THE EFFECTS OF SUPERSATURATED NITROGEN ON THE ENDANGERED FOUNTAIN DARTER AND THE SAN MARCOS SALAMANDER

THESIS

Presented to the Graduate Council of

Southwest Texas State University

in Partial Fulfillment of

the Requirements

For the Degree of

Master of SCIENCE

By

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San Marcos, Texas

May 2000

ACKNOWLEDGMENTS

Special thanks are in order to the U.S. Fish and Wildlife service for funding this project and in particular to the San Marcos National Fish Hatchery and Technology Center for the use of their facilities and equipment. I would also like to thank A. W. Groeger for his advice and suggestions. Another person I must thank is T. M. Brandt for his help throughout the project, for finding the time to work with me when there was no time and for encouraging me to enter graduate school in the first place. I must also thank B. G. Whiteside for his marathon editing sessions and for teaching me to be a better scientific writer. His fishin' stories weren't bad either. Many thank to J. N. Fries for his invaluable technical advice on designing experimental apparatus and for his computer wizardry. Thanks very much to the faculty, staff and students who have made this such a fun learning experience. And finally, thanks Mom and Dad for taking me fishing.

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ABSTRACT

A chronic and an acute toxicity test were conducted to determine the effects of dissolved nitrogen gas (DN) supersaturation on the fountain darter *Etheostoma fonticola* and the San Marcos salamander *Eurycea nana*. In the chronic toxicity test, fountain darter fry, juvenile and adults were exposed to DN saturation levels of 90, 104, 118 and 136% for 28 d. Adult fountain darters died from gas bubble trauma (GBT) only at 136% in the chronic toxicity test. Fry and juveniles showed no external signs of GBT at any of these levels. Adults produced eggs at all treatment levels in both tests with no significant difference in the number of eggs produced between treatments. In the acute toxicity test, fountain darter fry, fountain darter adults and adult San Marcos salamanders were exposed to DN saturation levels of 106, 124, 130 and 143% for 96 h. Only adult fountain darters died from GBT and only at 130 and 143% DN saturation during the acute toxicity test. Three out of 36 San Marcos salamanders and three fountain darter fry exhibited signs of GBT at 143% DN saturation but did not die. The 96-h LC50 for adult fountain darters was 138% DN saturation. The LT50's for adult fountain darters were 12 d in 136% DN saturation and 48 h in 143% DN saturation.

INTRODUCTION

The fountain darter (fish) *Etheostoma fonticola* and the San Marcos salamander *Eurycea nana* are federally listed endangered and threatened species. The fountain darter is found only in the San Marcos and Comal rivers of Central Texas and the San Marcos salamander is found only in the headwaters of the San Marcos River (U.S. Fish and Wildlife Service 1996). A number of these animals are being held in refugium at the National Fish Hatchery and Technology Center (NFHTC) in San Marcos, Texas. Refugium fountain darters were dying at a slow, steady rate during normal conditions and higher during times of high dissolved nitrogen gas (DN) supersaturated conditions. Refugium San Marcos salamanders were not dying at a steady rate under normal conditions like the fountain darters, but some death occurred during high DN saturation events.

Supersaturation of DN can occur in water when air becomes entrained in pipes (Rucker and Hodgeboom 1953) and there is sufficient pressure (in excess of barometric pressure) to cause the gases to dissolve into solution (Colt 1984). Supersaturation of DN can also occur in warm months when water temperatures increase as well water flows through pipes. Cooler water holds more dissolved gas, so as water temperatures increase, gases that would normally escape into the atmosphere are confined and remain in solution (Adair and Hains 1974). Water supplied to the refugium holding tanks at the NFHTC is pumped from two wells, 2.1 km and 1.0 km from the NFHTC. The water is pumped from the Edwards Aquifer, through a series of pipes and becomes supersaturated at times. When supersaturation of gases has occurred at the NFHTC, DN saturation levels reached

140% and remained high (125%) for as long as 3 weeks while dissolved oxygen (DO) levels typically stabilized in a few days to around 70%.

When fish are exposed to excessive levels of gas supersaturated water, they can develop gas bubble trauma (GBT) leading to a number of conditions that are harmful to them and can ultimately cause death. External signs of GBT in fish include bubbles forming in the loose connective tissue of the eyes, causing exophthalmia (bug-eye) and emphysemas (gas bubbles) in the buccal cavity, skin, fins and gills (Marsh and Gorham 1905; Dawley et al. 1976; Weitkamp 1976).

No literature was found documenting the effects of supersaturation on salamanders or other amphibians, however, there have been many studies documenting the effects of gas supersaturation on different fish species. Rucker and Hodgeboom (1953) found GBT in salmonid yolk sac fry reared in a spring water supply that had 70% DO and 120% DN saturation. Montgomery and Becker (1980) documented GBT in smallmouth bass *Micropterus dolomieu* and northern squawfish *Ptychochelius orogensis* in the lower Snake and mid-Columbia rivers where total dissolved gas (TDG) levels exceeded 115%. MacDonald and Hyatt (1973) reported GBT in Atlantic salmon *Salmo salar* and American eels *Anguilla rostrata* where DN saturation levels were 118-125% below the Mactaquac Dam on the St. John River. Fickeisen and Montgomery (1978) found that different species of fish have different tolerances to supersaturated conditions. Fishes used in their study were exposed for 10 d at levels up to 128% TDG saturation and are listed in increasing order of tolerance; mountain whitefish *Prosopium williamsoni,* cutthroat trout *Salmo clarki,* largescale sucker *Catostomus machrocheilus* and torrent sculpin *Cottus* *rhotheus*. Krise (1993) found that mortality of lake trout *Salvelinus namaycush* was significantly higher at 110% TDG saturation than at ≤ 108% TDG saturation after 28 d in a 1 year study.

In these previous studies, some authors examined the effects of TDG saturation and others examined DN saturation. There is some debate among authors as to whether GBT is induced more by DN supersaturation or TDG pressure (Weitkamp and Katz 1980). It was not my intent to determine which of these is the major cause of GBT. Since supersaturation events at the NFHTC involved high DN saturation levels and DO levels below 100%, the purpose of this study was to use a chronic and an acute toxicity test to determine what effect nitrogen supersaturated water has on health and survival of adult San Marcos salamanders and health and survival of fountain darter fry, juveniles and adults and fountain darter egg production.

METHODS

Two different tests were run: 1) a chronic toxicity test was conducted to determine any long-term effects of DN saturation on fountain darter fry, juveniles and adults and 2) an acute toxicity test was conducted to determine the short-term effects of DN saturation on fry and adult fountain darters and adult San Marcos salamanders. Calculations were made to determine fountain darter LT50's (median time to 50% mortality) for 136 and 143% DN saturation and fountain darter LC50 (median concentration to cause 50% mortality) for DN saturation. Each treatment system for the chronic and acute toxicity tests consisted of a 650-L fiberglass water reservoir tank (Living Stream Model LS-700, Frigid Units Inc., Toledo, Ohio) with 14 or 16 5-L aquaria mounted on top. Water recirculated within each system and flowed from the reservoir tank, through a degassing unit, through a 0.5-hp electric pump (Hayward Power-Flo II, Elizabeth, New Jersey), through a 0.5-hp chiller/1000-watt heater unit (Universal Marine Industries, Inc., San Leandro, California) and then the flow split. Most of the water flowed into a U-tube (Summerfelt 1996) into which nitrogen gas and compressed atmospheric air were injected, and then back into the reservoir tank. The rest of the water flowed into each of the aquaria equipped with standpipes which allowed the excess water to overflow into the reservoir tank.

The degasser was used as a means of regulating the TDG level. Each degasser consisted of a 1.3 cm PVC (polyvinyl chloride) valve installed on the water return drain line of the reservoir tank. The 1.3 cm valve was used to restrict water flow into the water return pipe and create a vacuum on the suction side of the pump (Herman 1995). This action draws gases out of solution. These undissolved gases are released into the atmosphere when water is discharged from the pipe. Degassing can be controlled by adjusting the valve opening.

The U-tube consisted of a 2.5 cm PVC pipe (inside diameter) inserted inside a 3 m long (except for the 146% DN treatment system where a 9 m long PVC pipe was used) 10.2 cm PVC pipe (inside diameter) to within 10 cm of the bottom of the 10.2 cm PVC pipe. Water entered the smaller pipe and flowed downward until it reached the bottom of the larger pipe. The water then flowed 2/3 of the way up the larger pipe until it reached a tee connection. The water then flowed out the tee through another pipe to the reservoir tank. A mixture of nitrogen gas from a cylinder and atmospheric air from an air compressor was injected into the smaller pipe at the top of the U-tube. The head pressure of the water and gas pressure in the top 1/3 of the U-tube forced most of the gases into solution. Oxygen and nitrogen gas saturation levels were manipulated by adjusting degassers and the input levels of the compressed air and nitrogen.

An instrument (Hydrolab Data Sonde 4, Austin, Texas) equipped to measure TDG, pH, temperature, DO and barometric pressure was used to record water quality daily in the four treatment systems. The instrument was swirled in the water manually until the TDG probe reached equilibrium and then a reading was taken. To ensure the most accurate reading possible, the instrument was shaken occasionally during the equilibration time to remove any bubbles from the sensor probes. A computer was used to compute the DN saturation level using information from the Data Sonde 4 and the following equation:

$$\% N_2 + Ar = [BP + \Delta P - (CO_2 BO_2)(0.5318) - PH_2O](100)/[(BP - PH_2O)(0.7902)]$$

(Colt, 1984) where:

$\% N_2 + Ar$ = percent concentration of nitrogen and trace argon in water,

BP = barometric pressure in mm mercury,

ΔP = differential gas pressure in mm mercury measured by membrane diffusion method,

CO_2 = dissolved oxygen concentration in mg/L,

BO_2 = Bunsen coefficient for oxygen as a function of temperature, and

PH_2O = vapor pressure of fresh water in mm mercury.

Prior to testing, fountain darters were maintained (since hatching) in flow-through holding tanks with water at 21 ± 3°C, a pH of 8.1 ± 0.3, DO at 85 ± 10% saturation and DN saturation levels below 95%. Fry and juvenile fish were fed pond harvested zooplankton that passed through a 425μm (850 μm for juveniles) mesh screen. Prior to feeding, all zooplankton samples were checked for cyclopoid copepods and discarded if any were found. These copepods have been found to be predaceous on fountain darter fry (Labay and Brandt 1994). Juvenile fish were also fed blackworms *Lumbriculus variegatus* (Aqualife; Friant, California) three times per week. Fry rearing tanks were cleaned daily and juvenile holding tanks were cleaned twice a week. Adult fish were fed three times per week with blackworms and their tanks were cleaned twice a week. Prior to testing, San Marcos salamanders were maintained in a flow-through aquarium with water at 21 ± 3°C, a pH of 7.8 ± 0.3, DO at 70 ± 10% saturation and DN saturation levels below 80% and fed blackworms three times per week.

Chronic toxicity test.--The chronic toxicity test for fountain darters was run for 28 d at four DN saturation levels. The four DN treatment levels were 90, 104, 118 and 136% saturation while water temperature was maintained at 21 ± 1°C, pH at 8.1 ± 0.4, DO at 70 ± 10% saturation. Each of the four treatments had 16 aquaria mounted on top of a reservoir tank. In each treatment, 5 aquaria had fry 24-48 h old (12 per aquarium), 5 had juveniles 15-22 mm total length (TL; 12 per aquarium) and 5 had adults ≥30 mm TL (12 per aquarium) for a total of 240 fry, 240 juveniles and 240 adult fountain darters for the test. Within each treatment, a number was taped to each aquarium and it was designated as a fry, juvenile or adult aquarium. Corresponding numbers were randomly drawn from a hat and aquaria were placed on top of the reservoir tank in the order the numbers were drawn to ensure random distribution of test fish. The ratio of male to female fish was not known for this test. On day one, fish were placed directly into their appropriate aquaria from the holding tanks with no acclimation time.

In each aquarium, fry and juvenile fish were fed zooplankton (adjusted daily to a density of 2-4 zooplankters per mL) using the same procedure that was used while they were being held in the flow-through holding troughs prior to testing. In addition to zooplankton, each aquarium of juveniles received 1 mL of blackworms twice per week. Each aquarium of adults was fed 2 ml of blackworms three times per week. Aquaria for fry and juveniles were siphoned clean three times per week and aquaria for adults were siphoned clean twice per week. There was one extra aquarium for each of the treatment systems without test animals in which water quality was measured twice daily. Water quality information was analyzed using a one-way analysis of variance (ANOVA: Statview PC statistical software, SAS Institute; Cary, North Carolina). When the ANOVA value was significant, multiple comparisons among means were made with Fisher's protected least significant difference test (PLSD). Statistical significance was determined at 5% ($P < 0.05$) for all analyses in the chronic and acute toxicity tests. Aquaria for adults contained short sections of PVC pipes that were cut longitudinally in half to serve as spawning substrate. Eggs were collected from PVC spawning substrate and the walls of adult aquaria twice per week. The total number of viable eggs (non-opaque eggs) collected from each treatment aquarium was recorded and analyzed using ANOVA for any significant difference between treatments. Fountain darters were observed twice per day for mortalities, behavioral changes, signs of GBT and any anomalies. Dead fountain darters were recorded, noting condition, sex, TL, date and the aquarium in which it was found. Mortality data was analyzed using ANOVA and PLSD to determine if there was a statistical difference between treatments.

Upon completion of the chronic toxicity test, all fry were counted and preserved in a 10% formalin solution. The TL of the preserved fry was measured with the aid of a dissecting microscope and a micrometer and analyzed using ANOVA and PLSD to determine if there was a statistical difference in the growth of fry between the four treatments. Juvenile fish were counted, measured and analyzed in the same manner as the fry.

Acute toxicity test.--The acute toxicity test for fountain darters and San Marcos salamanders was run for 96 h at four DN saturation levels. The four DN treatment levels were 106, 124, 130 and 143% saturation, while water temperature was maintained at 21 ± 2°C, pH at 8.1 ± 0.2 and DO at 65 ± 10% saturation. This test used 14 d old fountain darter fry, adult fountain darters ≥ 30 mm TL, and San Marcos salamanders ≥ 61 mm TL. Each of the four treatments had 14 aquaria mounted on top of a reservoir tank. In each treatment, 5 aquaria had fountain darter fry (7 per aquarium), 5 had fountain darter adults (12 per aquarium) and 3 had San Marcos salamanders (3 per aquarium) for a total of 140 fountain darter fry, 240 fountain darter adults and 36 San Marcos salamanders. Water quality was monitored in the extra aquarium and analyzed using the same procedure as in the chronic toxicity test. Placement of aquaria within each treatment was randomized using the same procedure as in the chronic toxicity test. There was a male to female ratio of 1.2:1 (fountain darters) per treatment at the start of the test. The number of male and female fish in each aquarium per treatment was not known. In each aquarium, fry were fed zooplankton daily using the same procedure as in the chronic toxicity test. Adult fish and San Marcos salamanders were fed blackworms on day two. Test animals were observed twice per day for mortalities, behavioral changes, signs of GBT and any anomalies. Dead test animals were recorded, noting condition, sex, TL, date and the aquarium in which it was found. Mortality data was analyzed using ANOVA and PLSD to determine if there was a statistical difference between treatments.

Fountain darter eggs (viable and non-viable) were collected from PVC spawning substrate and walls of adult aquaria on day two and day four and analyzed the same as in the chronic toxicity test. The Trimmed Spearman Karber method (EPA Probit Analysis Program, Version 1.5; Environmental Protection Agency, Washington D.C.) was used to determine the LC50 for adult fountain darters. A non-linear regression (Sigma Plot, Jandel Scientific; San Rafael, CA) was fitted to a graph of percent mortality and time to estimate LT50's for fountain darters in 136 (from chronic toxicity test) and 143% DN saturation.

RESULTS AND DISCUSSION

External signs of GBT

External signs of GBT were only found on fountain darters exposed to ≥130% DN saturation and included gas bubbles in the skin on the anterior dorsal surface, around paired fins and in the branchiostegal area, as well as bug-eye (Figure 1). Nebeker et al. (1980) found similar signs of GBT on the speckled dace *Rhinichthys osculus* after

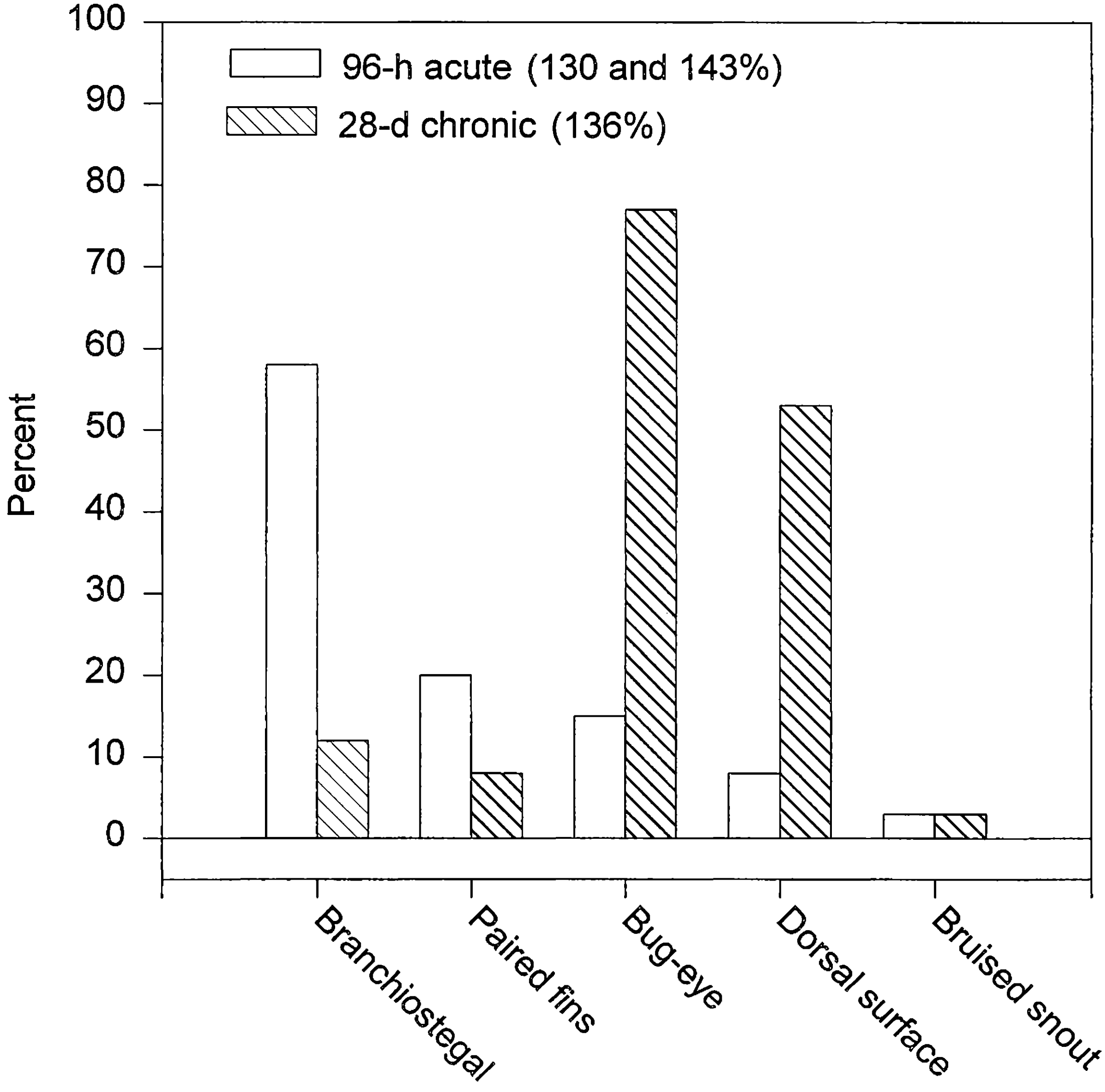

FIGURE 1.—Percent occurrence of gas bubble trauma signs found on dead fountain darters in chronic and acute toxicity tests. Some fish exhibited multiple GBT signs. The x-axis refers to the location of gas bubbles found on different body parts.

exposing them to TDG levels (% DN saturation was not given) up to 143%. I found the following progression of symptoms among fountain darters as the chronic and acute toxicity tests proceeded: 1) gas bubbles in the branchiostegal area; 2) gas bubbles on the anterior dorsal surface; 3) bug-eye; 4) and finally, gas bubbles around paired fins. Hemorrhaging snouts were found on 5% of dead fish in the chronic and 4% acute toxicity tests, but this was probably due to erratic swimming behavior which was observed in some fish and resulted in collisions with the aquarium glass. The effects of GBT often resulted in buoyancy in fountain darters (fountain darters lack swim bladders) and in one case in the San Marcos salamander causing these animals to float. External signs of GBT were only found on San Marcos salamanders exposed to 143% DN saturation and included gas-filled blisters along the tail and head.

Chronic toxicity test

The DO levels in the 104 and 136% DN saturation treatment tanks were significantly lower than in the other two tanks (Table 1). Although this is statistically significant, it is not likely biologically significant since this is above the minimum DO requirements of most warmwater fish (Doudoroff and Shumway 1967). The pH in the 104% DN saturation treatment tank was significantly lower (mean=8.2) than the other three treatment tanks (mean=8.4), but within the 6.5-8.5 limits that Stroud (1967) found to be best for good fish production. Temperature was significantly higher in the 136% DN saturation treatment tank (21.5°C) than in the other three treatment tanks (20.7-20.8°C; Table 1), but the 0.8°C mean difference is still within the optimum range for fountain darters (Bonner et al.1998) and should not have affected results. The DN

TABLE 1.—Mean water quality data (± SE) for the 28-d chronic toxicity test treatments; $N = 56$. Within each row, values followed by different letters are significantly different ($p < 0.05$).

Variable	Treatment (% dissolved nitrogen gas saturation)			
	90	104	118	136
Dissolved oxygen (mg/L)	6.24 ± 0.06t	5.94 ± 0.02p	6.34 ± 0.08t	5.84 ± 0.12p
pH	8.41 ± 0.01t	8.23 ± 0.02p	8.40 ± 0.02t	8.44 ± 0.01t
Temperature ^{0}C	20.74 ± 0.02t	20.80 ± 0.05t	20.81 ± 0.04t	21.45 ± 0.02p
Dissolved nitrogen gas saturation (%)	90.2 ± 0.50t	104.2 ± 0.37p	117.7 ± 0.31r	135.7 ± 0.39s

saturation levels varied slightly during the test but were within 10% of the their designated level (Table 1).

Only adult fountain darters exhibited external signs of GBT and only at 136% DN saturation. During the test, 63% of adult fountain darters died at 136% DN saturation (Figure 2). All of these fish were examined after death and had signs of GBT. Twenty-two percent of the fish died within the first 4 d of the test. Some fish retained gas bubbles for several days before dying. Other fish exhibiting signs of GBT did not die. Six fish that exhibited signs of GBT recovered with no signs of GBT after being put in degassed water after the completion of the test. Two of 60 adult fish with no external signs of GBT died of unknown causes at 104% DN saturation. No adult fish died at 90 or 118% DN saturation and no external signs of GBT were evident on adult fish at 118% DN saturation or below. Two juvenile fish with no external signs of GBT died (one in 90, one in 118% DN saturation treatment tanks) of unknown causes.

Fry mortality occurred in all treatment tanks with no significant difference ($F=0.59$; $df=3$) between treatments. Because of high and variable mortality within all treatments (Table 2), I do not think that DN saturation treatment levels were having an effect on fry mortality. High mortalities of fountain darter fry at the NFHTC has been common. Bonner et al. (1998) had a mortality of 74% with 24-72 h old fountain darter fry being reared under similar conditions in water with < 105% DN saturation. In addition to high mortalities of fry in my test, their small size made them difficult to monitor as the test proceeded. I was missing 15.8% of the fry from the entire test population at the end of the test (Table 2). To keep relatively constant DO and DN saturation levels requires a

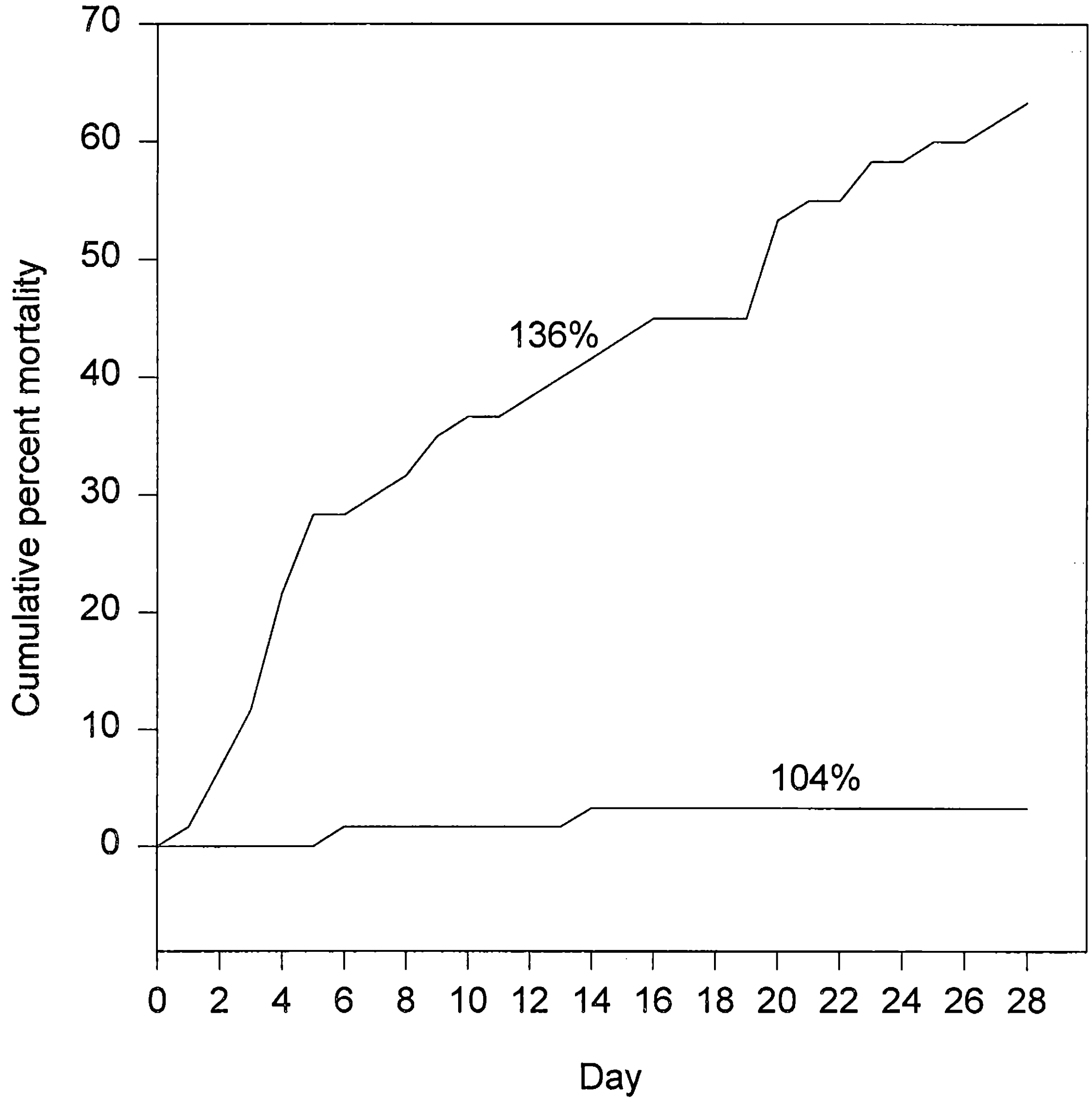

FIGURE 2.—Cumulative percent mortality of adult fountain darters in 104 and 136% dissolved nitrogen gas saturation treatment tanks during 28-d chronic toxicity test. There were no mortalities in the 90 or 118% dissolved nitrogen saturation treatment tanks.

TABLE 2.—Mean and range of fry, juvenile and adult fountain darter mortality percentages per aquarium during 28-d chronic toxicity test and 96-h acute toxicity test. The percent of fry mortalities per aquarium includes missing fry. There were five replicates per treatment. # = the percentage of fry that were missing per treatment at the end of the test.

Treatment level (% Dissolve nitrogen gas saturation)	Fry			Juveniles		Adults	
	Mean	Range	#	Mean	Range	Mean	Range
Chronic toxicity test							
90	35	8-75	20	2	0-8		
104	22	0-33	27	0		3	0-8
118	33	0-83	12	2	0-8	0	
136	22	8-33	5	0		63	33-83
Acute toxicity test							
106	14	0-57	14			0	
124	11	0-29	3			0	
130	11	0-29	6			5	0-17
143	9	0-29	3			75	58-83

high flow rate of water through the aquaria. Because of the rapid decomposition and small size of the fry, it is possible that some dead fry were broken up by the water current and passed through the screen on the aquarium standpipe between observations. Most of the dead fry that were recovered were on the standpipe screen.

Fry and juvenile fountain darters seem to be less susceptible than adults to DN supersaturation. No signs of GBT were evident on fry or juvenile fish at any DN saturation level in the chronic toxicity test. Other authors have shown that earlier life stages are less susceptible to GBT than later life stages. Krise (1991) found TDG supersaturated conditions to be more harmful to juvenile lake trout *Salvelinus namaycush* than to egg and larval stages. Counihan et al. (1998) found that earlier stages of white sturgeon larvae *Acipenser transmontanus* were more resistant to TDG supersaturated conditions than later larval stages. In 118% TDG saturation, white sturgeon larvae did not start dying from GBT until 34 d after hatching when their mouth and gills became functional.

Adult fish produced eggs at all DN saturation levels with great variation in egg production within replicate treatment aquaria (Table 3). There was no statistical difference in the number of eggs produced among the four treatments.

There was a significant difference in the TL of juvenile fountain darters at the completion of the test. Juvenile fish in lower DN saturation treatment levels grew more during the test than juveniles in higher DN saturation levels (Table 4). Fish were fed the same amount in all treatment aquaria and although no juvenile fish showed external signs of GBT, growth was affected. There was also a significant difference in the growth of fry,

TABLE 3.—Mean number (± SE) of viable and non-viable fountain darter eggs produced per aquarium per treatment during 28-d chronic toxicity test and 96-h acute toxicity test. Non-viable eggs were collected for acute toxicity test only. There were five replicates per treatment level.

Treatment level (% Dissolved nitrogen gas saturation)	Number of viable eggs	Number of non-viable eggs
Chronic toxicity test		
90	68.2 ± 8.3t	
104	93.6 ± 14.2t	
118	135.4 ± 12.7t	
136	91.6 ± 9.2t	
Acute toxicity test		
106	2.5 ± 0.3p	0.80 ± 0.8
124	9.6 ± 0.9p	0.21 ± 0.1
130	4.4 ± 1.0p	0.57 ± 0.2
143	18.0 ± 4.1p	0.60 ± 0.2

TABLE 4.—Mean total lengths in mm (± SE) for fry and juvenile fountain darters taken from 28-d chronic toxicity test treatment tanks. Means in a column followed by different letters are significantly different ($p < 0.05$).

Treatment level (% Dissolved nitrogen gas saturation)	Fry	Juveniles
90	0.40 ± 0.004t	21.1 ± 0.4t
104	0.40 ± 0.005t	21.0 ± 0.3t
118	0.42 ± 0.008p	19.6 ± 0.2p
136	0.42 ± 0.005p	19.4 ± 0.2p

however, it was the opposite of what was found for the juveniles. Fry grew more at higher DN saturation levels during the test (Table 4). Due to the difficult nature of maintaining test fry in this experiment, I have little confidence in the growth differences in fry found between treatments. More testing and different techniques are needed to determine the effects of DN supersaturation on fountain darter fry.

Acute Toxicity Test

There was no significant difference in DO among the four treatments (Table 5). There were significant differences in pH among the four treatments (range = 8.0 to 8.1), but pH was still within the 6.5-8.5 limits that Stroud (1967) found to be best for good fish production. Temperature was significantly lower in the 106% DN saturation treatment tank (mean = 20 °C) than in the others (mean = 21 °C) but this was still within their optimum temperature range (Table 5; Bonner et al. 1998).

During the test, 75% of adult fountain darters died at 143% DN saturation, 30% of which died within the first 24 h (Figure 3). All of the dead fish had signs of GBT. Three of 60 adult fountain darters died at 130% DN saturation, one per day on days 2, 3 and 4 (Figure 3). These fish had signs of GBT when they died. There were no mortalities and no signs of GBT in adult fountain darters at 106 or 124% DN saturation. Two-week old fry were used in this test to reduce the problem of missing 24-48 h old fry encountered in the chronic toxicity test. There were no significant differences ($F=0.49$; $df=3$) in fry mortality between treatments. However, 6.4% of the entire test population of fry in this test were unaccounted for (Table 2). Three of 35 fry exhibited signs of GBT at 143% DN saturation, but survived. These fry had a small bubble in their intestinal cavity

TABLE 5.—Mean water quality data (± SE) for the 96-h acute toxicity test treatments; $N = 8$. Within each row, values followed by different letters are significantly different ($p < 0.05$).

Variable	Treatment level (% dissolved nitrogen gas saturation)			
	106	124	130	143
Dissolved oxygen (mg/L)	5.84 ± 0.06t	5.93 ± 0.10t	5.61 ± 0.29t	5.58 ± 0.14t
pH	8.0 ± 0.01t	8.1 ± 0.01p	8.1 ± 0.00p	8.0 ± 0.00t
Temperature °C	20.3 ± 0.53t	21.9 ± 0.13p	21.1 ± 0.13p	21.9 ± 0.13p
Dissolved nitrogen gas saturation (%)	105.6 ± 0.72t	124.4 ± 0.37p	130.0 ± 0.31r	142.6 ± 0.39s

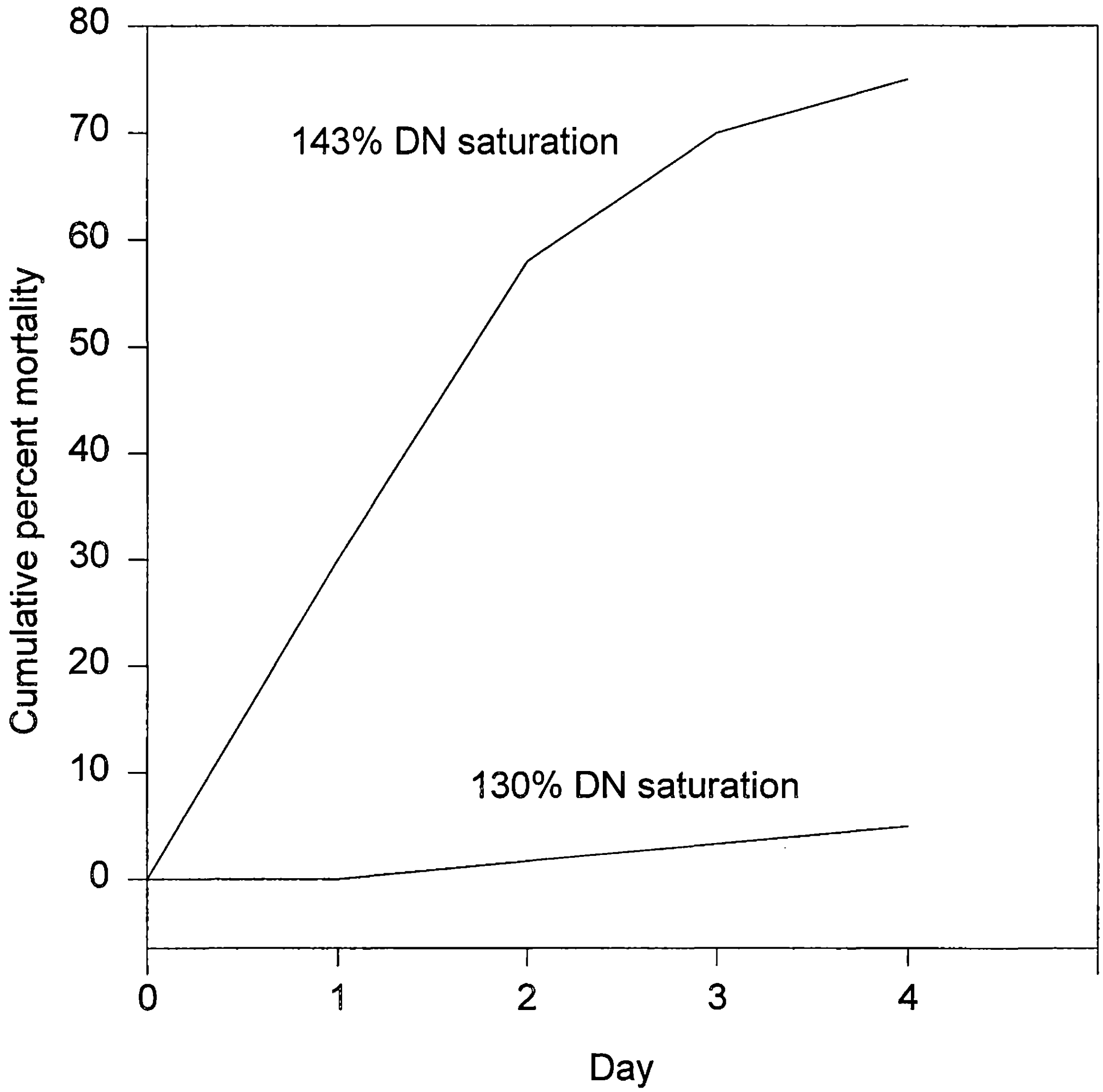

FIGURE 3.—Cumulative percent mortality of fountain darters in 130 and 143% dissolved nitrogen gas saturation treatment tanks during 96-h acute toxicity test. There were no mortalities in the 124 and 106% dissolved nitrogen saturation treatment tanks.

which caused them to float belly up. They were apparently able to pass this bubble because these same fry were seen later, swimming on the aquarium bottom without the bubble. No signs of GBT were evident in fry at lower levels of DN saturation.

The 96-h LC50 for adult fountain darters was 138% DN saturation with a 95% confidence interval of 137-140%. Nebeker et al. (1980) reported a 96-h LC50 of 140% TDG saturation (% DN saturation was not given) for the speckled dace and 119% for the cutthroat trout. The fountain darter's median times to death (LT50) were 12 d ($r^2 = 0.87$) in 136% DN saturation and 48 h ($r^2 = 0.99$) in 143% DN saturation. Fickeisen and Montgomery (1978) investigated LT50's for several fish species. They found the torrent sculpin was most tolerant of TDG supersaturation in their study and it was the closest to the fountain darter's DN saturation tolerance level. The torrent sculpin had an LT50 of 10 d at 128% TDG saturation (% DN saturation was not given) and at 116% TDG saturation there were no mortalities and just a few fish exhibiting signs of GBT. Westgard (1964) found GBT in chinook salmon *Oncorhynchus tshawytscha* exposed to 119% DN saturation in the McNary spawning channel of the Columbia River. Meekin and Allen (1975) found that mortality of salmonids in the Columbia River was higher at $\geq$ 120% DN saturation than at $\leq$ 112% DN saturation.

In the acute toxicity test, fountain darters produced eggs at all DN saturation treatment levels with great variation within replicate treatment aquaria. There was no significant difference in the number of eggs (viable and non-viable) produced per aquarium between treatments (Table 3).

There was no significant difference between the TL (mean = 38.6 for dead and

38.5 for living fish) of surviving adult fountain darters and those that died. The proportion of male to female adult fountain darters was 1.2:1 at the beginning of the test. The proportion of male to female adults that died during the test was 2.4:1, or about a 2:1 mortality rate of males to females. This difference may be from physiological differences between the sexes or from males expending energy defending breeding territory.

Only 3 of 12 San Marcos salamanders showed signs of GBT and this was at 143% DN saturation. These three salamanders survived the 96-h test and two recovered after being placed in degassed water at the end of the experiment. No LC50 was obtained for San Marcos salamanders because there was no mortality during the 96-h test. There was no statistical difference in the TL of affected and non-affected salamanders. The slow, steady mortality occurring with the refugium fountain darters at the NFHTC is not happening with the refugium San Marcos salamanders. The salamanders were added to the chronic toxicity test because nothing was known about how they would be affected by DN saturation. My preliminary results suggest that the adult San Marcos salamander is more resistant to DN supersaturation than the adult fountain darter.

The results of the chronic and acute toxicity tests indicate that the slow, steady rate of mortality of refugium fountain darters occurring at the NFHTC was not caused by DN supersaturation. After degassers were installed on the fountain darter refugium holding tank systems in January 1998, DN saturation levels were maintained at or below 105%, but the slow steady mortality of refugium fountain darters continued. These DN saturation levels were well below the levels found to be fatal in the chronic and acute toxicity tests and would not have contributed to this mortality.

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