min, and normal swimming and behavior in 18 to 24 min. Fish removed to fresh water after 15-min exposures recovered breathing motions in 16 to 45 min, equilibrium in 19 to 46 min, and normal behavior in 21 to 54 min. 800 mg/l fish averaged somewhat longer recovery times–normal swimming and behavior in up to 27 min for fish exposed for 5 min. and up to 60 min for fish exposed for 15 min.

With few exceptions, all mesolarvae survived these trials and appeared in good

condition 96 hours after trial exposure, even for 15 min at 800 mg/l. The exceptions were apparently anomalous and, as noted above, included two specimens in a 200-mg/l, 15-min exposure trial, a single specimen in an extended-exposure 100-mg/l, 60-min exposure trial, and all fish in another of the three 100-mg/l, 60-min exposure trials.

Protolarvae, 9-11 mm TL

At 50 mg/l tricaine, protolarvae only partially succumbed to the anesthetic (Table 3, Appendix 3). Even after 10 min (the initial 5 min limit for 100% loss of equilibrium plus 5 min subsequent exposure), some fish in all trials repeatedly turned upright and swam momentarily before again losing equilibrium. Similarly, loss of reactivity was only temporary. Some fish ceased to respond to touch, at least for a while, but all sporadically twitched and flexed throughout the exposure period. All fish continued breathing motions. During recovery, protolarvae lapsed back to loss of equilibrium ("resting" on their sides on the bottom of the dish) a few times before fully recovering, but full-batch recovery was generally faster than for mesolarvae exposed to 50 mg/l.

For most trials at 100 mg/l, protolarvae fully lost equilibrium and breathing motions in less time than mesolarvae with respective ranges of 0.7 to 0.8 min and 2.2 to 3.0 min, but loss of reflex reactivity (mobility) was incomplete. For at least half the fish in each trial, reflex reactivity was lost within 2 min. However, some of the remaining fish in each trial continued to occasionally flex and twitch even after they ceased responding to touch and breathing. Full-batch recovery times for 5 and 15-min exposures after loss of equilibrium were similar, ranging from 1.3 to 2.5 min for recovery of breathing motions, 3.3 to 4.3 min for recovery of equilibrium, and 5.0 to 6.5 min for recovery of normal swimming and behavioral responses. These recovery times were notably less than for mesolarvae, especially those exposed to tricaine for 15-min after loss of equilibrium.

At 200 mg/l, protolarvae required just slightly more time than most mesolarvae to succumb to the anesthetic–0.3 to 0.6 min for loss of equilibrium, and 0.5 to 0.7 min for nearly simultaneous loss of reactivity and breathing motions. Full-batch recovery times for protolarvae subjected to 5 and 15-min exposures were again similar with overall ranges of 3.0 to 4.5 min for recovery of breathing motions, 6 to 7 min for recovery of equilibrium, and 8 to 10 min for recovery of normal behavioral. As for trials at lesser concentrations, these recovery times were notably less than for mesolarvae, 32 to 62% less for protolarvae subjected to 5-min exposures and 64 to 76% less for those subjected to 15-min exposures.

All but one specimen (200-mg/l, 5-min exposure) survived and appeared to be in good condition 96 hr after exposure. The exception died during the fourth day of monitoring, probably of causes not directly related to anesthetic treatment.

Juveniles, 28-35 mm TL

Relative to protolarvae and mesolarvae, early juveniles tested at comparable tricaine concentrations (Table 3, Appendix 4) were generally less sensitive to the anesthetic; that is, they required more time to succumb to its effects. Unlike protolarvae and mesolarvae, loss of reflex reactivity occurred simultaneously with, or very shortly after, loss of equilibrium. Recovery times for batches subjected to 50 mg/l for 15-min and 100 mg/l for 5 min generally took notably less time than

for mesolarvae (same times as for protolarvae at 100 mg/l for 5 min). In contrast, recovery times for juvenile fish exposed to 200 mg/l for 5 min after loss of equilibrium and for solitary survivors exposed to 200 mg/l for 15 min and 400 mg/l for 5 min were similar to those for mesolarvae but greater than for protolarvae. Unlike larvae, survival beyond the first hour after exposure was a problem for fish subjected to 5-min exposures at 400 mg/l and 15-min exposures at concentrations as low as 100 mg/l.

At 50 mg/l, continuous anesthetic responses (beyond sedation) were not observed for all early juveniles in any of the three trials. Upon transfer to tricaine solution, fish seemed to calm a bit and did not swim as much as before. After 1 to 2 min, some became a bit tipsy and were not very responsive to touch. Beyond a few minutes, some fish repeatedly lost equilibrium for a brief period of time then regained it and swam again. Rather than remove half the fish to fresh water 5 min after an initial 5 min exposure (10 min total exposure), as for most other treatments, I maintained all fish in the anesthetic for 15 min beyond the initial 5 min (20 min total), but divided the fish upon transfer to fresh water to avoid crowding.

Throughout this 15-min exposure period, one quarter to one half of the fish in each trial were still upright and swimming at any one time. Fish that fully lost equilibrium while in the anesthetic also failed to respond to touch or prodding. Even those that repeatedly regained equilibrium were not very responsive to touch and could be easily grasped by flexible forceps. All fish continued breathing. After transfer to fresh water, all fish fully recovered equilibrium in about 1 min and normal swimming and behavioral responses in 2.0 to 3.0 min. All fish survived and appeared in good condition 4 d later, including one which was injured during handling and initially appeared pale with hemorrhage between the eyes.

At 100 mg/l, early juveniles succumbed simultaneously (or nearly so) to loss of both equilibrium and reflex reactivity in 1.3 to 2.0 min. Upon transfer to anesthetic, breathing motions increased rapidly then gradually became shallow, almost to a quiver. After about 3 min, some specimens repeatedly stopped breathing temporarily then resumed breathing motions with mouths and gill covers quivering. Breathing never completely ceased for some specimens in any one trial. Upon transfer to fresh water, most fish exposed for 5 min beyond loss of equilibrium recovered normal breathing motions within 15 s, but one specimen took 4.7 min. Full recovery of equilibrium was achieved in 2.0 to 5.0 minutes and normal swimming and behavior in 4.0 to 7.5 min. All fish were living and appeared in good condition 96 h later.

For fish exposed for 15 min beyond loss of equilibrium, two specimens in one trial and one in another which had fully ceased to breathe (but for which hearts continued to beat), failed to recover breathing motions and died within an hour of exposure. Among the remaining juveniles, all of which survived through 96 h, breathing motions were regained in 5.5 to 8 min, equilibrium in 6.7 to 10.5 min, and normal behavior in 8 to 11.7 min.

At 200 mg/l, early juveniles succumbed to equilibrium and reflex reactivity in 0.6 to 0.7 min. Breathing ceased within 2.0 to 2.2 minutes, except for some small mouth movements and an occasional flare of the gills which also ceased within the next minute (3 min total). Upon transfer to fresh water, batches of fish exposed for 5 min appeared to have normal heart beats and recovered breathing motions in 10.5 to 11.5 min, equilibrium in 10.5 to 12 min, and normal

swimming and behavioral responses in 13 to 13.5 min. All fish survived and appeared in good condition 96 h later.

Juveniles exposed to 200 mg/l for 15 min had slow, very shallow heart beats which were still detectable in most specimens 1 h after removal to freshwater. All but one fish in one trial died within the next hour. The solitary survivor recovered equilibrium in about 30 min and normal behavior in about 38 min and appeared normal 96 h later.

At 400 mg/l, early juveniles lost equilibrium and reflex reactivity in 0.3 to 0.7 min and ceased breathing in 1.0 to 1.3 min. For 5-min and 15-min exposures, all but one 5-min exposure fish failed to recover breathing motions and died within an hour of transfer to fresh water. The lone survivor recovered equilibrium in about 20 min and normal swimming and responses in about 25 min.

Observations on Euthanasia of Larvae

At the conclusion of each set of experiments, surviving fish were euthanized, fixed, and preserved. Prior to these experiments, we expected 1 to 5-min (sometimes longer) exposures to an overdose of tricaine (800 mg/l or greater) to kill these fish humanely (quickly without pain) or keep them anesthetized as they were killed by submersion in 10% formalin. However, shortly after transfer to formalin, and regardless of the anesthetic concentrations up to 1,600 mg/l, the larvae appeared to quickly awaken in formalin then contort themselves for a few seconds before finally dying.

Discussion and Conclusions

At the onset of these experiments, I hoped to determine suitable concentrations of tricaine for anesthetic immobilization (temporary paralysis, loss of reflex reactivity) in less than a minute and recovery to normal swimming and behavioral responses in less than 10 min for 5 to 15-min exposures beyond loss of equilibrium. For tested life stages and concentrations, these criteria were met only by protolarvae tested at 200 mg/l (Table 3). At 100 mg/l, recovery times for protolarvae were less much less than 10 min, but immobilization was not entirely achieved. Protolarvae tested at concentrations between 150 and 200 mg/l (and perhaps somewhat less) would probably have also met these criteria.

For mesolarvae, trials at 100 mg/l averaged less than 10 min for recovery after 5-min exposures, but more than twice the desired time for immobilization (2.2 min) and about a third longer than desired for recovery after a 15-min exposure (Table 2). In 200 mg/l trials, immobilization of mesolarvae occurred well within the 1 min criterium (0.4 min) but recover times were 1.4 and 2.6 times longer than the maximum time desired for 5 and 15-min exposures, respectively. Concentrations around 150 mg/l might have met or come very close to the desired criteria for both immobilization and recovery after 5-min exposures.

For field conditions approximating the water chemistry and temperatures of these experiments (e.g., those in the Upper Colorado River Basin in late spring and early summer), a tricaine concentration of about 150 mg/l is recommended for collections including both razorback sucker protolarvae and mesolarvae. However, optimal concentrations are likely to vary with water chemistry and temperature (Summerfelt and Smith 1990). Accordingly, some on-site experimentation may be necessary to verify or determine the specific concentration optimal for each situation.

Concentrations much greater than 200 mg/l (e.g., 400 to 800 mg/l) will also safely and

almost immediately immobilize mesolarvae (Table 2), and presumably protolarvae, but recovery times will be much longer. However, it might be possible and advantageous to instantaneously immobilize larvae at such high concentrations, remove them to freshwater after a couple minutes and have sufficient time to complete examination in the fresh water before the larvae begin to respond to handling.

At 100 mg/l, mesolarvae may be safely retained in the anesthetic for at least 30 min, but recovery times also will be much longer (Table 2). Even longer exposures may be safe, but further testing is recommended since my results for 60-min exposures at 100 mg/l are mixed and questionable (Appendix 2). Extended-exposure trials were not conducted at higher concentrations or with protolarvae. However, 30-min exposures at 100 mg/l are probably safe for protolarvae as well as mesolarvae.

Except for 15-min exposures, which were not safe for juveniles subjected to tricaine concentrations of 100 mg/l or greater, results for early juveniles at both 100 and 200 mg/l were similar to those for mesolarvae (Table 3). At 100 mg/l, the average 5-min exposure time was well within the desired 10-min limit, but average time to immobilization (1.7 min) was nearly twice as long as desired. At 200 mg/l, immobilization times for early juveniles met the optimal criterium of less than 1 min but now recovery times for 5-min exposures were greater than desired. Limiting exposure to 5 min (perhaps somewhat longer, but less than 15 min), a concentration of about 150 mg/l might, as for larvae, meet the desired criteria for both induction and recovery times. If longer exposure periods (e.g., 15 min) are needed for juveniles and longer than desired immobilization times are acceptable, tricaine concentrations somewhat less than 100 mg/l (perhaps as low as 75 mg/l) might be effective.

Survival for early juveniles was poor to nil at concentrations much greater than 200 mg/l (e.g., 400 mg/l even for 5-min exposures) and for longer exposures at concentrations as low as 100 mg/l (Table 3). Probable cause of death was asphyxiation among those fish that ceased to breathe.

Although breathing ceased within a few minutes for larvae in tricaine solutions of at least 100 mg/l, survival for these earlier life stages was not a problem, even for 15-min exposures at 800 mg/l (Tables 2 and 3). The gills of larvae are developing and much respiration still occurs passively directly through the skin. Consequently, loss of breathing motions by larvae does not result in asphyxiation.

A corollary to these observations is that tricaine concentrations up to at least 800 mg/l (and probably 1,600 mg/l based on my undocumented observations) are not useful for euthanasia of razorback sucker protolarvae or mesolarvae. For juveniles, and probably metalarvae, the mechanism of euthanasia by overdose of anesthetic is probably quiet asphyxiation. But for larvae not yet relying largely on gills for respiration, overdose of tricaine does not result in asphyxiation or quick death by other means. Upon transfer to formalin fixative, anesthetized larvae appeared to quickly awaken and physically react to the formalin as do larvae that are not anesthetized. Indeed, it may be just as humane to quickly kill the protolarvae and mesolarvae by direct transfer to 5 to 10% formalin. A mixture of tricaine in high concentration with formalin might be effective for euthanasia, but tests of such need to be run. The matter of larval (and even older) fish euthanasia deserves thorough investigation for establishment of meaningful procedural guidelines.

Potential alternatives to use of tricaine as an anesthetic include subjecting the larvae (or

early juveniles) to cold temperatures, transfer to solutions with high concentrations of CO_2, and other chemical anesthetics (Summerfelt and Smith 1990). Of these, CO_2 is probably the most practical alternative for investigation in a comparable set of future experiments. Anecdotal reports of its use for fish larvae suggest that it may be just as effective as tricaine. It's potential for purposes of euthanasia might also be worthy of investigation. Application of cold temperatures might be more difficult to control and use in the field. However, the possibility of combining cold and CO_2, perhaps by adding very small pieces of dry ice to a dish with fish larvae is intriguing.

Acknowledgments

This investigation was funded by the National Park Service, U.S. Department of the Interior, Project GLCA-R94-0001 under Cooperative Agreement CA1268-2-9004 (Work Order CSU-48). Research was conducted under Federal Endangered Species permit PRT-704930 (held by F. Pfeifer, U.S. Fish and Wildlife Service [verify]) and with the approval of the Colorado State University Animal Care and Use Committee (Protocol 94-066A-01). Fertilized razorback sucker eggs were provided by R. Hamman and B. Jensen of Dexter National Fish Hatchery and Technology Center (New Mexico) and by T. Pruitt of Ouray National Fish Hatchery (Utah). Fish were reared and experiments conducted in the Aquatic Research Laboratory, indoor wet lab facilities of the Department of Fishery and Wildlife Biology, Colorado State University, managed by Daniel W. Beyers. D. L. Miller, C. L. Bjork, X. Yu, and S. M. Meismer, helped rear the fish. C. L. Bjork drew the fish illustrated in Figure 1; she also measured specimens for this investigation. E. J. Wick, D. L. Miller, and M. B. Snyder reviewed or proofed drafts of the report.

Literature Cited

Argent Chemical Laboratories. 1987. Finquel® (MS-222). Product information brochure (revised 10/87), Argent Chemical Laboratories Inc., Redmond, WA.

Barton, B. A., and R. E. Peter. 1982. Plasma cortisol stress response in fingerling rainbow trout, *Salmo gairdneri*, Richardson, to various transport conditions, anaesthesia, and cold shock. Journal of Fish Biology 20:39-51.

Brandt, T. M., K. G. Graves, C. S. Berkhouse, T. P. Simon, and B. G. Whitehouse. 1993. Laboratory spawning and rearing of the endangered fountain darter. The Progressive Fish-Culturist 55:149-156.

Burke, T. 1995. Rearing wild razorback sucker larvae in lake-side backwaters, Lake Mohave, Arizona/Nevada (Abstract). Proceedings of the Desert Fishes Council 26:35-36.

Chapman, D. C., W. A. Hubert, and U. T. Jackson. 1988a. Influence of access to air and of salinity on gas bladder inflation in striped bass. The Progressive Fish-Culturist 50:23-27.

Chapman, D. C., U. T. Jackson, and W. A. Hubert. 1988b. Method for separating normal striped bass larvae from those with uninflated gas bladders. The Progressive Fish-Culturist 50:166-169.

Henderson-Arzapalo, A., C. Lemm, J. Hawkinson, and P. Keyes. 1992. Tricaine used to separate phase-I striped bass with uninflated gas bladders from normal fish. The Progressive Fish-Culturist 54:133-135.

Marking, L. L., and F. P. Meyer. 1985. Are better anesthetics needed in fisheries? Fisheries 10(6):2-5.

Massee, K. C., M. B .Rust, R. W. Hardy, and R. R. Stickney. 1995. The effectiveness of tricaine, quinaldine sulfate and metomidate as anesthetics for larval fish. Aquaculture 134:351-359.

Muth, R. T., and E. J. Wick. 1997. Field studies on larval razorback sucker in Canyonlands National Park and Glen Canyon National Recreation Area, 1993-1995. Final Report of the Colorado State University Larval Fish Laboratory and U.S. National Park Service, Fort Collins, Colorado.

Schnick, R. A., F. P. Meyer, and D. L. Gray. 1989. A guide to approved chemicals in fish production and fishery resource management. U.S. Fish and Wildlife Service National Fisheries Research Laboratory, La Cross, Wisconsin, and University of Arkansas Cooperative Extension Service MP241-11M-1-86, Little Rock.

Snyder, D. E., and R. T. Muth. 1990. Description and identification of razorback, flannelmouth, white, Utah, bluehead, and mountain sucker larvae and early juveniles. Colorado Division of Wildlife Technical Publication 38.

Summerfelt, R. C., and L. S. Smith. 1990. Anesthesia, surgery, and related techniques. Pages 213-272 *in* C. B. Schreck and P. B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

APPENDIX 1.–Results of individual trials to determine safe and effective concentrations of tricaine for anesthetization of razorback sucker mesolarvae, 12-14 mm TL, 0 (control)-100 mg/l. Trials were conducted on 4 April 1994; stock solutions of tricaine were prepared on 2 April. Abbreviation "na" indicates the pertinent observation was not applicable.

Treatment–tricaine concentration (mg/l):	0	6.25	12.5	25				50				100			
Replicate:	1	1[b]	1[b]	1	2	3	$\bar{x}$	1	2	3	$\bar{x}$	1	2	3	$\bar{x}$
Anesthetic induction times (min, 5-min limit; N=10) for:															
Full loss of equilibrium (on side or back)															
First fish:	-	-	-[c]	-[d]	-[d]	-[d]	-[d]	0.1	0.1	0.3	0.2	0.3	0.3	0.3	0.3
50%:	-	-	-[c]	-[d]	-[d]	-[d]	-[d]	1.3	1.5	1.5	1.4	0.4	0.5	0.4	0.4
100%:	-	-	-	-	-	-	-	2.0	2.5	2.5	2.3	0.9	1.5	0.6	1.0
Full loss of reflex reactivity (mobility)															
First fish:	-	-	-	-[d]	-[d]	-[d]	-[d]	1.0	1.5	1.0	1.2	?[f]	?[f]	?[f]	?[f]
50%:	-	-	-	-	-	-	-	2.0	2.5	2.5	2.3	1.0	1.0	1.0	1.0
100%:	-	-	-	-	-	-	-	-[e]	-[e]	-[e]	-[e]	2.0	2.5	2.0	2.2
Full loss of breathing motions (jaw movement)															
First fish:	-	-	-	-	-	-	-	-	-	-	-	?[f]	?[f]	?[f]	?[f]
50%:	-	-	-	-	-	-	-	-	-	-	-	?[f]	3.0	2.0	2.5
100%:	-	-	-	-	-	-	-	-	-	-	-	?[f]	4.0	3.5	3.8
Recovery times (min, N=5 per exposure period):															
5-min exposure after loss of equilibrium by all fish in trial[a]															
Breathing motions (jaw movement)															
40% (2 of 5):	na	na	na	na	na	na	na	na	na	na	na	1.5	2.0	1.5	1.7
100%:	na	na	na	na	na	na	na	na	na	na	na	2.0	4.0	2.5	2.8
Equilibrium re-established (upright)															
40% (2 of 5):	na	na	na	na	na	na	na	2.5	1.5	1.5	1.8	3.5	4.5	4.0	4.0
100%:	na	na	na	0.3	0.5	1.3	0.7	4.0	3.5	2.5	3.3	4.5	7.0	5.5	5.7
Normal swimming and behavioral responses															
40% (2 of 5):	na	na	na	na	na	na	na	4.5	3.5	3.0	3.7	5.0	8.0	5.0	6.0
100%:	na	na	na[c]	1.5	1.5	3.5	2.2	7.0	5.0	4.5	5.5	7.0	10.0	7.5	8.2
15-min exposure after loss of equilibrium by all fish in trial[a]															
Breathing motions (jaw movement)															
40% (2 of 5):	na			na	na	na	na	na	na	na	na	4.0	7.0	4.0	5.0
100%:	na			na	na	na	na	na	na	na	na	5.0	8.0	6.5	6.5
Equilibrium re-established (upright)															
40% (2 of 5):	na			na	na	na	na	2.5	3.0	3.0	2.8	5.5	8.5	6.0	6.7
100%:	na			0.5	0.6	0.8	0.6	3.5	4.5	5.0	4.3	9.0	11	10	10.0
Normal swimming and behavioral responses															
40% (2 of 5):	na			na	na	na	na	4.0	4.0	4.0	4.0	8.5	12	8.0	9.9
100%:	na			1	1.5	1.5	1.3	6.0	7.0	6.0	6.3	13	14	13	13.3
Survival after exposure (%)															
5-min exposure fish															
0 hour:	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1 hour:	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
24 hours:	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
48 hours:	100	100	100	100	100	80	93	100	100	100	100	100	100	100	100
72 hours:	100	100	100	100	100	80	93	100	100	100	100	100	100	100	100
96 hours:	100	100	100	100	100	80	93	100	100	100	100	100	100	100	100
15-min exposure fish															
0 hour:	100			100	100	100	100	100	100	100	100	100	100	100	100
1 hour:	100			100	100	100	100	100	100	100	100	100	100	100	100
24 hours:	100			100	100	100	100	100	100	100	100	100	100	100	100
48 hours:	100			100	100	100	100	100	100	100	100	100	100	100	100
72 hours:	100			100	100	100	100	100	100	100	100	100	100	100	100
96 hours:	100			100	100	100	100	100	100	100	100	100	100	100	100

Continued

APPENDIX 1.–Continued.

Table Footnotes:

[a] Or after an initial 5-min exposure if condition not achieved within that time.

[b] Single trial with only a 5-min exposure beyond the initial 5 min (10 min total exposure), N=5.

[c] Fish seemed irritated upon initial exposure, then normal except for a couple which were lethargic but remained upright and responsive; the latter fully recovered within a minute of transfer to fresh water.

[d] Some fish appeared unaffected, some were lethargic, but others (half the fish), after 50-80 s of exposure, repeatedly lost equilibrium for a short time then recovered; within 1-2 min, a few of these fish also were immobilized (lost reflex reactivity) but only temporarily.

[e] By end of 5 and 15 min exposure periods after loss of equilibrium of all fish in these trials, some fish in each trial still occasionally twitched with spurts of upside-down swimming.

[f] Observation(s) missed or time(s) not recorded.

APPENDIX 2.–Results of individual trials to determine safe and effective concentrations of tricaine for anesthetization of razorback sucker mesolarvae, 12-14 mm TL; 200-800 mg/l and 100 mg/l with extended exposures.[a] Standard-exposure trials were conducted on 2-3 April 1994 and extended-exposure trials on 7 April; stock solutions of tricaine were prepared on 2 April.

Treatment–tricaine concentration (mg/l):	200				400				800				100[a]			
Replicate:	1	2	3	$\bar{x}$	1	2	3	$\bar{x}$	1	2	3	$\bar{x}$	1	2	3	$\bar{x}$
Anesthetic induction times (min, 5-min limit; N=10) for:																
Full loss of equilibrium (on side or back)																
First fish:	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.4	0.3	0.4
50%:	0.1	0.1	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.8	0.5	0.7
100%:	0.3	0.2	0.4	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.1	1.0	1.0
Full loss of reflex reactivity (mobility)																
First fish:	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	?[c]	?[c]	?[c]	?[c]
50%:	0.1	0.1	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	?[c]	<1.1	<1.0	<1.1
100%:	0.3	0.2	0.6	0.4	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	2.0	4.0	2.5	2.8
Full loss of breathing motions (jaw movement)																
First fish:	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	?[c]	?[c]	?[c]	?[c]
50%:	0.1	0.1	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	?[c]	<2.0	<1.0	<1.5
100%:	0.3	0.2	0.6	0.4	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	3.0	5.0	3.5	3.8
Recovery times (min, N=5 per exposure period):																
5-min exposure after loss of equilibrium by all fish in trial[b]													(30-min exposure)[a]			
Breathing motions (jaw movement)																
40% (2 of 5):	7.0	7.5	4.5	6.3	12	15	12	13.0	?[c]	15.5	17.5	16.5	10.5	>7[c]	>6[c]	
100%:	10.5	9.5	7.5	9.2	14	17	13	14.7	24	21	22	22.3	<25[d]	40.0	<21[c]	
Equilibrium re-established (upright)																
40% (2 of 5):	8.5	10.0	5.5	8.0	14	18	13	15.0	17	19	18	18.0	19	22	11	17.3
100%:	13.5	13.5	9.0	12.0	16	20	14	16.7	24.5	22.5	24	23.7	25	40	21.5	28.8
Normal swimming and behavioral responses																
40% (2 of 5):	10.5	13	7.5	10.3	16	20	14	16.7	18	22	21	20.3	21	25	15	20.3
100%:	15	16	12	14.3	19	24	18	20.3	27	25	26	26.0	29	55	25	36.3
15-min exposure after loss of equilibrium by all fish in trial[b]													(60-min exposure)[a]			
Breathing motions (jaw movement)	[e]												[g]			
40% (2 of 5):	15	13.5	7.0	11.8	19	20	<16[c]	<18[c]	20	20	24.5	21.5	14	[f]	7.0	
100%:	(21)	26	9.5	17.8	45	24	16	28.3	33	26	37	32.0	22	[f]	(<12)	
Equilibrium re-established (upright)	[e]												[g]			
40% (2 of 5):	18	17	10	15.0	20	24	16	20.0	25.5	23	30	26.2	15	[f]	8.0	
100%:	(22)	30	12	21.0	46	28	19	31.0	36.5	30	42	36.2	24	[f]	(12)	
Normal swimming and behavioral responses	[e]												[g]			
40% (2 of 5):	21	22	12	18.3	25	26	18	23.0	40	29	32	33.7	20	[f]	11	
100%:	(26)	38	14	26.0	54	34	21	36.3	60	32	52	48	30	[f]	(30)	
Survival after exposure (%)																
5-min exposure fish													(30-min exposure)[a]			
0 hour:	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1 hour:	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
24 hours:	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
48 hours:	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
72 hours:	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
96 hours:	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
15-min exposure fish													(60-min exposure)[a]			
0 hour:	100	100	100	100	100	100	100	100	100	100	100	100	100	100[f]	100[g]	100
1 hour:	100	100	100	100	100	100	100	100	100	100	100	100	100	100[f]	100[g]	100
24 hours:	80	100	100	93	100	100	100	100	100	100	100	100	100	20[f]	100[g]	73
48 hours:	80	100	100	93	100	100	100	100	100	100	100	100	100	0	75[g]	58
72 hours:	60	100	100	87	100	100	100	100	100	100	100	100	100	0	75[g]	58
96 hours:	60	100	100	87	100	100	100	100	100	100	100	100	100	0	75[g]	58

Continued

APPENDIX 2.–Continued.

Table Footnotes:

[a] A special treatment at 100 mg/l was run with 30 and 60-min rather than the usual 5 and 15-min exposure periods after loss of equilibrium.

[b] Or after an initial 5-min exposure if condition not achieved within that time.

[c] Observation(s) missed or time(s) not recorded.

[d] Between 22 and 25 min.

[e] One 15-min-exposure specimen recovered very slow breathing motions 2.5 h after exposure, then occasionally twitched and swam on it's back for a few seconds but never recovered equilibrium, and died (heart ceased to beat) 4-17 h after exposure. Times in parentheses are for the remaining four specimens and are not included in computation of treatment means.

[f] All fish in the 60-min-exposure batch of this trial failed to recover breathing motions but their hearts continued to beat strongly; four died (hearts stopped beating) within 24 h and the fifth within 48 h of exposure.

[g] One fish in the 60-min exposure batch of this trial failed to recover breathing motions but it's heart continued to beat strongly; at 24 h after exposure, it's eyes were swollen or outwardly displaced (popped) and between 24 and 48 h it died (heart stopped beating). Data in parentheses represent maximum times for recovery of the remainder of the batch. Another specimen in this batch was accidentally injured after it had recovered equilibrium and began to swim; the specimen was removed, preserved, and excluded from data for recovery of normal swimming and behavioral responses and for survival during the next 96 h.

APPENDIX 3.–Results of individual trials to determine safe and effective concentrations of tricaine for anesthetization of razorback sucker protolarvae, 9-11 mm TL; 0 (control)-200 mg/l. Trials were conducted on 18 May 1994; stock solutions of tricaine were prepared a couple hours before the first trials. Abbreviation "na" indicates the pertinent observation was not applicable.

Treatment–tricaine concentration (mg/l):	0[a]				50				100				200			
Replicate:	1	2	3	$\bar{x}$	1[c]	2	3[c]	$\bar{x}$	1[d]	2	3	$\bar{x}$	1	2	3	$\bar{x}$
Anesthetic induction times (min, 5-min limit; N=8) for:																
Full loss of equilibrium (on side or back)																
First fish:	-	-	-	-	-[e]	-[e]	-[e]	-[e]	0.4	0.4	0.4	0.4	0.3	0.1	0.2	0.2
50%:	-	-	-	-	-[e]	-[e]	-[e]	-[e]	0.5	0.6	0.6	0.6	0.3	0.2	0.3	0.3
100%:	-	-	-	-	-[e]	-[e]	-[e]	-[e]	0.7	0.8	0.7	0.7	0.6	0.3	0.4	0.5
Full loss of reflex reactivity (mobility)									[g]	[g]						
First fish:	-	-	-	-	-[e]	-[e]	-[e]	-[e]	1.0	1.3	?[h]	1.2	?[h]	?[h]	?[h]	?[h]
50%:	-	-	-	-	-[e]	-[e]	-[e]	-[e]	1.7	2.0	<2[h]	1.8	?[h]	?[h]	?[h]	?[h]
100%:	-	-	-	-	-[e]	-[e]	-[e]	-[e]	(4.5)	(>5)	>5[h]		0.6[i]	0.5	0.7	0.6
Full loss of breathing motions (jaw movement)																
First fish:	-	-	-	-	-	-	-	-	1.0	1.3	?[h]	1.2	?[h]	?[h]	?[h]	?[h]
50%:	-	-	-	-	-	-	-	-	1.7	2.0	<2[h]	1.8	?[h]	?[h]	?[h]	?[h]
100%:	-	-	-	-	-	-	-	-	2.7	3.0	2.2	2.6	0.6[i]	0.5	0.7	0.6
Recovery times (min, N=4 per exposure period):																
5-min exposure after loss of equilibrium by all fish in trial[b]																
Breathing motions (jaw movement)																
50% (2 of 4):					na	na	na	na	1.3	1.0	1.0	1.1	2.8	2.7	3.7	3.1
100%:					na	na	na	na	2.5	1.3	1.3	1.7	3.4	3.0	4.2	3.5
Equilibrium re-established (upright)																
50% (2 of 4):					1.5[f]	0.5[f]	0.5[f]	0.8[f]	3.0	2.7	2.2	2.6	5.0	5.0	5.8	5.3
100%:					2.5[f]	4.0[f]	1.5[f]	2.7[f]	3.7	3.3	4.0	3.7	6.0	6.0	6.8	6.3
Normal swimming and behavioral responses																
50% (2 of 4):					3.5	4.3	3.0	3.6	4.5	5.0	4.5	4.7	7.0	7.5	7.5	7.3
100%:					4.5	5.5	4.0	4.7	5.0	5.5	6.5	5.7	9.5	10.0	9.5	9.7
15-min exposure after loss of equilibrium by all fish in trial[b]													[j]	[j]		
Breathing motions (jaw movement)																
50% (2 of 4):	na	na	na	na	na	na	na	na	2.3	1.3	1.5	1.7	?[h]	4.0	3.8	3.9
100%:	na	na	na	na	na	na	na	na	2.5	1.7	1.8	2.0	4.5	4.3	4.0	4.3
Equilibrium re-established (upright)																
50% (2 of 4):	na	na	na	na	1.0[f]	0.5[f]	1.2[f]	0.9[f]	4.0	3.3	3.0	3.4	5.7	5.5	5.5	5.6
100%:	na	na	na	na	1.3[f]	3.5[f]	2.0[f]	2.3[f]	4.3	4.0	4.0	4.1	6.5	7.0	6.3	6.6
Normal swimming and behavioral responses																
50% (2 of 4):	na	na	na	na	2.0	3.0	3.0	2.7	5.8	4.8	4.5	5.0	7.0	7.0	6.8	6.9
100%:	na	na	na	na	3.0	5.0	4.0	4.0	6.3	5.0	5.2	5.5	8.5	9.5	8.0	8.7
Survival after exposure (%)																
5-min exposure fish																
0 hour:					100	100	100	100	100	100	100	100	100	100	100	100
1 hour:					100	100	100	100	100	100	100	100	100	100	100	100
24 hours:					100	100	100	100	100	100	100	100	100	100	100	100
48 hours:					100	100	100	100	100	100	100	100	100	100	100	100
72 hours:					100	100	100	100	100	100	100	100	100	100	100	100
96 hours:					100	100	100	100	100	100	100	100	100	75	100	92
15-min exposure fish																
0 hour:	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1 hour:	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
24 hours:	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
48 hours:	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
72 hours:	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
96 hours:	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Continued

APPENDIX 3.–Continued.

Table Footnotes:

[a] Control–N=4 per trail, all treated as a 15-min exposure.

[b] Or after an initial 5-min exposure if condition not achieved within that time.

[c] N=7 instead of eight, divided to N=4 for 5-min-exposure batch and N=3 for 15-min-exposure batch.

[d] N=6 instead of eight razorback sucker, N=3 for each exposure period. Two of the original eight specimens in this trial were misplaced bonytail *Gila elegans* flexion mesolarvae about 9 mm TL discovered after all had lost equilibrium. The bonytail succumbed to the anesthetic within the times recorded for razorback sucker; the specimen subjected to 5-min exposure recovered breathing motions in 3.0 min, equilibrium in 3.8 min and normal swimming and behavioral responses in 5.5 min whereas the specimen subjected to 15-min exposure recovered in 3.4, 4.7, and 6.5 min, respectively.

[e] All fish experienced loss of equilibrium within about 3 min and loss of mobility (reflex reactivity) withing 4.5 min, but all responses were temporary with fish sporadically twitching and flexing, swimming or quivering upside down, and most occasionally righting themselves and swimming upright momentarily. Some specimens are believed to have fully lost equilibrium but such individuals could not be continually distinguished from those that periodically turned upright.

[f] Recovering fish seemed quite lethargic; many recovered equilibrium momentarily then rolled over again and would lie on their sides on the bottom of the dish, perhaps resting before trying to upright themselves again. Perhaps partial anesthesia is more stressful than quick and full anesthesia.

[g] One or two specimens occasionally twitched even as they stopped responding to touch or prodding (latter times in parentheses) and were therefore not quite fully immobilized.

[h] Observation(s) missed or time(s) not recorded.

[I] Single anomalous specimen continued to quiver despite loss of equilibrium, response to touch, and breathing motions; recorded time for loss of reflex reactivity excludes this specimen which recovered as well as others in the trial.

[j] Batch exposed for only 10 min after loss of equilibrium but treated as a 15-min exposure.

APPENDIX 4.–Results of individual trials to determine safe and effective concentrations of tricaine for anesthetization of razorback sucker juveniles, 28-35 mm TL; 50-200 mg/l (Control, 0 mg/l, results same as for protolarvae, Appendix 3). Trials were conducted and stock solutions prepared on 5 August 1994. Abbreviation "na" indicates the pertinent observation was not applicable.

Treatment–tricaine concentration (mg/l):	50[b]				100				200				400			
Replicate:	1	2	3	x̄	1	2	3	x̄	1	2	3	x̄	1	2	3	x̄
Anesthetic induction times (min, 5-min limit; N=8) for:																
Full loss of equilibrium (on side or back)	[c]	[c]	[c]	[c]												
First fish:	1.8	3.0	1.8	2.2	0.3	0.6	0.4	0.4	0.3	0.3	0.3	0.3	0.1	0.2	0.2	0.1
50%:	5.5	4.3	4.0	4.6	0.8	1.3	0.8	0.9	0.5	0.5	0.6	0.5	0.2	0.2	0.3	0.2
100%:	-	-	-	-	1.8	2.0	1.3	1.7	0.6	0.6	0.7	0.6	0.3	0.3	0.7	0.4
Full loss of reflex reactivity (mobility)	[c]	[c]	[c]	[c]	[f]	[f]	[f]	[f]	[f]	[f]	[f]	[f]	[f]	[f]	[f]	[f]
First fish:	1.8	3.0	1.8	2.2	0.3	0.6	0.4	0.4	0.3	0.3	0.3	0.3	0.1	0.2	0.2	0.1
50%:	5.5	4.3	4.0	4.6	0.8	1.3	0.8	0.9	0.5	0.5	0.6	0.5	0.2	0.2	0.3	0.2
100%:	-	-	-	-	1.8	2.0	1.3	1.7	0.6	0.6	0.7	0.6	0.3	0.3	0.7	0.4
Full loss of breathing motions (jaw movement)					[g]	[g]	[g]	[g]								
First fish:	-	-	-	-	3.4	3.8	3.0	3.4	1.3	1.1	1.3	1.2	0.6	0.4	0.6	0.5
50%:	-	-	-	-	4.6	4.7	4.0	4.4	1.5	1.8	1.7	1.6	0.8	0.6	0.8	0.7
100%:	-	-	-	-	(~5)	(~6)	(6.5)	(5.8)	3.0[j]	3.0[j]	3.0[j]	3.0[j]	1.2	1.0	1.3	1.1
Recovery times (min, N=4 per exposure period):																
5-min exposure after loss of equilibrium by all fish in trial[a]									[k]							
Breathing motions (jaw movement)																
50% (2 of 4):					0.3	0.2	0.5	0.3	8.0[l]	7.0	9.5	8.2				
100%:					0.3	0.3	4.7	1.8	10.5	11.5	11.0	11.0				
Equilibrium re-established (upright)																
50% (2 of 4):					2.5	1.3	1.5	1.8	9.5	9.0	10.0	9.5				
100%:					4.0	2.0	5.0	3.7	12	12	10.5	11.5				
Normal swimming and behavioral responses																
50% (2 of 4):					3.0	2.5	2.5	2.7	11.5	11	11.5	11.3				
100%:					6.0	4.0	7.5	5.8	13	13	13.5	13.2				
15-min exposure after loss of equilibrium by all fish in trial[a]									[m]							
Breathing motions (jaw movement)																
50% (2 of 4):	na	na	na	na	8.0	2.0	2.3	4.1	[n]				[o]			
100%:	na	na	na	na	[h]	5.5	(6)[l]									
Equilibrium re-established (upright)																
50% (2 of 4):	?[d]	?[d]	?[d]	?[d]	10.5	3.5	4.0	6.0	[n]				[o]			
100%:	1.0	1.0	1.0	1.0	[h]	6.7	(8.3)[l]									
Normal swimming and behavioral responses																
50% (2 of 4):	2.0	1.5	2.0	1.8	11.7	6.0	5.5	7.7	[n]				[o]			
100%:	3.0	2.0	2.5	2.3[e]	[h]	8.0	(11)[l]									
Survival after exposure (%)																
5-min exposure fish									[k]							
0 hour:					100	100	100	100	100	100	100	100	100	100	100	100
1 hour:					100	100	100	100	100	100	100	100	0	25[o]	0	8
24 hours:					100	100	100	100	100	100	100	100	0	25[o]	0	8
48 hours:					100	100	100	100	100	100	100	100	0	25[o]	0	8
72 hours:					100	100	100	100	100	100	100	100	0	25[o]	0	8
96 hours:					100	100	100	100	100	100	100	100	0	25[o]	0	8
15-min exposure fish									[m]							
0 hour:	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1 hour:	100	100	100	100	50[h]	100	75[i]	75	100	100	100	100	0	0	0	0
24 hours:	100	100	100	100	50[h]	100	75[i]	75	0	0	25[n]	8	0	0	0	0
48 hours:	100	100	100	100	50[h]	100	75[i]	75	0	0	25[n]	8	0	0	0	0
72 hours:	100	100	100	100	50[h]	100	75[i]	75	0	0	25[n]	8	0	0	0	0
96 hours:	100	100	100	100	50[h]	100	75[i]	75	0	0	25[n]	8	0	0	0	0

Continued

APPENDIX 4.–Continued.

Table Footnotes:

[a] Or after an initial 5-min exposure if condition not achieved within that time.

[b] In each trial (replicate), all eight test specimens were exposed to anesthetic solution for 15-min beyond an initial 5-min exposure (equilibrium was not continuously lost by all fish in any one trial), however the fish were still split into two separate containers to better monitor recovery and avoid crowding.

[c] Upon immersion in anesthetic solution, fish calmed a bit and swam less. After 1-2 min fish seemed lethargic, a bit tipsy and not very responsive to touch, and began to lose equilibrium. When equilibrium was fully lost, the fish were also fully immobilized and did not respond to probing. However, for some fish, loss of equilibrium was only temporary–they repeatedly rolled over onto their sides or backs then righted themselves and swam again. By the end of the 15-min exposure period, two to four fish in each trial either had not lost equilibrium or had returned to an upright position and were swimming again. Even so, these upright fish remained lethargic, not very responsive to touch, and could be easily grasped by forceps.

[d] Observation(s) missed or time(s) not recorded.

[e] One specimen was injured by handling and despite appearing to swim and behavior normally, was pale (melanophores contracted more than other specimens) and had a hemorrhage between these eyes–it was still alive and behaving normally 96 h later.

[f] Reflex reactivity lost with or very shortly after loss of equilibrium.

[g] For all specimens, breathing motions increased rapidly upon introduction to anesthetic solution then gradually became shallow, almost to a quiver, before ceasing at least temporarily. Breathing motions were never continuously lost by some specimens which repeatedly stopped breathing then resumed in atypical form with gill covers and mouths shallowly quivering. For loss by 100% of the specimens, times are in parentheses to indicate times by which all specimens at least temporarily lost breathing motions.

[h] Two 15-min-exposure specimens in replicate 1 failed to recover breathing motions and died (hearts ceased beating) within an hour after exposure. Times in parentheses for trial 3 are for recovery by three of the four specimens.

[i] One 15-min-exposure specimens in trial (replicate) 3 failed to recover breathing motions and died within an hour after exposure. Recovery times in parentheses are for three of the four specimens rather than 100% of the specimens.

[j] For remaining fish between 2 and 3 minutes of addition of the anesthetic, mouth movements were shallow and gills occasionally flared.

[k] Heart beats seemed normal for all 5-min-exposure fish upon transfer to freshwater.

[l] Two largest specimens began sporadic gill movements about 5 min after transfer to freshwater but did not recover normal breathing motions until 3 min later.

[m] Heart beats very shallow and slow (hard to see) upon transfer to freshwater and barely detectable 1 h later; fish in replicate 1 recorded as dead (hearts ceased beating within the next hour (probably same or shortly thereafter for non-surviving fish in replicates 2 and 3, but not recorded as such).

[n] One 15-min-exposure specimen in replicate 3 recovered equilibrium at about 30 min and normal swimming and behavioral responses about 38 min after transfer to freshwater; the fish was still doing well 96 h later (missed time for recovery of breathing motions).

[o] One 5-min-exposure specimen in replicate 2 recovered equilibrium at about 20 min and normal swimming and behavioral responses about 25 min after transfer to fresh water; the fish was still doing well 96 h later (missed time for recovery of breathing motions).