EFFECTS OF GAS SUPERSATURATED WATER ON JUVENILE BROWN AND RAINBOW TROUT

by

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APPROVAL

of a thesis submitted by

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VITA

William Paul Connor was born to Bonnie and Fred Connor on January 13, 1960 in Harrisburg, Pennsylvania. He attended and graduated from Cumberland Valley High School, Mechanicsburg, Pennsylvania in June 1978 and West Virginia University, Morgantown, West Virginia in May 1984 with a Bachelor of Science degree in Fisheries Resources. He was accepted to graduate school at Montana State University in January 1986 and since then has been working on a Master of Science degree in Fish and Wildlife Management.

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ABSTRACT

Six bioassays were conducted at the Bozeman Fish Technology Center using juvenile brown trout (Salmo trutta) and rainbow trout (Salmo gairdneri) to determine the influence of fish size and species to gas supersaturated water. Juveniles of both species were exposed for 30-d to 104 ± 1.2% (control), 112 ± 1.0% (medium treatment), and 125 ± 0.7% (high treatment) atmospheric gas supersaturated water. No significant mortality occurred in the 112% treatment except during two tests when percent total gas pressure exceeded 113%, indicating that a critical threshold exists above this pressure. As fish grew they became more susceptible to 125% gas supersaturation. Total mortality and mortality rate were less when fish were small. The frequency at which emphysema occurred by body region also varied with fish size and smaller fish died with different external symptoms than larger fish. Larger fish were more likely to die faster than smaller fish and with no external symptoms. Daily mortality of brown trout exposed to 125% gas supersaturation was always significantly greater than for rainbow trout of similar size. Juvenile brown trout that survived exposure to 112% supersaturation showed greater incidence of external symptoms of GBT than juvenile rainbow trout of similar size. Juvenile rainbow and brown trout were exposed to supersaturations from 112% - 118% total gas pressure to test for recovery. Recovery varied according to external symptom severity. Fish that did not recover usually had severe exopthalmia. Juvenile brown trout, repeatedly exposed to 118% gas supersaturated water, given 30-d to recover between exposures, developed more severe symptoms with each exposure. Sublethal effects of exposure to gas supersaturated water on growth, predation, and microbial infection were tested using fish exposed to supersaturations from 112 - 118% total gas pressure. Surviving fish developed new symptoms more often than recurring symptoms. Growth of survivors of 30-d exposure to 112% total gas pressure was not different from control fish. There was no difference in vulnerability to predation in circular tanks between juvenile brown and rainbow trout caused by exposure to gas supersaturation. Predation tests conducted in an artificial stream suggested some difference in susceptibility may be present, but data were not conclusive. Bacterial challenges indicated that juvenile brown trout exposed to 118% gas supersaturated water for 5-d were more susceptible to infection by Aeromonas hydrophila than non-exposed fish.

INTRODUCTION

Supersaturation of water with atmospheric gases is directly responsible for occurrence of Gas Bubble Trauma (GBT) in fishes. Bouck (1980) and Fidler (1985) described GBT as a noninfectious process in fishes and invertebrates promoted by pressure disequilibrium between liquid and gas phases that produces primary lesions in blood (emboli) and in tissues (emphysema) and subsequent dysfunctions. Recent literature reviews thoroughly examine history and causes of GBT (Bouck 1980, Weitkamp and Katz 1980). Gas bubble trauma is commonly a problem in river-reaches below dams. This is the case on the Bighorn River downstream from Yellowtail Afterbay Dam.

The Yellowtail Afterbay Dam reregulates peaking discharges from Yellowtail Dam and provides uniform daily discharge into the Bighorn River. Supersaturation of dissolved gases results from entrainment of air as water passes through the gates of the Afterbay Dam, particularly the sluiceway gates.

Several supersaturation studies on the Bighorn River (Swedburg 1973, Porter and Viel 1980, Curry and Curry 1981, and White et al. 1987) have expanded our knowledge of effects of supersaturation of dissolved gases on large river fisheries. However, there are still many factors that complicate our understanding of GBT in fishes. These

factors include possible differential susceptibility according to fish species and size, the ability of salmonids to recover from GBT, and the indirect effects of exposure to GBT on juvenile brown and rainbow trout.

Variations in fish response to gas supersaturated water have been noted for different sizes and species of fish. Jensen et al. (1986) predicted that larger fish would be more sensitive to gas supersaturated water than smaller fish. White et al. (1987) found that large brown trout in the Bighorn River have more severe symptoms of GBT than smaller brown trout. In general it is believed that susceptibility differs among life stages and sizes within a species (Weitkamp and Katz 1980).

Some salmonid species may be less tolerant of gas supersaturated water than others. Fredenberg (1985) and White et al. (1987) observed that Bighorn River brown trout (Salmo trutta) were more susceptible to GBT than rainbow trout (Salmo gairdneri) of similar size.

An early study on effects of gas supersaturated water on marine fishes showed external signs of GBT regressed within 24-h after removal to unsaturated water (Gorham 1901). Information pertaining to brown and rainbow trout recovery from GBT is scarce. Subsequent to extended periods of low percent total gas pressure (TGP), emphysema was superseded by dark patches of scar tissue on brown trout of the Bighorn River (George Liknes pers.

comm.). However, the time needed for this recovery is uncertain.

Repeated exposure may influence the time of recovery after initial exposure to gas supersaturated water. Weitkamp and Katz (1980) implied that intermittent exposure may increase the level of supersaturation fish can tolerate by increasing the time over which a specific exposure accumulates. Alternatively, scar tissue formed during recovery from prior exposure could accelerate formation of GBT symptoms by providing nucleation sites with larger surface area. Also, stress associated with the physiological insult brought on by gas supersaturated water and the subsequent recovery response could lead to indirect mortality.

Indirect mortality resulting from sublethal effects of GBT have not been thoroughly investigated and may be a factor influencing fish species composition of rivers with gas supersaturation problems. This phenomenon may occur on the Bighorn River where brown trout number over 7,700 fish/km in the 1.6 km below afterbay dam, while rainbow trout number approximately 350 fish/km (White et al. 1987). Indirect mortality could be manifested as increased susceptibility to predation or disease.

Swimming ability can also be affected by exposure to gas supersaturated water. Juvenile chinook salmon (Oncorhynchus tschawytscha) exposed to 106% - 120% gas

supersaturated water swam less efficiently than control fish in an artificial channel having a water velocity of 1.28 m/s (Scheiwe 1974). Such reduced physical fitness can result in increased vulnerability of prey fish (Bams 1967, Hertig and Witt 1967, Hatfield and Anderson 1972, Sylvester 1972, Coutant 1973, Kania and O'Hara 1974). Emphysema on fish skin may facilitate microbial invasion (Sniesko 1974). Indirect mortality associated with GBT is often mentioned in the literature but documentation is rare.

Based upon the need for better information on effects of gas supersaturated water on brown and rainbow trout in the Bighorn River, this study was designed to:

(1) determine the relationship between juvenile brown and rainbow trout size and susceptibility to GBT;

(2) compare the relative sensitivity of juvenile brown and rainbow trout to GBT;

(3) evaluate the ability of juvenile brown and rainbow trout to recover from GBT at various levels of severity;

(4) determine the effect of repeated exposure to gas supersaturated water on juvenile brown trout; and

(5) investigate the sublethal effects of exposure to gas supersaturated water on fish growth, predator-prey relationships, and susceptibility to microbial invasion.

METHODS

Gas Supersaturated Water Production and Measurement

This study was conducted in laboratory facilities at the United States Fish and Wildlife Service, Bozeman Fish Technology Center. Gas supersaturated water was produced by a method similar to that described by Bouck and King (1983). An air compressor was used to force air into a water pipeline. Increased turbulence and pressure forced air into solution (Figure 1). Six 30-d exposure tests were conducted in nine fiberglas tanks (57 cm long, 33 cm deep, 43 cm wide), each subdivided into two equal sections by a wooden frame covered by 400.0 cm^2 of plastic screening. Water depth was 22.0 cm. Equal flow of water was provided through PVC pipe to each side of each tank. Mixing was complete throughout the tank; no gradient of gas levels was present.

Two levels of gas supersaturated water were produced (mean of six tests = 112 $\pm$ 1.0% and 125 $\pm$ 0.7%) by mixing artificially supersaturated water with 102 - 104% gas supersaturated spring water. Daily monitoring of delta P (pressure in mm in excess of atmospheric pressure) was accomplished using a Weis saturometer for the control (mean of six tests = 104 $\pm$ 1.2%) and Bouck gasometers (Bouck 1982) for the two test treatments; meters were

calibrated weekly by taking measurements in the same treatment tank with both kinds of instruments. Dissolved oxygen (DO) was measured using a YSI dissolved oxygen meter, standardized weekly by Winkler's procedure (APHA 1976). Temperature was measured in degrees centigrade (^{o}C) using digital thermometer calibrated with a mercury thermometer. Gas measurements were taken daily from one tank of each gas treatment. Daily temperature and dissolved oxygen (DO) measurements were taken on both sides of each tank. A rotation schedule ensured equal distribution of measurements. Barometric pressure was measured daily using a Princo Nova, fortin-type mercury barometer.

Gas levels fluctuated somewhat during the first three tests in the medium gas treatment and during tests 2 and 3 of the high gas treatment. The problem was corrected in subsequent tests by overhauling the measuring equipment and by installing a constant differential pressure regulator on the air compressor (Table 1, Figures 2 - 7).

Dissolved oxygen concentrations and water temperature were relatively constant between tests except during test 4 when temperature was lower (Table 2). This problem was corrected by using more warm spring water in the system.

After each 30-d test, values for daily delta P, DO, water temperature, and barometric pressure were used in Colt's formulas (1984) to calculate mean 30-d gas levels.

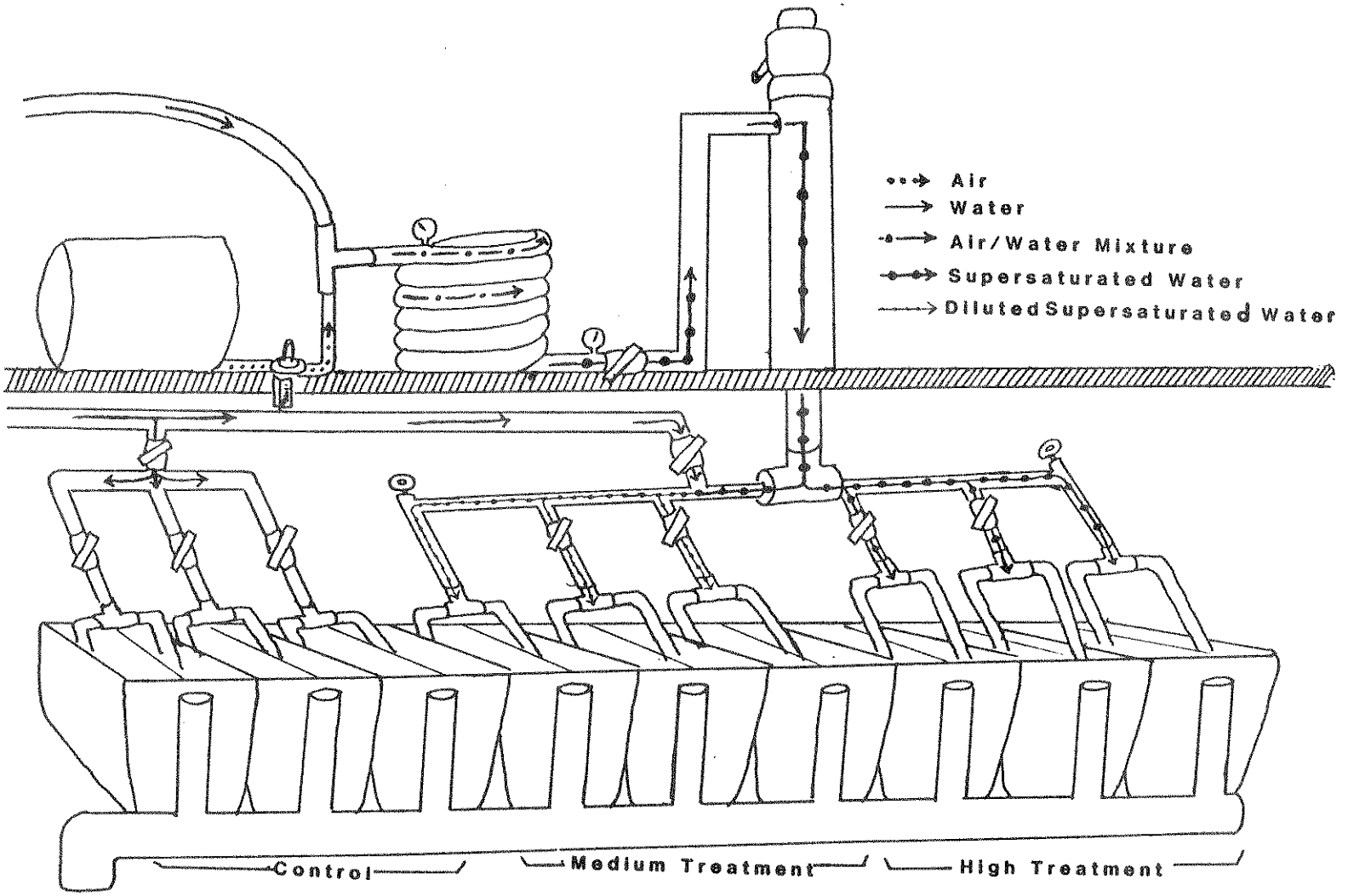

Figure 1. Laboratory apparatus used to test effects of three levels of gas supersaturation on juvenile brown and rainbow trout.

Table 1. Mean delta P values (mm) for 30-day exposure tests 1 - 6.

Test	Treatment	Delta P values ± SD
1	High	155 ± 6
2	High	153 ± 15
3	High	162 ± 13
4	High	167 ± 6
5	High	160 + 6
6	High	160 ± 9
1	Medium	82 ± 20
2	Medium	78 ± 22
3	Medium	69 ± 15
4	Medium	83 ± 7
5	Medium	72 ± 11
6	Medium	84 ± 7
1	Control	16 ± 2
2	Control	38 ± 3
3	Control	29 ± 4
4	Control	20 ± 3
5	Control	26 ± 8
6	Control	25 ± 5

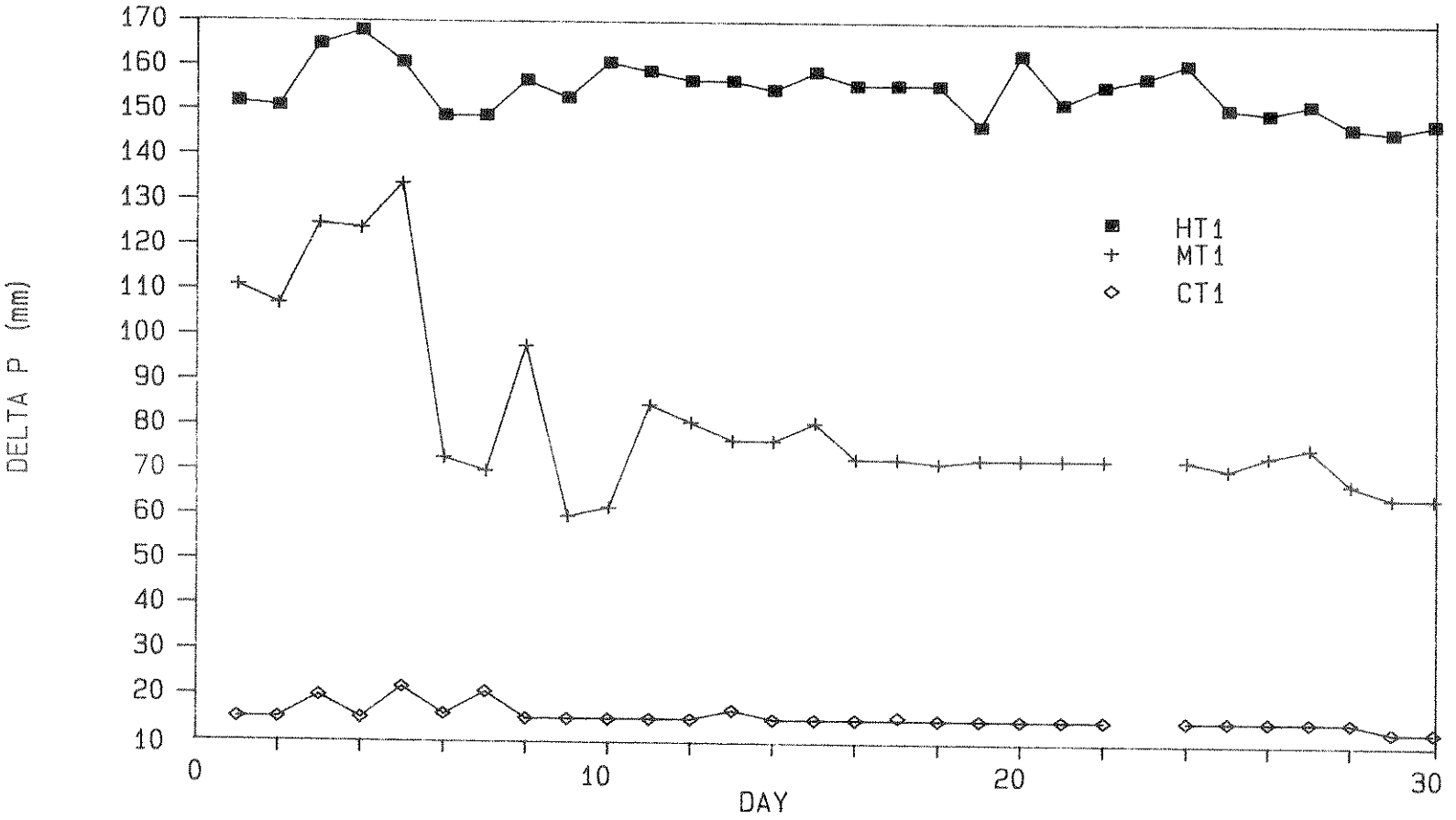

Figure 2. Delta P of water for the high (HT)(mean ^P = 155 mm and mean percent total saturation = 124.3), medium (MT)(mean ^P = 82 mm and mean percent total saturation = 112.8), and control treatments (CT)(mean ^P = 16 mm and mean percent total saturation = 102.5), test 1, March 15-April 15, 1986.

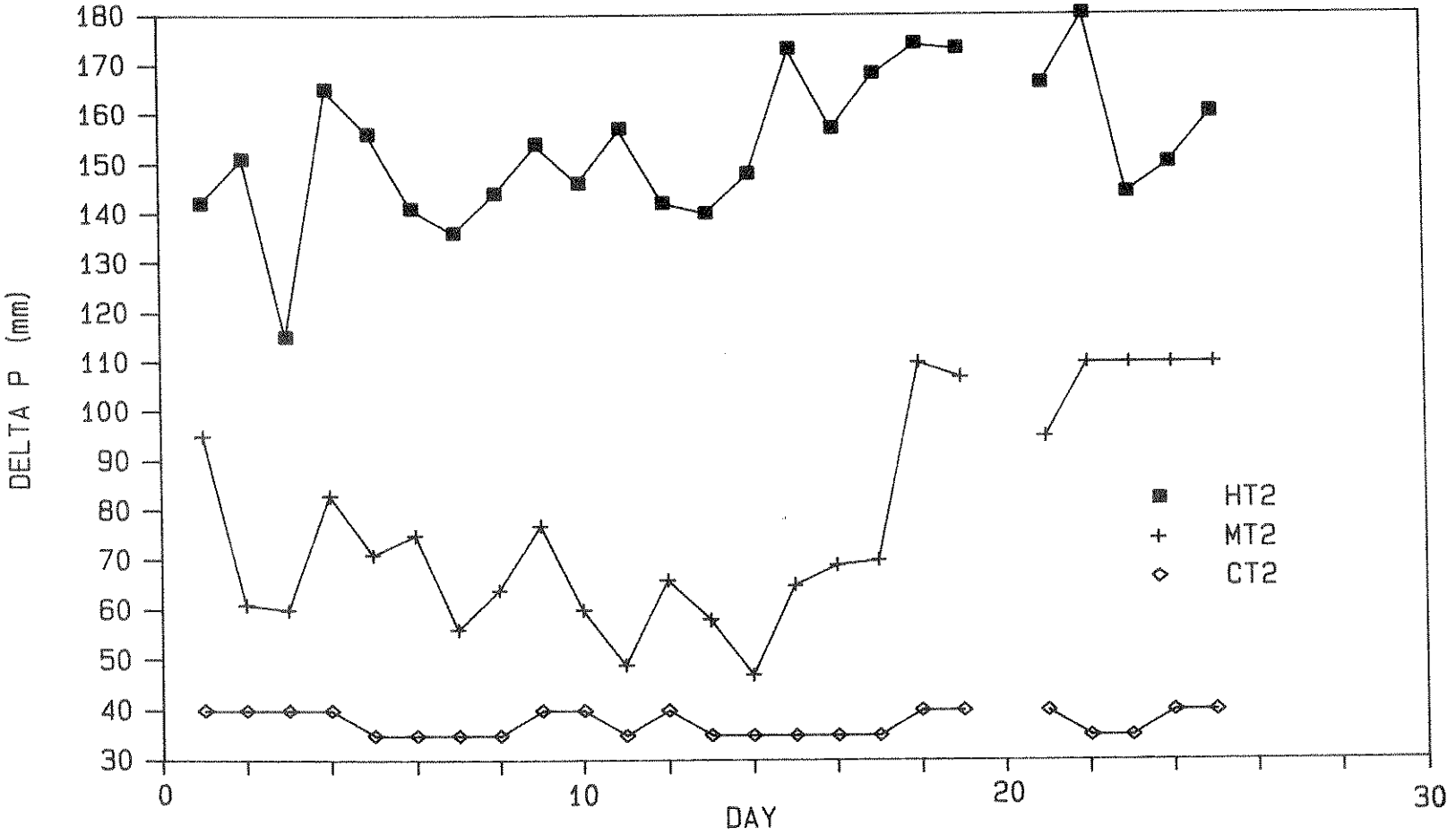

Figure 3. Delta P of water for the high (HT)(mean ^P = 153 mm and mean percent total saturation = 124.0), medium (MT)(mean ^P = 78 mm and mean percent total saturation = 112.3), and control treatments (CT)(mean ^P = 38 mm and mean percent total saturation = 105.9), test 2, May 18-June 11, 1986.

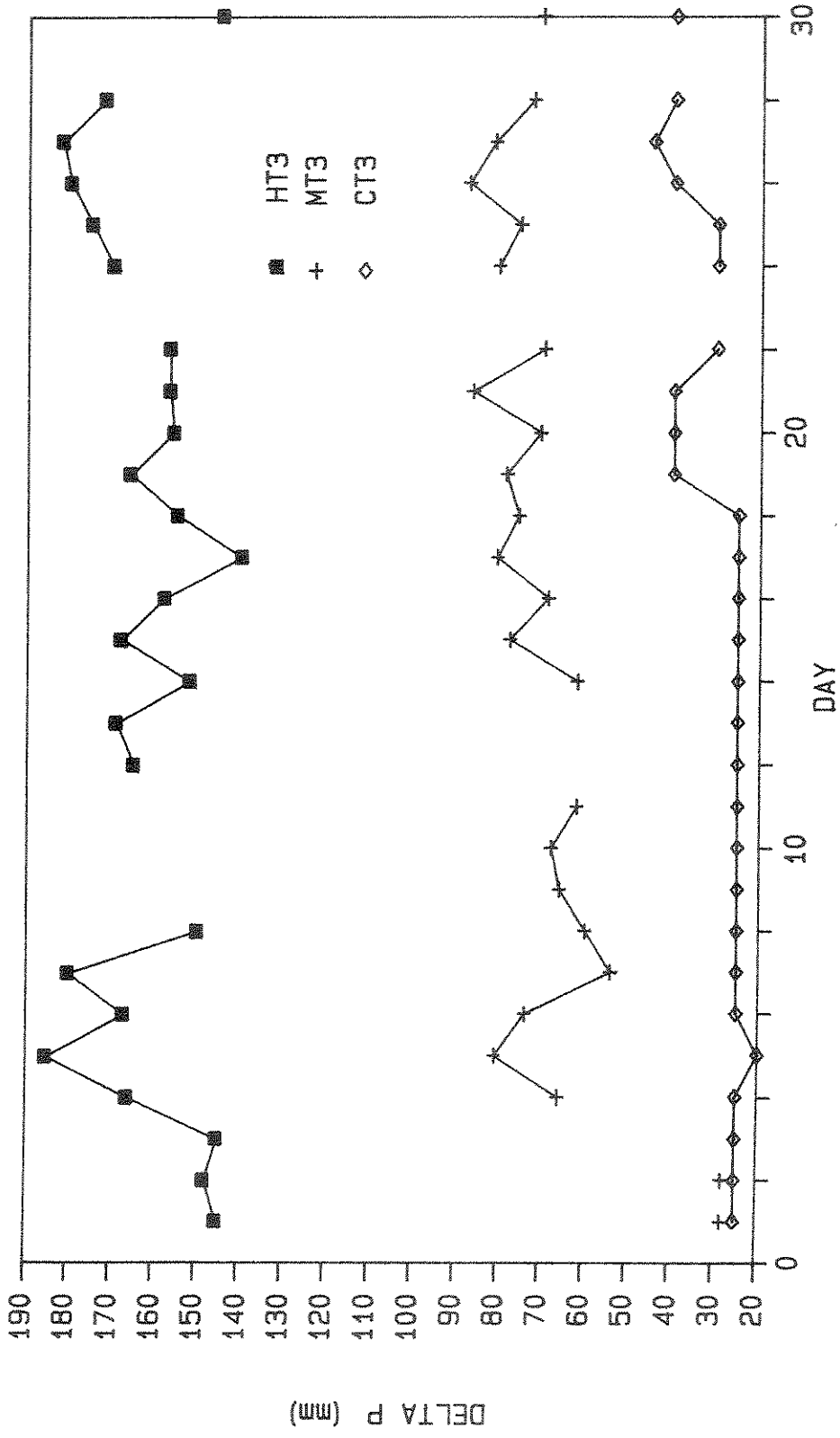

Figure 4. Delta P of water for the high (HT)(mean ^P = 162 mm and mean percent total saturation = 125.4), medium (MT)(mean ^P = 69 mm and mean percent total saturation = 110.8), and control treatments (CT)(mean ^P = 29 mm and mean percent total saturation = 104.5), test 3, July 17-August 17, 1986.

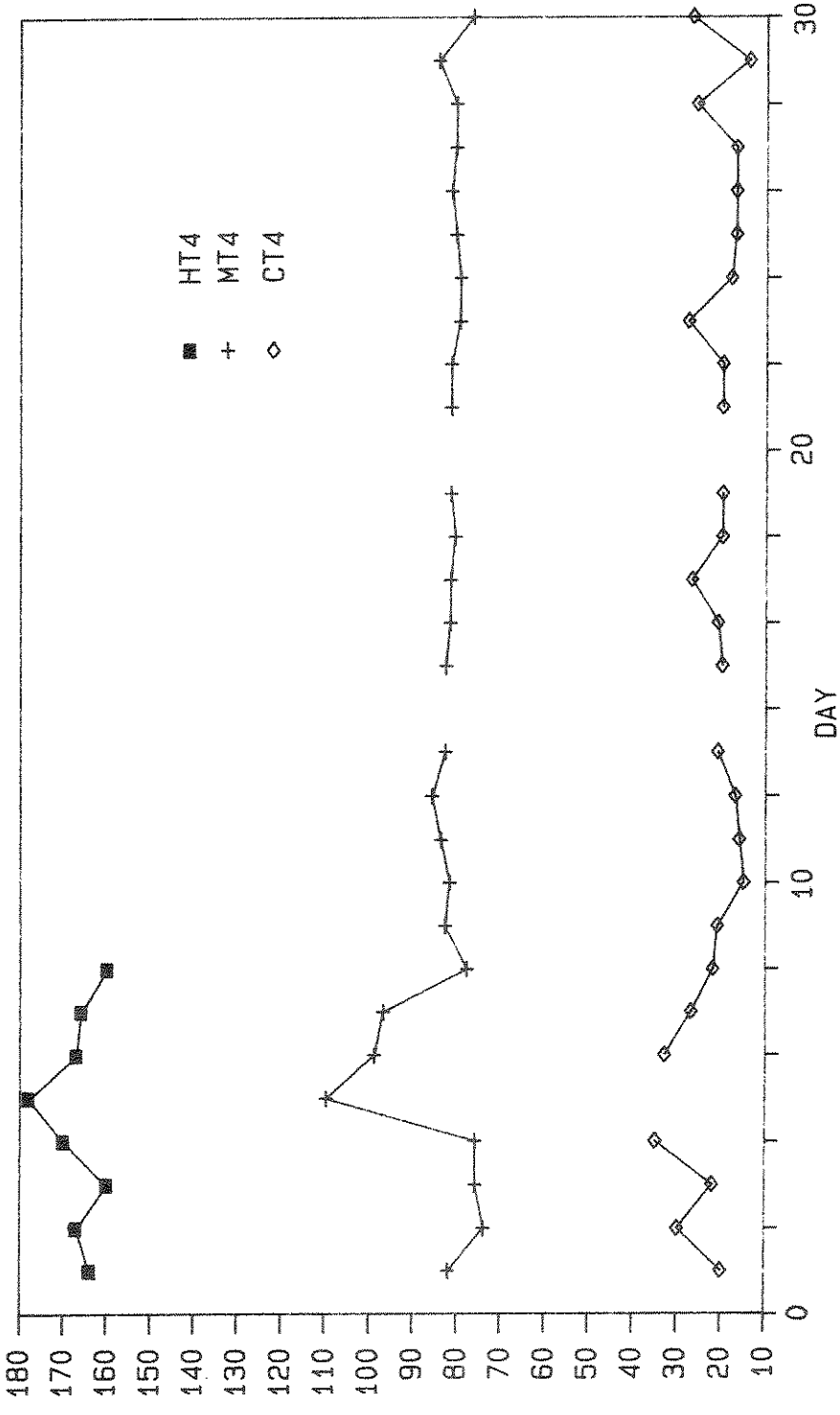

Figure 5. Delta P of water for the high (HT)(mean ^P = 167 mm and mean percent total saturation = 126.1), medium (MT)(mean ^P = 83 mm and mean percent total saturation = 113.0), and control treatments (CT)(mean ^P = 20 mm and mean percent total saturation = 103.3), test 4, September 28-October 29.

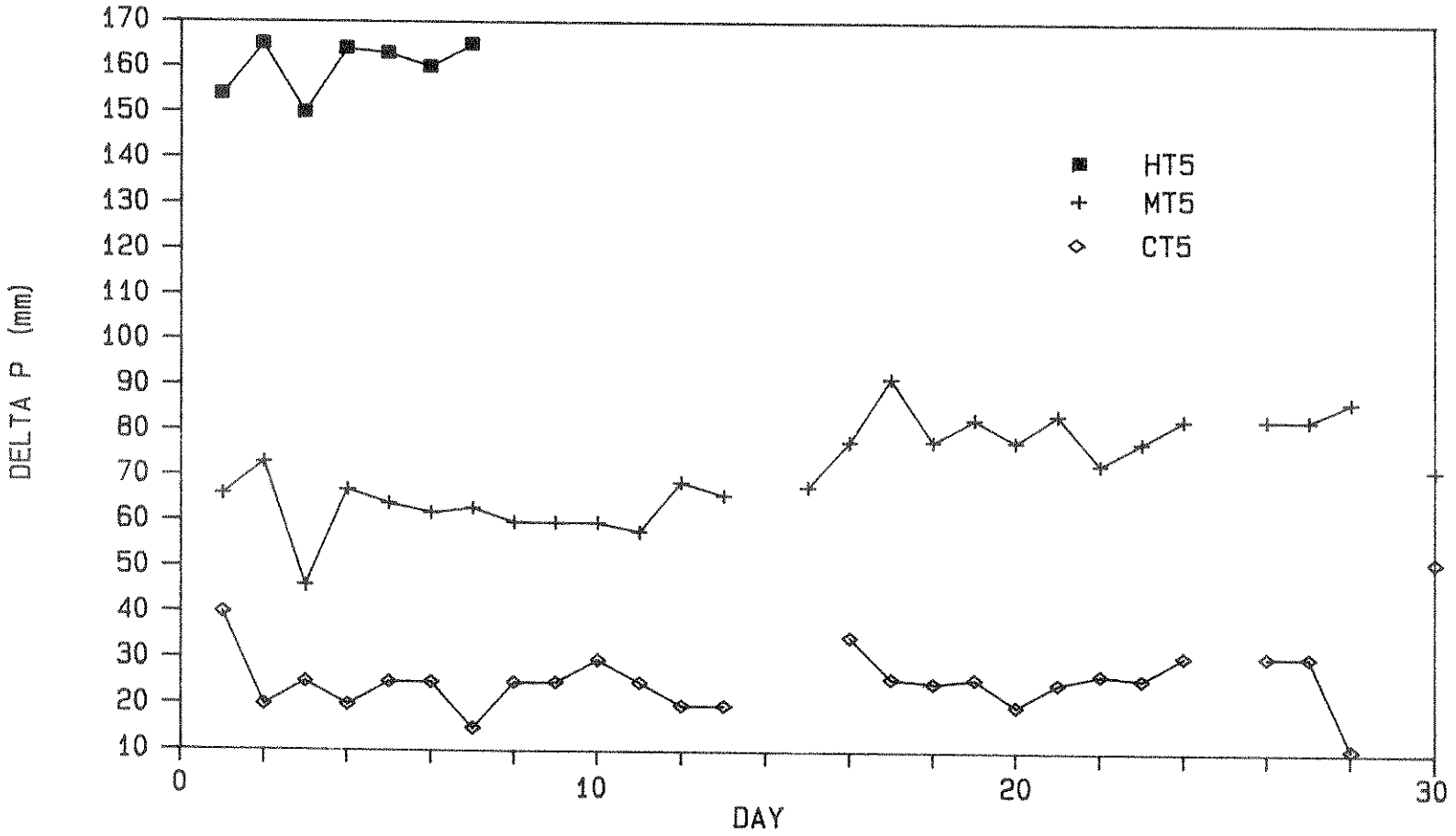

Figure 6. Delta P of water for the high (HT)(mean ^P = 160 mm and mean percent total saturation = 125.0), medium (MT)(mean ^P = 72 mm and mean percent total saturation = 111.2), and control treatments (CT)(mean ^P = 26 mm and mean percent total saturation = 104.1), test 5, January 9-February 9.

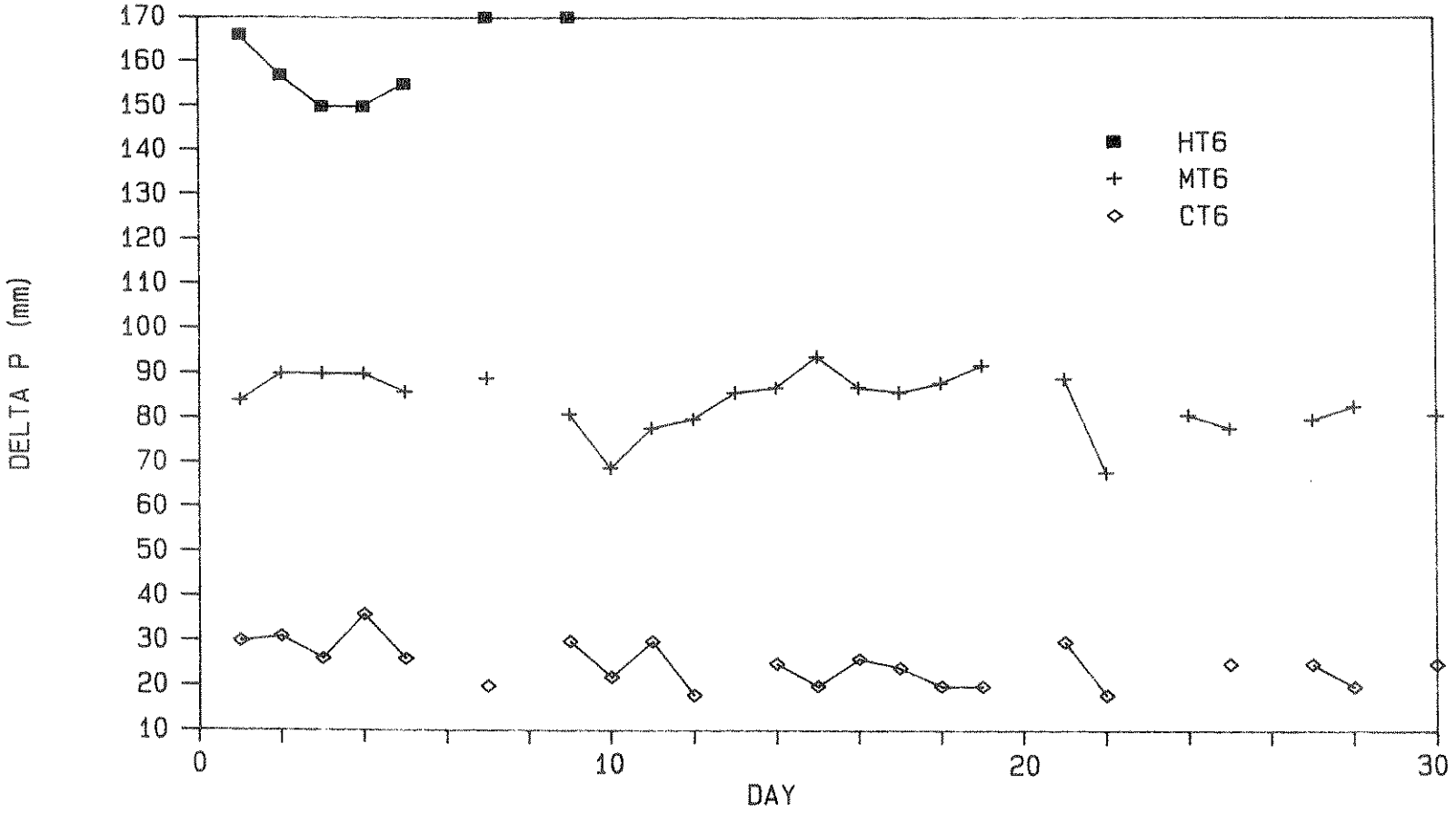

Figure 7. Delta P of water for the high (HT)(mean ^P = 160 mm and mean percent total saturation = 125.1), medium (MT)(mean ^P = 84 mm and mean percent total saturation = 113.2), and control treatments (CT)(mean ^P = 25 mm and mean percent total saturation = 103.9), test 5, February 19-March 19.

Table 2. Mean dissolved oxygen concentrations and water temperatures during tests 1-6.

Test	Treatment	Dissolved Oxygen (mg/l)	Temperature (^{o}C)
1	High	10.5 ± 0.4	10.2 ± 0.4
2	High	11.4 ± 0.4	9.7 ± 0.4
3	High	11.3 ± 0.3	9.7 ± 0.3
4	High	11.6 ± 0.4	8.6 ± 0.1
5	High	10.7 ± 0.8	10.4 ± 0.3
6	High	10.6 ± 0.5	11.0 ± 0.1
1	Medium	9.6 ± 0.3	10.2 ± 0.4
2	Medium	10.6 ± 0.4	9.8 ± 0.2
3	Medium	9.9 ± 0.3	10.0 ± 0.2
4	Medium	10.4 ± 0.4	8.1 ± 0.1
5	Medium	9.4 ± 0.3	10.0 ± 0.4
6	Medium	9.5 ± 0.4	10.2 ± 0.5
1	Control	8.9 ± 0.2	10.1 ± 0.2
2	Control	9.6 ± 0.4	10.1 ± 0.5
3	Control	9.2 ± 0.2	10.0 ± 0.2
4	Control	9.4 ± 0.4	7.9 ± 0.1
5	Control	8.6 ± 0.3	9.8 ± 0.3
6	Control	8.8 ± 0.3	9.9 ± 0.6

Fish Care

Brown trout eggs were obtained from wild stock of the Bighorn River in early December, 1985. At the same time, domestic Shasta strain rainbow trout were spawned at the Ennis Fish Hatchery.

Eggs were water hardened, treated with 100 ppm alpha caps-7 betadine and transported to the Bozeman Fish Technology Center where they were incubated in Heath trays supplied with spring water (19.0 L/min at 10±1 ^{o}C). Eggs were treated five times each week with a solution of 1:600

100% formalin. Eggs hatched in approximately 30-d. At the end of January, fish were transferred to 1.8 m circular fiberglas tanks supplied with 42 - 49 L/min of 10 ± 1 ^{o}C spring water. Fish were fed a diet of Silver Cup Salmon Pellets (Murry Elevators, Murry, UT) of appropriate size. Feed rations were calculated using the formula of Piper et al. (1982) on the first day of feeding and every 30-d thereafter.

Because Bighorn River brown trout were hesitant to feed on pellets or to feed at a predictable rate, the food ration was reduced until brown trout consumed all food offered. Bacterial infection (A gram-negative flagellate identified in September, 1986) was controlled with periodic drip treatments of 11.0 ml/h of hyamine and adding Terramycin to the diet. Growth of fish was controlled by adjusting rearing tank water temperature.

Test Fish Selection

Mean fish length was determined before each experiment by randomly selecting 25 - 100 fish, measuring each to the nearest 1 mm, and returning them to the rearing tanks (Table 3). Fish of each species were then randomly selected from the rearing tanks, weighed, and placed into each experimental tank (Table 4). The number of fish used varied by test because as fish grew the number of fish each tank could support decreased (Table 3). Fish size

increased with each test, except rainbow trout used in test 6 were smaller than rainbow trout used in test 5.

Table 3. Pre-exposure mean lengths of juvenile rainbow and brown trout computed using an aggregate of fish from high, medium, and control gas treatments.

Species	Test	Mean total length (mm ± SD)	Number of fish measured
Brown	1	35 *	25
Brown	2	53 *	25
Brown	3	62 ± 6	102
Brown	4	91 ± 8	73
Brown	5	126 ± 6	100
Brown	6	173 ± 12	90
Rainbow	1	34 *	25
Rainbow	2	71 *	25
Rainbow	3	91 ± 11	95
Rainbow	4	131 ± 14	39
Rainbow	5	194 ± 20	100
Rainbow	6	179 ± 14	90

* Data not available for standard deviation calculations

Table 4. Mean weight and number of juvenile rainbow and brown trout exposed to high, medium, and control levels of gas supersaturation during tests 1, 2, 3, 4, 5, and 6.

Species	Exposure test	Gas treatment	Total number of fish exposed	Mean weight (g/fish)
Brown	1	High	150	0.3
Brown	1	Medium	150	0.3
Brown	1	Control	150	0.3
Brown	2	High	75	1.3
Brown	2	Medium	75	1.2
Brown	2	Control	75	1.2
Brown	3	High	75	2.9
Brown	3	Medium	75	2.9
Brown	3	Control	75	2.9
Brown	4	High	75	8.3
Brown	4	Medium	75	8.3
Brown	4	Control	75	7.9
Brown	5	High	45	22.9
Brown	5	Medium	45	23.0
Brown	5	Control	45	21.3
Brown	6	High	30	61.5
Brown	6	Medium	30	59.7
Brown	6	Control	30	59.7
Rainbow	1	High	150	0.4
Rainbow	1	Medium	150	0.4
Rainbow	1	Control	150	0.4
Rainbow	2	High	75	3.4
Rainbow	2	Medium	75	3.6
Rainbow	2	Control	75	3.6
Rainbow	3	High	75	8.9
Rainbow	3	Medium	75	8.4
Rainbow	3	Control	75	8.2
Rainbow	4	High	45	26.3
Rainbow	4	Medium	45	26.7
Rainbow	4	Control	45	26.1
Rainbow	5	High	45	79.3
Rainbow	5	Medium	45	89.5
Rainbow	5	Control	45	83.5
Rainbow	6	High	30	60.5
Rainbow	6	Medium	30	60.3
Rainbow	6	Control	30	57.8

Gas Supersaturation Bioassay Data Analysis

The influence of fish length and weight on susceptibility of juvenile brown and rainbow trout to GBT was evaluated and relative sensitivity was compared. Percent cumulative mortality was calculated for control, medium, and high treatment fish of both species for all tests. Where: Percent Cumulative Mortality =

$$\frac{\text{(Day x mortality) + (Day x..n mortality)}}{\text{Fish number at day 0}}$$

multiplied x 100

Significant differences in mortality were tested by comparing mortality of control fish to mortality of medium and high gas treatment fish using Biomedical Data processing (BMDP) software (Dixon et al. 1983). BMDP employs the non-parametric Breslow rank test to generate p-values. The effect of size on susceptibility to GBT was then determined by comparing intraspecific percent cumulative mortality from the high treatment of the six tests (Table 5). Relative sensitivity of juvenile brown and rainbow trout was compared by testing for significant difference in percent cumulative mortality between high treatment rainbow and brown trout of similar size (Table 5).

Table 5. Intra- and interspecific mortality comparisons for juvenile brown (BT) and rainbow trout (RBT) exposed to the high gas treatment during tests 1 - 6.

Brown trout mortality comparison	Rainbow trout mortality comparison	Interspecific mortality comparison
BT1 vs BT2	RBT1 vs RBT2	BT1 vs RBT1
BT2 vs BT3	RBT2 vs RBT3	BT3 vs RBT2
BT3 vs BT4	RBT3 vs RBT4	BT4 vs RBT3
BT4 vs BT5	RBT4 vs RBT6	BT5 vs RBT4
BT5 vs BT6	RBT6 vs RBT5	BT6 vs RBT6

Evaluation of External Symptoms of GBT

Each juvenile brown and rainbow trout that died in the high treatment of experiments 1 - 4 was examined for external symptoms of GBT to determine the relationship between fish size and body region of GBT occurrence. All symptoms were recorded in detail during these tests. Surviving juvenile brown and rainbow trout of the medium treatment from tests 3, 4, 5, and 6 were examined to compare the frequency of external symptom difference between the two species.

Recovery Tests

A different gas supersaturating system was developed for recovery studies (Figure 8). Both rainbow and brown trout were placed into two, 4-m diameter tanks and exposed to 118% supersaturated water for 120-h.

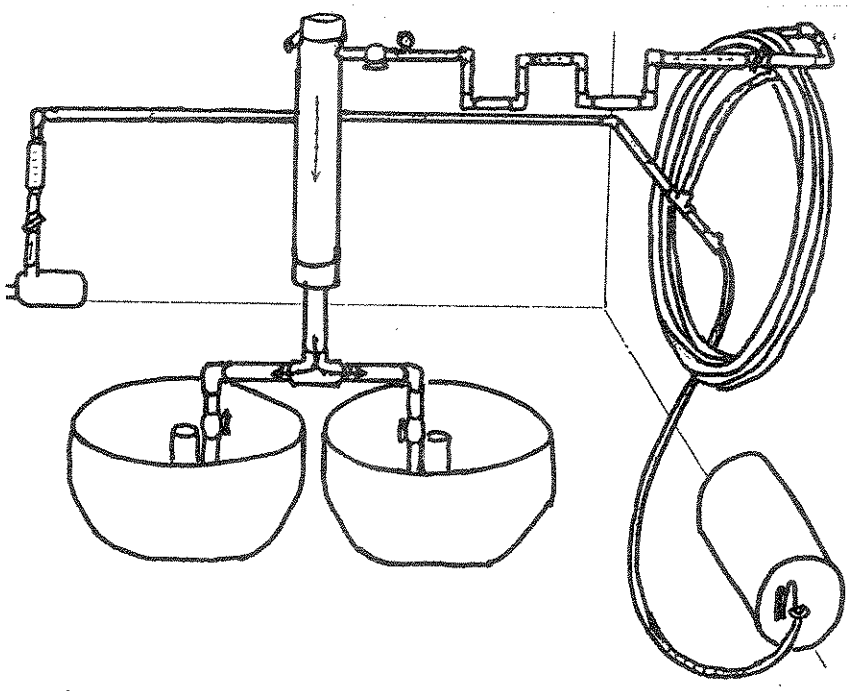

Figure 8. Laboratory apparatus used to produce gas bubble trauma in juvenile brown and rainbow trout for recovery tests.

Affected fish were removed from treatment tanks, symptoms recorded, and each fish was assigned a status rating of 0, 1, 2, or 3 (Table 6). Fish were then transferred to 104% supersaturated water and recovery was monitored every other day for 30-d.

A second series of recovery tests was conducted to investigate the effects of repeated exposure of juvenile brown and rainbow trout to gas supersaturated water. The procedure was identical to other recovery tests except fish were exposed initially, survivors were given 30-d to recover, and then were exposed a second and third time, with a 30-d recovery period in between. One additional

GBT rating (4) was used in this part of the study to describe fish that did not survive repeat exposure to gas supersaturated water (Table 6). Fisher's Exact Test (Hintze 1987) was used to test for changes in symptom severity between exposures.

Table 6. Gas bubble trauma description and other criteria used to rank laboratory recovery data.

Description and Status	Rating
-No visible external symptoms. Fish have developed no external symptoms of GBT during exposure to gas supersaturated water or have lost all previously acquired symptoms during recovery	0
-Emphysema present in one to four of the following regions: Buccal cavity, mandible, maxillary, left opercle, right opercle, left eye, right eye, head, left lateral line, right lateral line, left pectoral fin, right pectoral fin, anal fin, adipose fin, caudal fin, trunk. Fish may display minor stress	1
-Emphysema present on more than four of the above regions. Mild exopthalmia in one or both eyes, but no evidence of impaired swimming. Impaired vision combined with reduced appetite may reduce feeding activity and lead to poor nutritional status	2
-Severe emphysema and exopthalmia in one or both eyes. Eyes may be cloudy and hemorrhaging. May lack response to visual stimuli. Noticeable swimming impairment. Obvious loss of equilibrium, spastic swimming may be evident. Blinded fish physiologically deteriorate (embedded scales and weight loss) and may starve. Acute mortality is possible in some cases	3
-Fish that died during the second or third exposure to gas supersaturated water	4

Growth of Fish Exposed to Sublethal Levels of Gas Supersaturation

Brown and rainbow trout juveniles from control and medium gas treatment tests 1, 3, 5, and 6 were used to determine the effect of exposure to sublethal levels of gas supersaturation on fish growth. Weights of juvenile brown and rainbow trout from any high treatment or from medium treatments of tests 2 and 4 were not used to test for sublethal effects on growth because mortality attributable to GBT occurred in these tests.

Growth effects were examined by comparing control fish weight to treatment fish weight. Fish weights taken on days 1 and 30 from tests 1, 3, 5, and 6 were pooled to provided a sample size of twelve mean weights (four tests @ three replicates/test) (Table 7). Average day 1 and 30 control and medium treatment weights were calculated using these 12 weights and a comparisons were made using the Man-Whitney nonparametric rank test (Hintze 1987).

Table 7. Sample sizes used to test for the effects of gas supersaturated water on juvenile brown (BT) and rainbow trout (RBT) growth.

Test	Treatment	Number of samples	Number of fish weighed/sample			
			BTDAY1[a]	BTDAY30[b]	RBTDAY1[c]	RBTDAY[d]
1	Control	3	150	139	150	145
3	Control	3	75	73	75	71
5	Control	3	45	45	30	28
6	Control	3	30	29	30	26
1	Medium	3	150	140	150	145
3	Medium	3	75	66	75	69
5	Medium	3	45	45	30	27
6	Medium	3	30	30	30	30

a = Brown trout weighed on day 1
b = Brown trout weighed on day 30
c = Rainbow trout weighed on day 1
d = Rainbow trout weighed on day 30

Predation Susceptibility

Tank Tests

Sublethal effects of GBT on juvenile brown and rainbow trout were evaluated during predation susceptibility tests similar to those described by Bams (1967). The experimental procedure (Figure 9) was repeated 14 times.

One hour before testing, eight Arlee hatchery rainbow trout ranging in size from 200-270 mm (four during the first four tests) were transferred from a 1.8 m circular fiberglass rearing tank (80 cm deep) to three replicate 1.2 m tanks (65 cm deep). Each tank was supplied with a

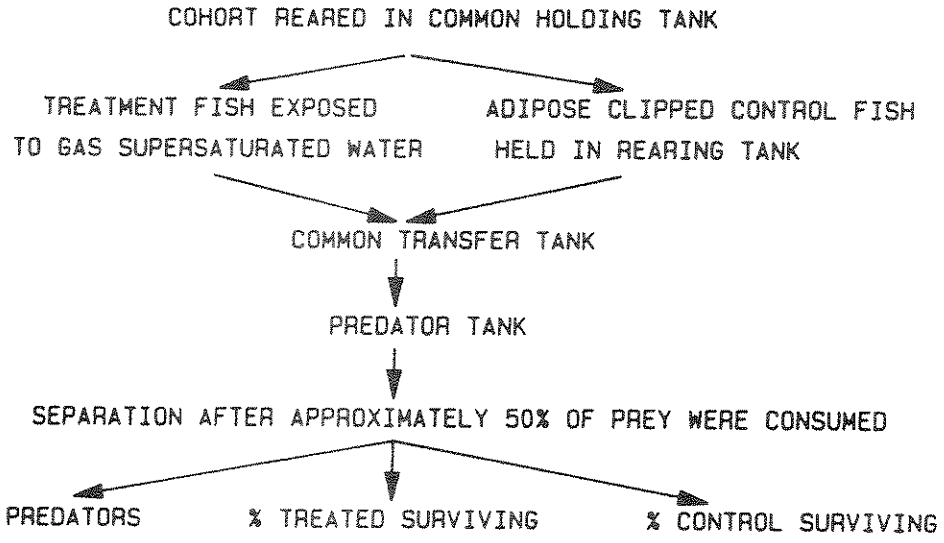

Figure 9. Flow diagram for tests to determine relative vulnerability to predation between fish exposed to gas supersaturated water and unexposed fish.

constant flow of water which exited via a single stand-pipe. Different prey fish were used during each test. Juvenile rainbow (mean length = 46 $\pm$ 8 mm) and brown trout (mean length = 42 $\pm$ 4 mm) that had survived exposure to 124% supersaturated water for 30-d were used during the first four tests. Larger rainbow (mean length = 77 $\pm$ 6 mm) and brown trout (mean length = 56 $\pm$ 7 mm) that had been exposed for 30-d to 112% supersaturated water were used for the second four tests. Brown trout exposed for 30-d to 110 % were used for two tests. Brown trout exposed 13 hours to 130% super-saturated water were used during the remaining four tests.

Three days prior to predator avoidance tests, numbers

of control fish equal to numbers of experimental fish were adipose clipped. Bams (1967) found that fin clipping control fish created no bias in testing. Control fish were from the same cohort, fed the same food, and reared at the same water temperature, but had not been exposed to supersaturated water.

Prey (25 control and 25 test) were placed in a 4 L plastic bowl and gently stirred into the predator occupied water. When predators had ingested approximately 50% of the prey, they were removed from the tanks. Test results were discarded if more than 35 or less than 15 prey remained; five tests were invalidated. Surviving prey were counted, measured, and sorted according to experimental history. Instantaneous mortality ratios (Bams 1967) were calculated using the following equation:

$$dp = \frac{-\log_e (i1)}{-\log_e (i2)}$$

where:

dp = instantaneous mortality ratio
i1 = surviving treatment fish/starting # of treatment fish
i2 = surviving control fish/ starting # of control fish

When the instantaneous mortality rate of fish exposed to gas supersaturated water is greater than the instantaneous mortality rate of control fish, dp will be greater than 1. When the predation ratio is equal, dp = 1. The chi-square

correction for continuity (Zar 1984) was used to test for statistical significance.

Experimental Stream Tests

Seven predation tests were conducted in an experimental stream to determine if juvenile rainbow and brown trout exposed to high gas supersaturation levels are more susceptible to predation than non-exposed fish. The rectangular study section within an oval experimental stream channel was 5.1 m long, 0.6 m wide, 0.4 m deep; water depth was 0.39 m (Figure 10). Plastic-mesh screening formed upstream and downstream boundaries and metal screening placed on top of the channel kept fish from jumping out. Gravel substrate (mean diameter = 1.9 cm), 0.3 m deep, was used to try to simulate natural conditions. The stream channel was provided with 8 L/min of 16.6 ^{o}C water containing 6.2 ppm DO. Water depth was regulated at 39.0 cm by a single stand pipe. A Sears electric outboard motor (with 30 pounds thrust) created a mean stream velocity of 7 cm/sec. Three types of escape cover were provided for the prey (Figure 10). Two trapezoidal wooden slabs (30.0 cm x 21.0 cm x 4.0 cm x 10.5 cm and 33.5 cm x 26.0 cm x 4.0 cm x 9.0 cm) and a straight white plastic cylinder (37.5 cm long, 6.0 cm diameter) and an elbowed white plastic cylinder (22.0 cm long, 6.5 cm diameter) provided overhead cover.

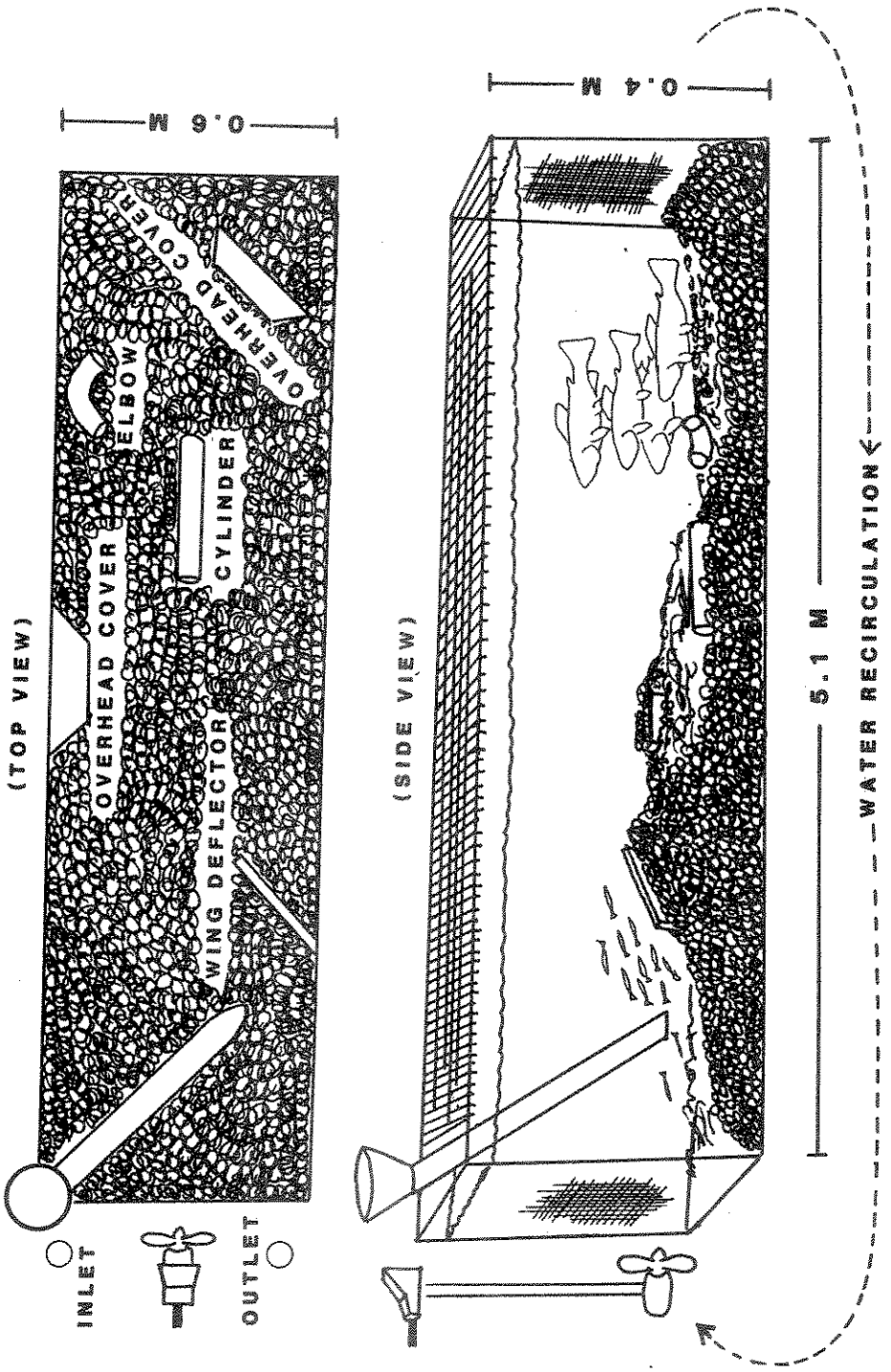

Figure 10. Experimental stream used in the second series of predation tests May 1 - 21, 1987.

The experimental procedure for the seven experimental stream tests was similar to that of tank tests except treatment fish with external symptoms of GBT were selected over those without external symptoms and predators were maintained in the channel between tests. The predators (four Arlee hatchery rainbow trout) ranged in size from 270-286 mm total length. Different prey fish were used during each test. An equal number of treatment prey and control prey (except during rainbow test 2 when 14 treatment and 15 control fish were used) were placed in a 10 L bucket. The electric outboard motor was turned on, and prey were introduced into the channel through a funnel located in the upstream portion of study section (Figure 10). An observer watched the predators consume prey from behind a black curtain until approximately one-half the prey were consumed. This time ranged from 34 min 47 sec to 88 min 54 sec. The predators were then removed and surviving prey were captured, separated into treatment and control groups, and measured. Because these stream tests were not repeated, Fisher's exact test (Hintze 1987) was used to test for significant difference in predation on treatment and control fish.

Juvenile brown trout (mean total length = 46 $\pm$ 3 mm) were used as prey fish in the first five tests. Gas bubble trauma symptoms were induced in prey fish by exposure to 118% gas supersaturated water for 4 - 7-d;

control prey were not exposed to gas supersaturated conditions. Control fish were marked with an adipose clip 1-h prior to testing. Fish showing external symptoms of GBT were intentionally selected. All fish captured (up to five fish per dip including fish without external signs of GBT) were placed in a container with control fish until 25 treatment prey were obtained.

Two tests were conducted using rainbow trout (mean total length 60 ± 6 mm) as prey. One-half of the treatment rainbow trout prey in the first test had external symptoms of GBT induced by exposure to 118% gas supersaturation and the remaining 12 fish were randomly sampled from the exposure tank. During the second test the water level in exposure tank 1 was held at 10 cm and the water level in exposure tank 2 was raised to 45 cm (Figure 11). For a period of 4-d all fish in tank 1 were examined for external signs of GBT. Those with external signs were transferred to tank 2 to allow for compensation but not recovery. Fourteen rainbow trout prey with external signs of GBT were obtained in this manner.

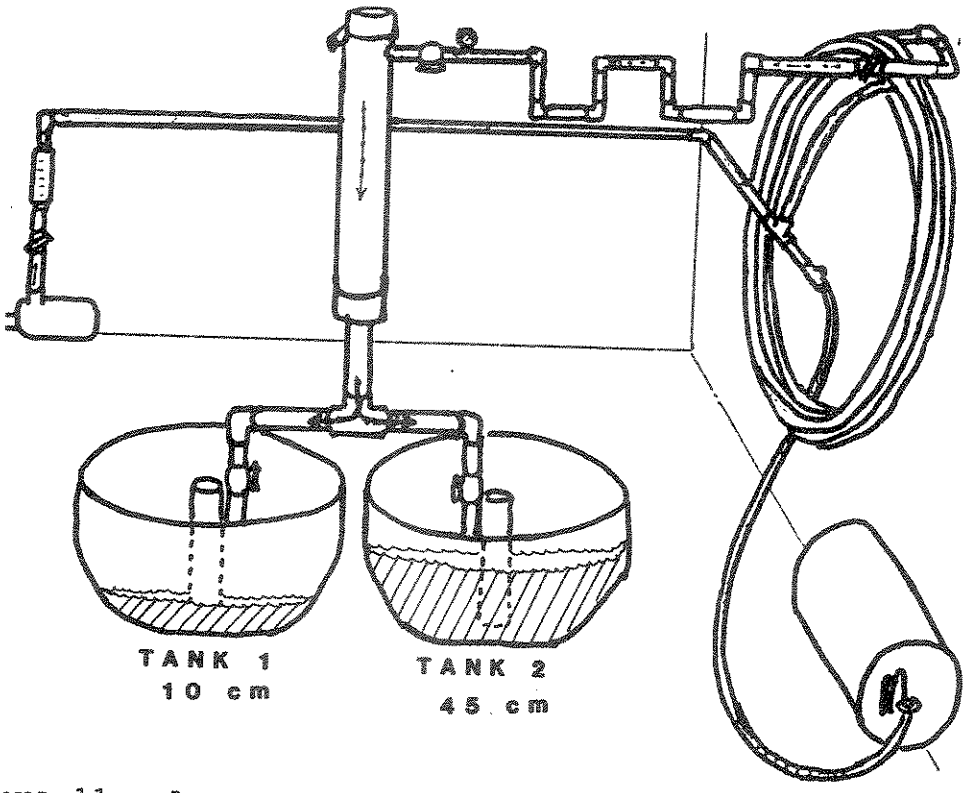

Figure 11. Apparatus and water level system used to induce external symptoms of GBT in rainbow trout for predation tests May 16 - 21, 1987.

Bacterial Challenge

The effects of GBT on the susceptibility of juvenile rainbow and brown trout to the pathogen Aeromonas hydrophila was tested. A. hydrophila CDC Strain III A-20 was obtained from the Wyoming Department of Game and Fish, Laramie, WY. Test protocol involved: 1) antiserum production; 2) pre-treatment serial dilution; 3) challenge application; 4) post-treatment serial dilution; 5) fluorescent antibody technique application; 6) enumeration and statistical analysis of bacterial infection of the kidney using a two sample T-test (Hintze 1987).

Preparation of the antigen was completed using the method of Garvey et al. (1977). Five hundred milliliters of Tripticate Soy Broth (TSB) were inoculated with broth culture of A. hydrophila that had incubated at 28 ^{o}C for 18-h. At the end of the incubation period, an equal volume of 0.6% formalinized saline was added. This preparation was stored at room temperature for 4-d. Sterility of the bacterial suspension was checked by inoculating a plate of Triptic Soy Agar (TSA), incubating for 48-h at 28 ^{o}C, and examining the plate for growth.

Bacteria were collected using centrifugation at approximately 3000 rpm for 0.5-h. The supernatant fluid was discarded and the bacteria resuspended in 100 ml of 0.85% saline. At 4-d intervals, a 2.5 kg rabbit was intravenously injected with 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, and 6.0 ml of the A. hydrophila antigen preparation diluted 50% with physiological saline. The rabbit was rested for 6-d after the last injection. A small serum sample was then obtained by venous puncture and its titer was tested by the agglutination reaction. Specificity of the antiserum was confirmed by testing it against A. salmonicida and Yersinia ruckeri. The rabbit was bled at 3-week intervals. Whole blood was centrifuged at 3000 rpm for 15 min and the serum was removed with a syringe. The antiserum was preserved with 0.2% sodium azide and frozen.

Inoculum was prepared independently for two replicate challenges. Seventy-two hours prior to the challenge a brown trout (approximately 180 mm) was injected with 1.0 cc of a 1.0 x 10^8 suspension of A. hydrophila and placed in 20 ^{o}C water. Mortality occurred within 24-h. The kidney of the dead fish was punctured with an inoculating loop which was then used to inoculate a test tube of TSB. The TSB was incubated at 20 ^{o}C for 24-h. One tenth milliliter of this suspension was used to inoculate 200 ml of TSB which was then incubated at 37 ^{o}C for 12-h.

A pre-treatment serial dilution was made in triplicate to determine the viability of challenge inoculum. One-tenth milliliter of the 12-h challenge inoculum of A. hydrophila was pipetted into 9.9 ml of distilled water to give 1:10^2 dilution. One milliliter of this suspension was transferred to a second test tube containing 9.0 ml of distilled water to give 1:10^3 dilution. This procedure was followed for three more suspensions until a tube with a dilution factor of 1:10^6 was achieved. One hundred microliters of the 1:10^6 dilution were pipetted onto petri dishes containing TSA and incubated at 20 ^{o}C for 48-h. Colonies were counted after incubation to verify inoculum viability and to insure that there were approximately 1.1 x 10.0^8 viable cells in the challenge inoculum.

Six juvenile brown trout (180 - 210 mm) were placed in each of five plastic buckets containing 10.0 L of 15.9

^{O}C water with 7.4 mg/L DO. Three fish in each bucket had GBT as a result of exposure to 118% gas supersaturated water for 120-h with no recovery time. The remaining three fish had no previous exposure to gas supersaturated water. Nine milliliters of 12-h challenge inoculum of A. hydrophila were introduced into four of the buckets. The fifth bucket was treated with 9.0 ml of sterile TSB. The challenge times ranged from 10 - 40 min. After the challenge was complete, fish from each bucket were placed into a separate tank that received 2.4 L/min of 15.9 ^{O}C water with 7.6 mg/L oxygen. The negative control treatment was removed at 20 min.

After 96-h fish in each bucket were euthanized with MS-222 and refrigerated at 0 ^{O}C. Fish were measured and weighed, and kidneys were removed and homogenized in 5 ml of distilled water using a tissue homogenizer. One milliliter of homogenized solution was pipetted onto a circle (d = 1.5 cm) on a fluorescent antibody technique slide and heat fixed. This procedure was followed once for each of the 30 fish. One-tenth milliliter of rabbit antiserum was then applied to the slide for 1-h, then rinsed with physiological saline. Commercially prepared fluoroscein-conjugated goat antisera to rabbit immunoglobulins was applied to the slide for 0.5-h and then rinsed off with physiological saline.

A fluorescent phase contrast microscope (400x objective) was used to count all bacteria in the circle of each slide. The number of bacteria counted on control and treatment slides were compared using the two sample t-test (Hintze 1987).

RESULTS AND DISCUSSION

Brown and Rainbow Trout Mortality

Significant mortality of medium treatment fish in the 30-d bioassays occurred only during tests 2 and 4 (Table 8). No brown trout mortality occurred in control or medium treatments during tests 5 or 6 and no rainbow trout mortality occurred in test 5.

Table 8. Breslow rank analysis of medium gas treatment percent cumulative mortality for juvenile brown (BT) and rainbow trout (RBT) during tests 1, 2, 3, 4, 5, and 6.

Mortality Comparison					
Control treatment			Medium treatment		p-value
Species	Test	outcome	Species	Test	
BT	1	=	BT	1	0.6287
BT	2	<	BT	2	< 0.0001*
BT	3	=	BT	3	0.1583
BT	4	<	BT	4	< 0.0001*
BT	5	=	BT	5	-------
BT	6	=	BT	6	-------
RBT	1	=	RBT	1	0.3999
RBT	2	<	RBT	2	< 0.0001*
RBT	3	=	RBT	3	0.3173
RBT	4	<	RBT	4	0.0351
RBT	5	=	RBT	5	-------
RBT	6	=	RBT	6	0.3255

* Statistically different at alpha = 0.05

Approximately 30% of mortality in test 2 occurred when delta P values were higher than planned for 10-d

(Figure 12). During test 4, all mortality of both species in the medium treatment coincided with a 3-d period of elevated delta P values (Figure 13).

Research has shown that critical levels of gas supersaturation exist for different species of fish (Weitkamp and Katz 1980). The critical level is defined as the maximum level of supersaturation that allows for survival and propagation of aquatic biota. Cutthroat, rainbow, and steelhead trout fry (9.5 cm) were tolerant to water containing 110% saturation of nitrogen, but died when exposed to 115% nitrogen supersaturation (Rucker and Hodgeboom 1953). Juvenile steelhead (180-200 mm) survived exposure to 115% gas supersaturation, but 120% supersaturation caused 100% mortality (Stroud and Nebeker 1976). Apparently a tolerance threshold for juvenile steelhead trout (180 -200 mm) exists between 115 and 120% supersaturation. Data from my study indicate a similar threshold exists for juvenile brown and rainbow trout. Critical levels of gas supersaturation for brown and rainbow trout were surpassed during tests 2 and 4. The critical threshold required to cause acute mortality in juvenile rainbow and brown trout (53. -131 mm) appears to be between 113-117%. Medium treatment levels never exceeded the critical threshold long enough to cause mortality in tests 1, 3, 5, and 6.

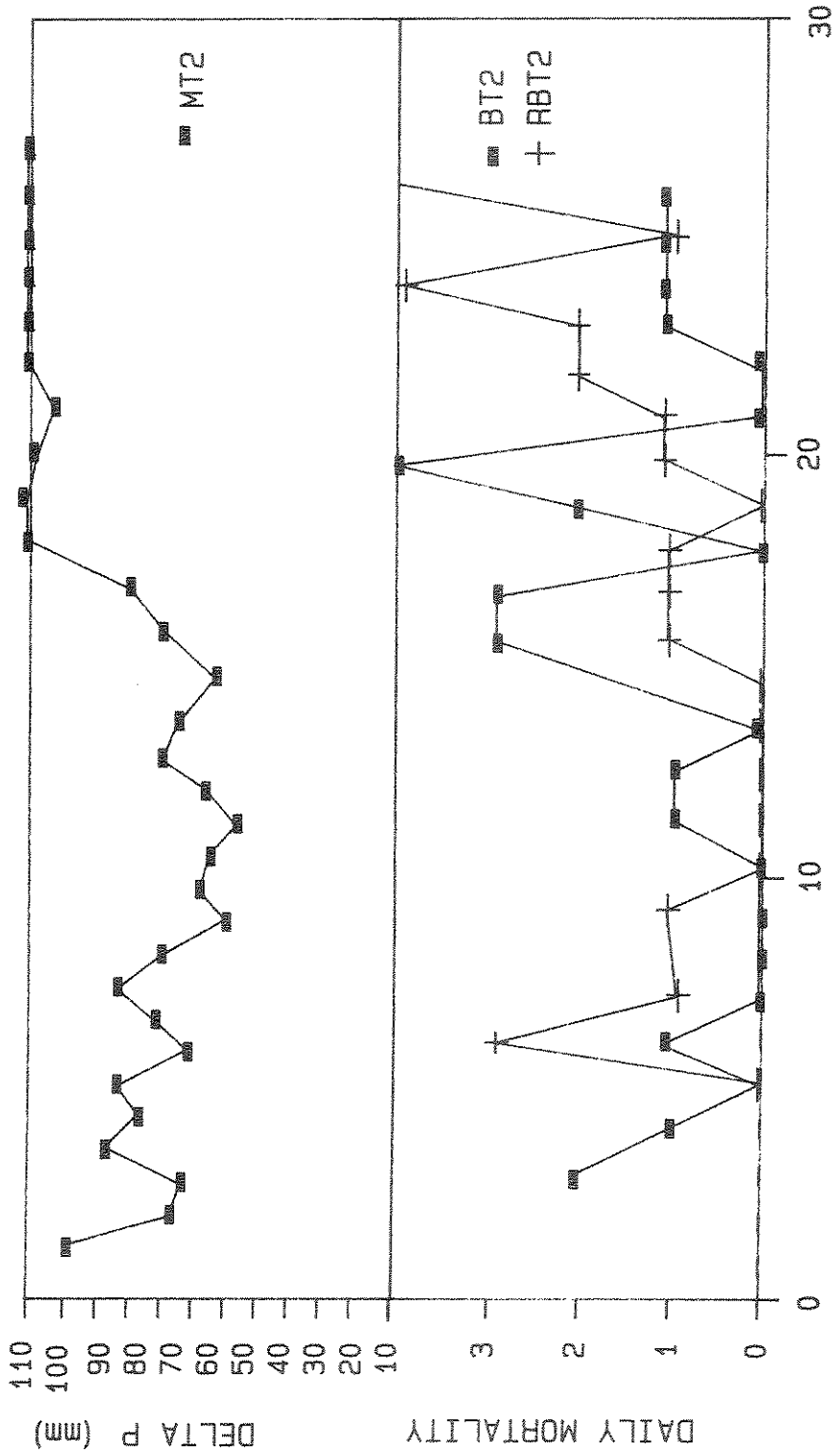

Figure 12. Comparison of medium treatment (MT) juvenile brown (BT) and rainbow trout (RBT) daily mortality and associated ^P values by day during test 2, May 18 - June 11, 1986.

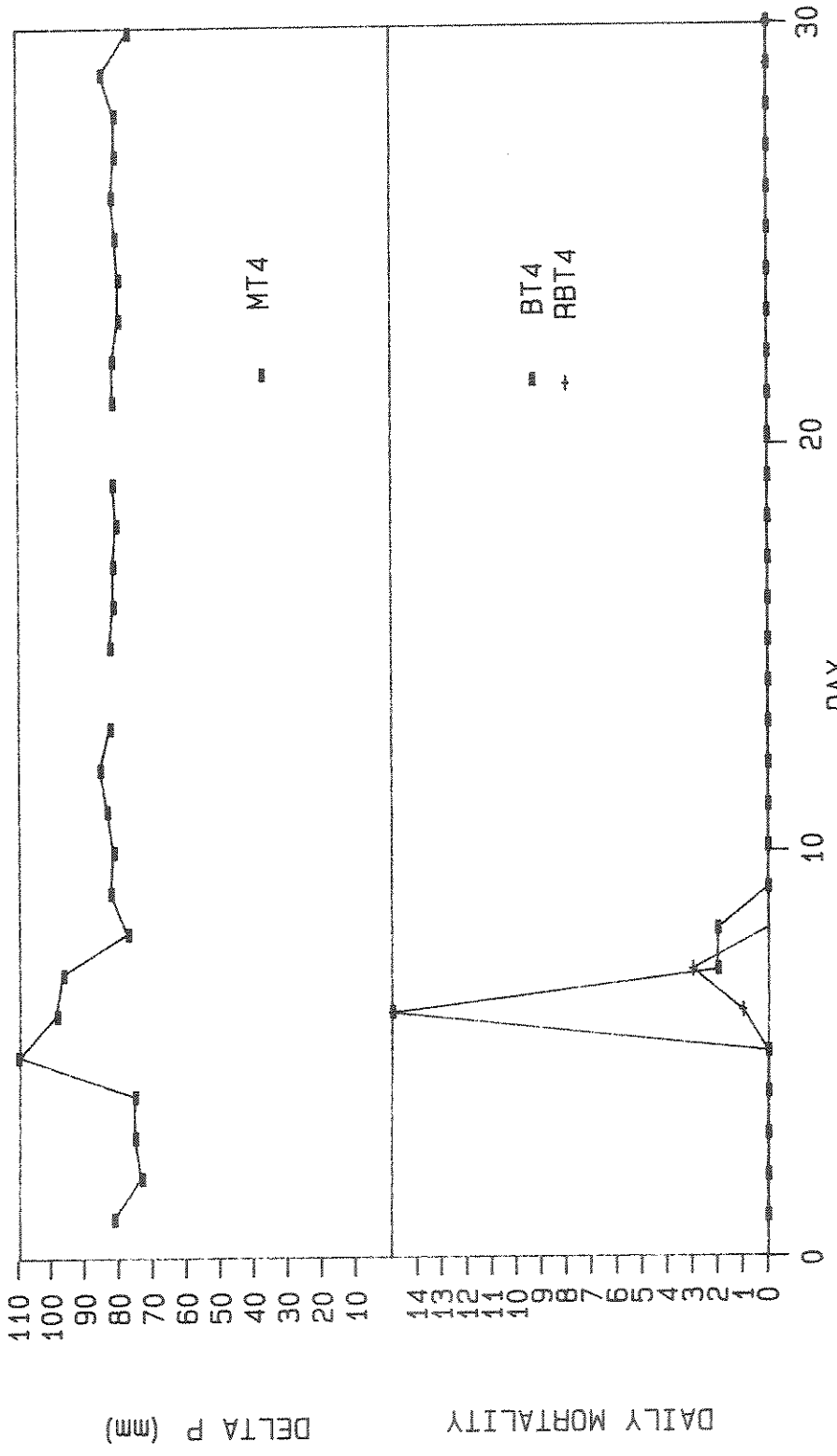

Figure 13. Comparison of medium treatment (MT) juvenile brown (BT) and rainbow trout (RBT) daily mortality and associated ^P values by day during test 4, September 28 - October 29, 1986.

Mortality of rainbow and brown trout of the high gas treatment was significantly greater than that of control fish in all cases except for test 1 rainbow trout (Table 9). Several test 1 control treatment rainbow trout fry died and high treatment rainbow trout mortality was low. The relevance of these data is discussed later.

Table 9. Breslow rank analysis of medium gas treatment percent cumulative mortality for juvenile brown (BT) and rainbow trout (RBT) during tests 1, 2, 3, 4, 5, and 6.

Mortality Comparison					
Control treatment			Medium treatment		p-value
species	test	outcome	species	test	
BT	1	<	BT	1	< 0.0001*
BT	2	<	BT	2	< 0.0001*
BT	3	<	BT	3	< 0.0001*
BT	4	<	BT	4	< 0.0001*
BT	5	<	BT	5	< 0.0001*
BT	6	<	BT	6	< 0.0001*
RBT	1	=	RBT	1	0.7760
RBT	2	<	RBT	2	< 0.0001*
RBT	3	<	RBT	3	< 0.0001*
RBT	4	<	RBT	4	< 0.0001*
RBT	5	<	RBT	5	< 0.0001*
RBT	6	<	RBT	6	< 0.0001*

* Statistically different at alpha = 0.05

Influence of Length and Weight on Susceptibility to GBT

Length and weight of juvenile brown and rainbow trout had a significant influence on susceptibility to super-saturated water. During test 1 percent cumulative mortality for both species exposed to the high gas treatment was significantly less for small fish than for large fish (Table 10, Figures 14 and 15). However, there was no statistical difference in brown trout mortality between tests 4 and 5 or rainbow trout mortality between tests 4 and 6 (Table 10).

Table 10. Breslow rank analysis of high gas treatment percent cumulative mortality for juvenile brown and rainbow trout exposed to the high gas treatment during tests 1, 2, 3, 4, 5, and 6.

Mortality comparison outcome	p-value
BT1 < BT2	< 0.0001*
BT2 < BT3	< 0.0001*
BT3 < BT4	< 0.0001*
BT4 = BT5	0.7009
BT5 < BT6	0.0019*
RBT1 < RBT2	< 0.0001*
RBT2 < RBT3	< 0.0001*
RBT3 < RBT4	< 0.0001*
RBT4 = RBT6	0.1778
RBT6 < RBT5	0.0295*

* Statistically different at alpha = 0.05

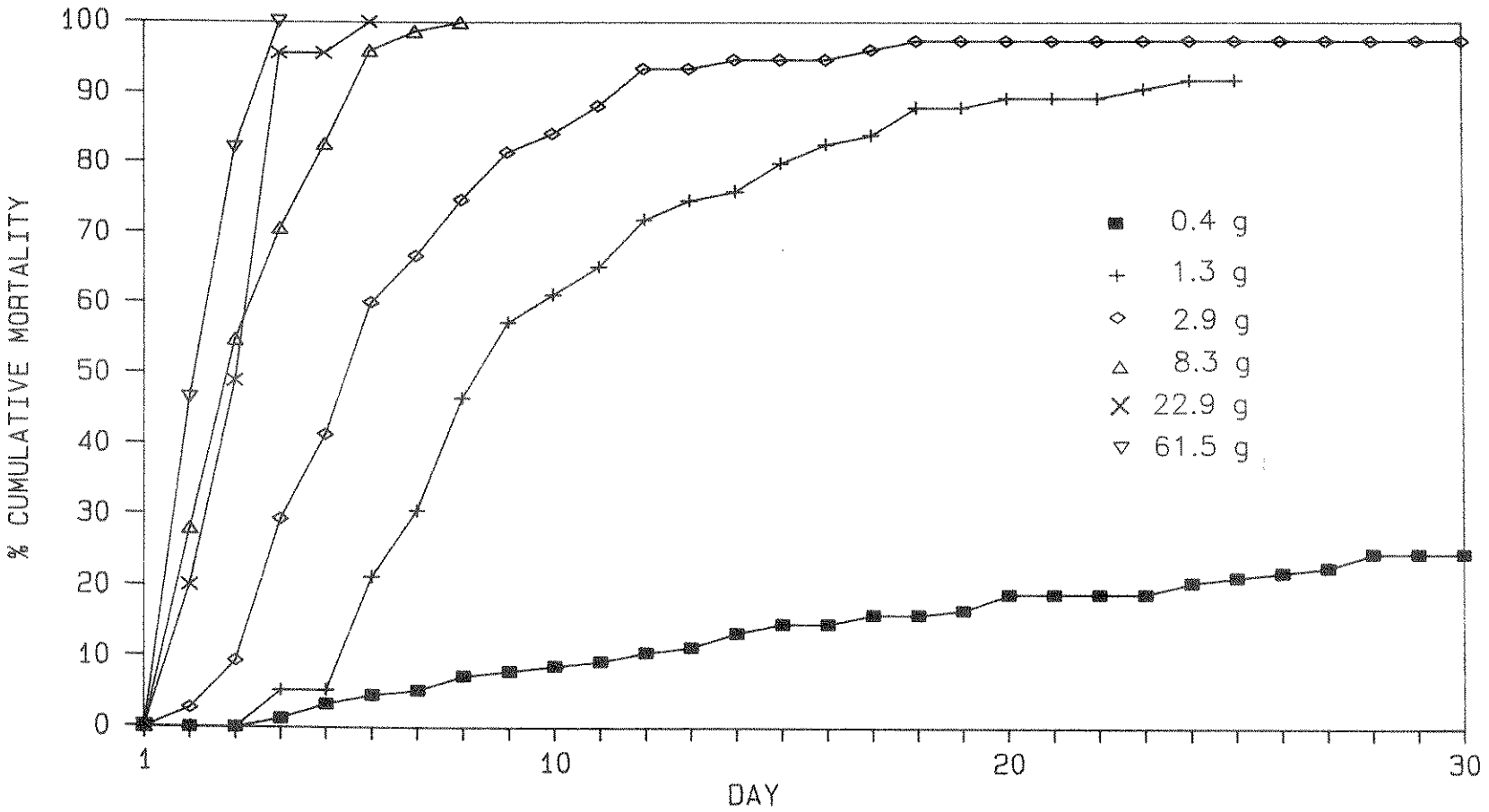

Figure 14. High treatment (125%) juvenile brown trout percent cumulative mortality by day, during tests 1, 2, 3, 4, 5, and 6 (BT1 = 35.2 mm and 0.4 g, BT2 = 53.1 mm and 1.3 g, BT3 = 62.2 mm and 2.9 g, BT4 = 90.7 mm and 8.3 g, BT5 = 126.3 mm and 22.9 g, BT6 = 172.8 mm and 61.5 g).

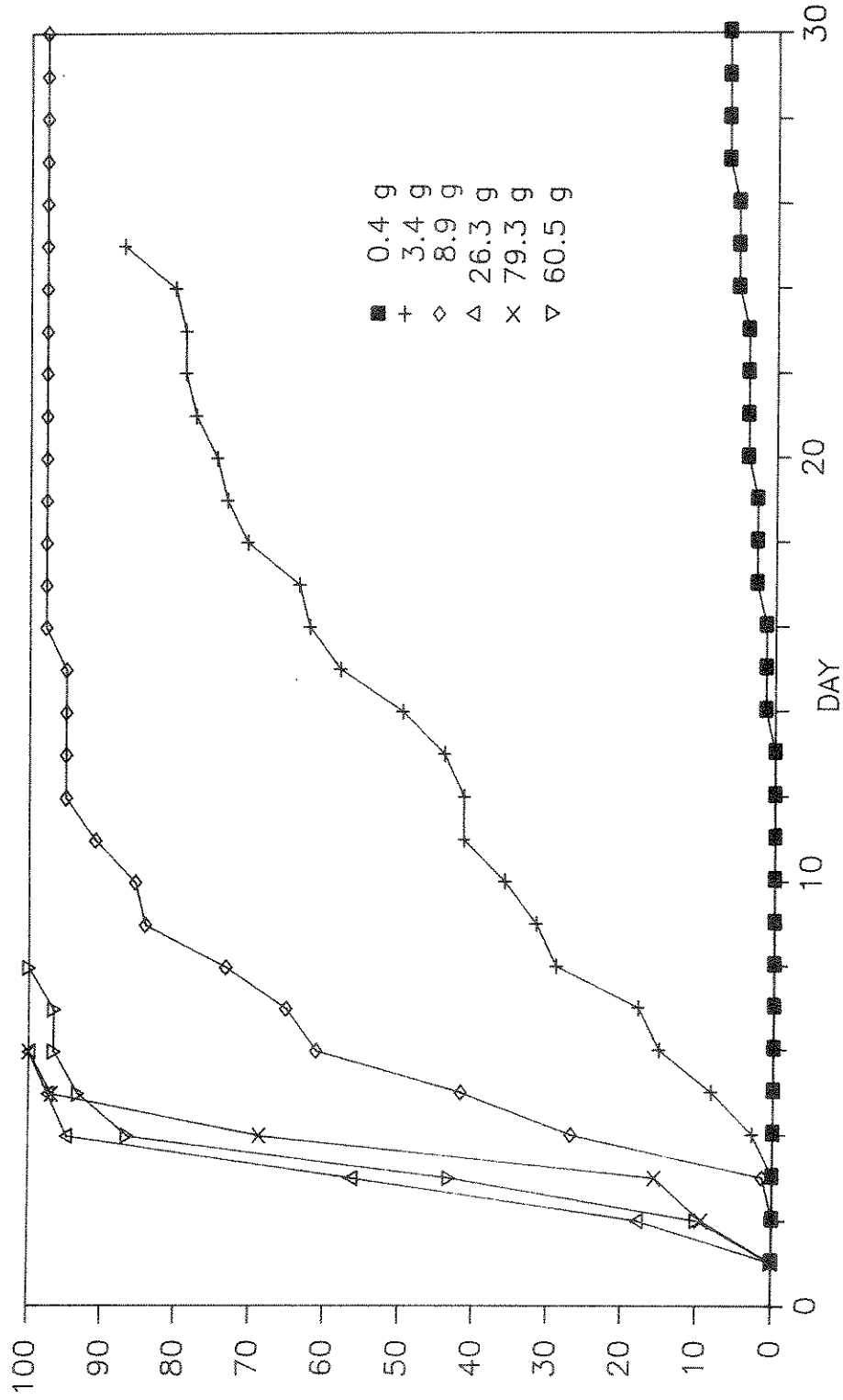

Figure 15. High treatment (125%) juvenile rainbow trout percent cumulative mortality by day, during tests 1, 2, 3, 4, 5, and 6 (RBT1 = 34.0 mm and 0.4 g, RBT2 = 70.5 mm and 3.4 g, RBT3 = 90.6 mm and 8.9 g, RBT4 = 130.5 mm and 26.3 g, RBT5 = 194.1 mm and 79.3 g, RBT6 = 178.7 mm and 60.5 g)

Available literature indicates that fish become more sensitive to gas supersaturated water as they increase in size. Meekin and Turner (1974) found large juvenile chinook salmon exposed to 124% supersaturation suffered greater mortality than did smaller fish of these species. Rucker (1976) tested two size groups of coho salmon (3.8 - 6.0 cm and 8.0 - 10.0 cm) in 119% gas supersaturated water and concluded that larger fingerlings were more subject to harm than smaller fish. Jensen et al. (1986) predicted that larger anadromous juvenile salmonids should be more sensitive to effects of gas supersaturated water than are smaller fish.

The basis for increased susceptibility to gas supersaturated water with size remains unclear. The mechanics of bubble formation (emboli) may offer an explanation for increasing sensitivity to gas supersaturated water with increasing fish size. Harvey et al. (1944) explained the mechanism of emboli formation in the blood stream of vertebrates. Undissolved gases in the blood stream begin to form emboli when they adhere to "nucleation sites" (rough or irregular regions in the vascular system or body tissue). As the delta P of the blood increases, the radius of a gas bubble on the nucleation site expands as gas diffuses into it. If supersaturated conditions continue long enough at a high enough level the buoyant force of the bubble becomes

sufficient for it to dislodge from the nucleation site.

Fidler (1985) hypothesized that increased susceptibility is closely associated to the radius and number of nucleation sites in a fish. Two reasons were given as support for this hypothesis. The gas level threshold required for bubble formation is lower for large nucleation sites. Consequently, smaller fish (with smaller nucleations sites) would suffer less severe emboli formation than a larger fish at a given percent TGP. Secondly, larger fish may have greater discontinuity in tissue and vascular wall structure. This may provided a greater number of nucleation sites and result in more emboli.

My results generally support Fidler's (1985) theory with results of test 4 being the only exception. Total gas pressure was 1% higher during test 4 than in any other test and may have influenced mortality.

External symptoms of GBT in fishes have been thoroughly described (Woodbury 1941, Renfro 1963, Beiningen and Ebel 1970, Harvey 1975, Dawley et al. 1976, Nebeker and Brett 1976, Stroud and Nebeker 1976, Thorn et al. 1978, and Weitkamp and Katz 1980). The progression of symptoms with increasing fish size, however, has not been completely investigated. In my study, emphysema occurrence on certain regions of the body varied with fish size (Figures 16 and 17). Oral emphysema (buccal cavity,

mandibles, and maxillaries), the most common external symptom in small trout, and opercular emphysema occurred less frequently as fish grew. Exopthalmia was uncommon except in brown trout of test 1 (35 mm and 0.4 g). Emphysema in the lateral line was also rare except in rainbow trout in test 3 (91 mm and 8.9 g). Emphysema on the fins became more prominent as fish size increased, especially for rainbow trout in tests 3 and 4. As the size of the brown trout increased, more died without external symptoms (Figure 16). Rainbow trout did not follow this trend (Figure 17).

The higher frequency of emphysema in and around the buccal cavity of smaller brown (0.3 and 1.3 g) and rainbow trout (0.4 and 3.6 g) (Figures 16 and 17) may have been related to duration of exposure to gas supersaturated water. Dawley et al. (1976) suggested that emphysema in these areas requires more time to develop than other symptoms. In my study smaller fish survived longer than larger fish, therefore they were exposed longer. Exop-thalmia was also more frequent in the smallest brown trout, which survived the longest.

Bubble formation in the lateral line is reported to be the earliest external symptom of GBT in juvenile salmonids (Schiewe and Weber 1976). Results of my study do not concur with this finding. However, I may have missed bubbles since I used only a disecting scope to

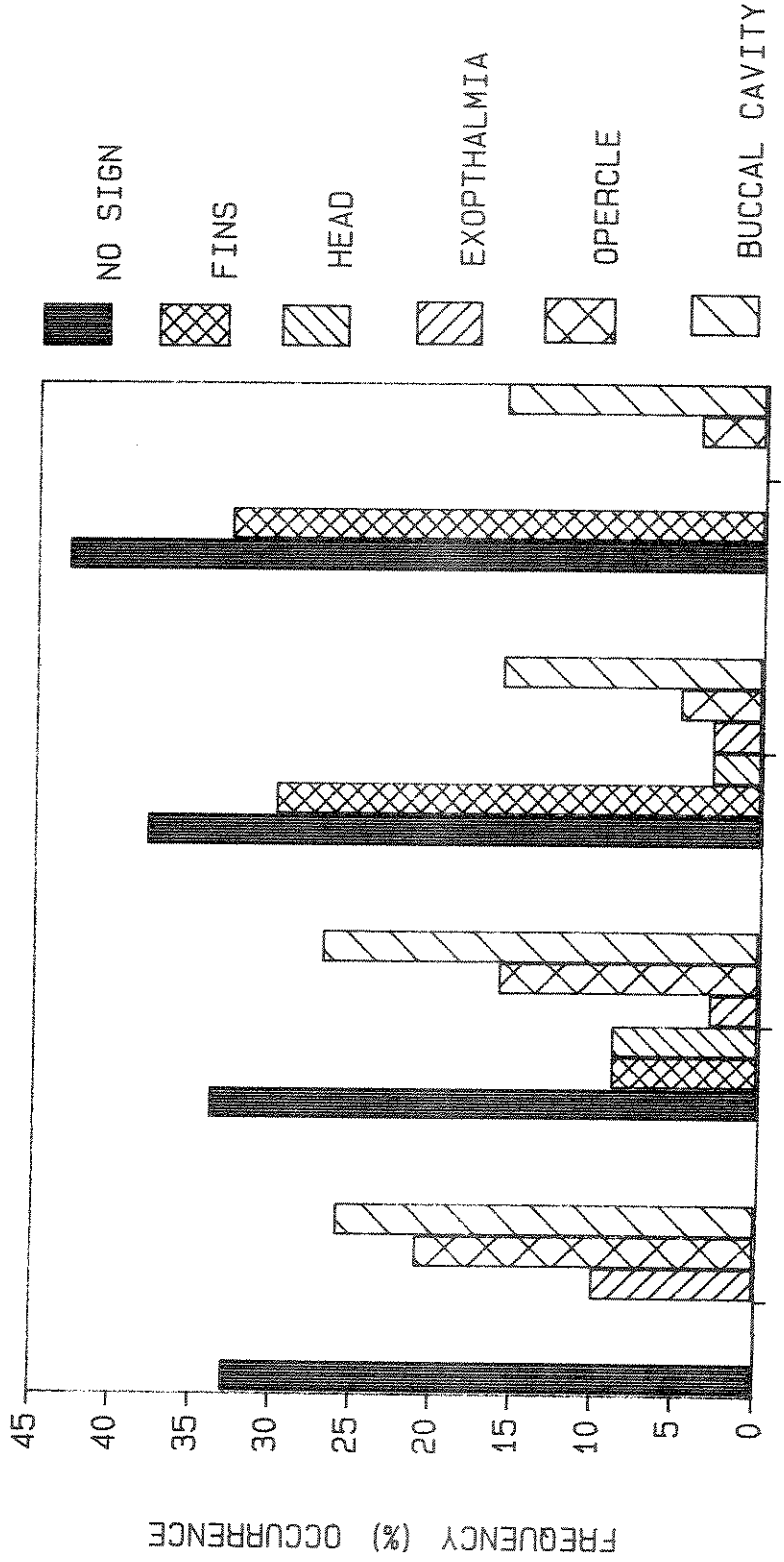

Figure 16. Frequency (%) of dead juvenile brown trout with external signs of GBT resulting from exposure to 125 % gas supersaturated water.

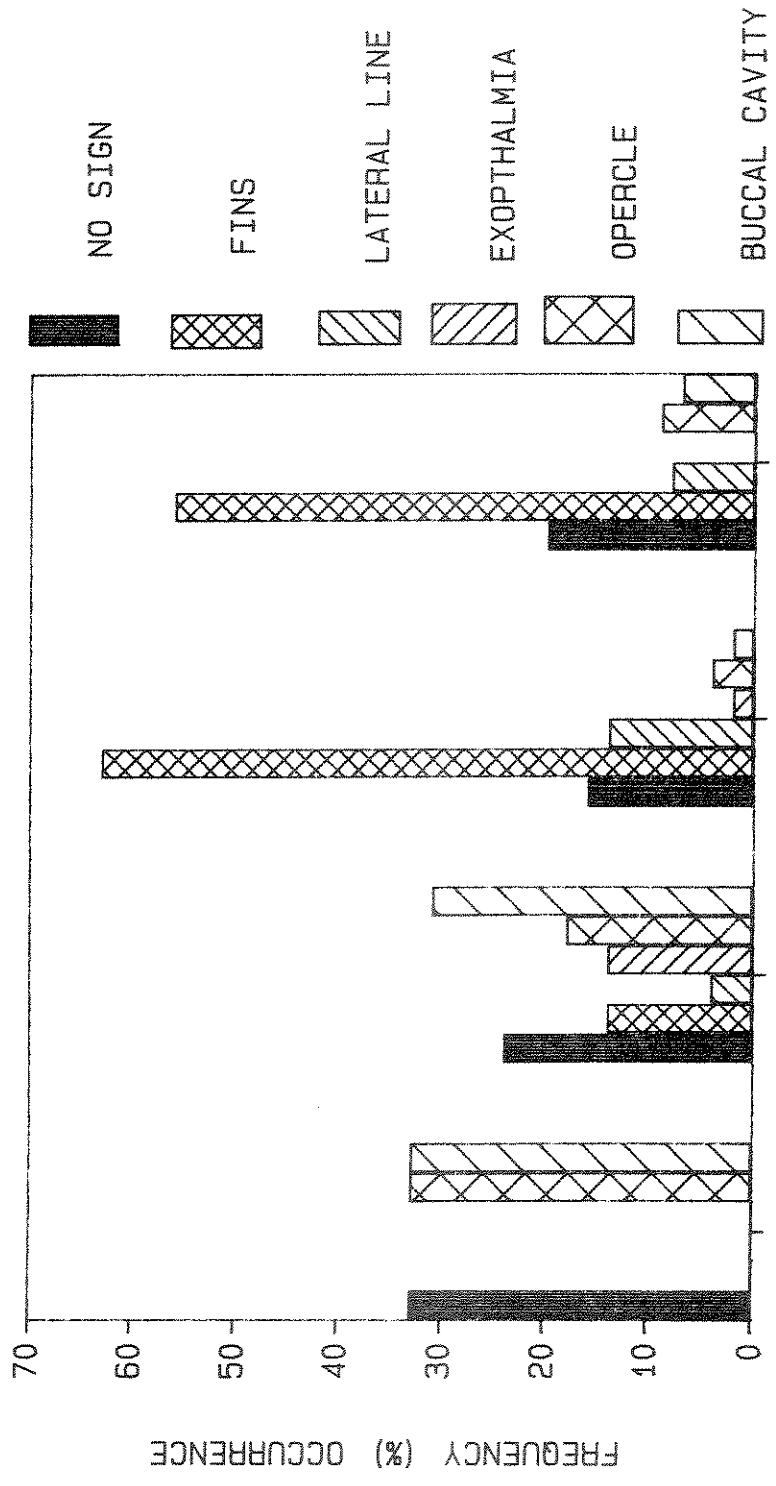

Figure 17. Frequency (%) of dead juvenile rainbow trout with external signs of GBT resulting from exposure to 125 % gas supersaturated water.

examine fish during early tests and lateral line bubbles are small and may require a microscope to detect. Also, bubbles in the lateral line may have disappeared before daily removal and examination of dead fish, since they do not persist long after death (Weitkamp and Katz 1980).

The percentage of brown trout that died without external symptoms increased from 33 - 43% in tests using 0.3 to 8.3 g fish (Figure 16). This may have been due to larger brown trout dying before external symptoms formed.

Conversely, 58 - 62% of dead rainbow trout (8.9 and 26.3 g) exhibited emphysema on the fins (Figure 17). Weitkamp (1976) reported similar results for 97-112 mm juvenile chinook salmon indicating that the body region of emphysema occurrence may vary with species.

Species Influence on Susceptibility to Gas Supersaturation

Mortality rate of brown trout exposed to high gas levels (125%) was significantly greater than that of rainbow trout of similar size (Table 11). The difference in susceptibility was greatest when fish were smallest (Figure 18) and became progressively less evident as fish grew (Figures 19, 20,and 21).

Variations in susceptibility to GBT have been reported for different species of fish. Coho salmon (*O. kisutch*) were more tolerant to water supersaturated with

nitrogen in the presence of decreasing water temperatures than were chinook salmon or steelhead trout (Ebel et al. 1971). Coho salmon were also more tolerant (LC_{50} = 116.2%) of gas supersaturated water than were steelhead trout (LC_{50} = 114.0%) or sockeye salmon (LC_{50} = 113.9%), (Nebeker and Brett 1976). Fickeisen and Montgomery (1978) found the median time to death caused by exposure to gas supersaturated water to be longest for whitefish (Prosopium williamsoni), followed in descending order by cutthroat trout (Salmo clarki), large scaled sucker (Catostomous macrocheilus), and torrent sculpin (Cottus rhotheus). Thorn et al. (1978) exposed rainbow and brown trout to 122%, 127%, and 129% gas supersaturated water for 7-h. They reported higher brown trout mortality in the

Table 11. Breslow rank analysis comparing the rate of mortality for juvenile brown and rainbow trout of similar size in the high gas treatment.

Mortality comparison outcome	p-value
BT1 > RBT1	< 0.0001*
BT3 > RBT2	< 0.0001*
BT4 > RBT3	< 0.0001*
BT6 > RBT6	< 0.0002*

* Statistically different at alpha = 0.05

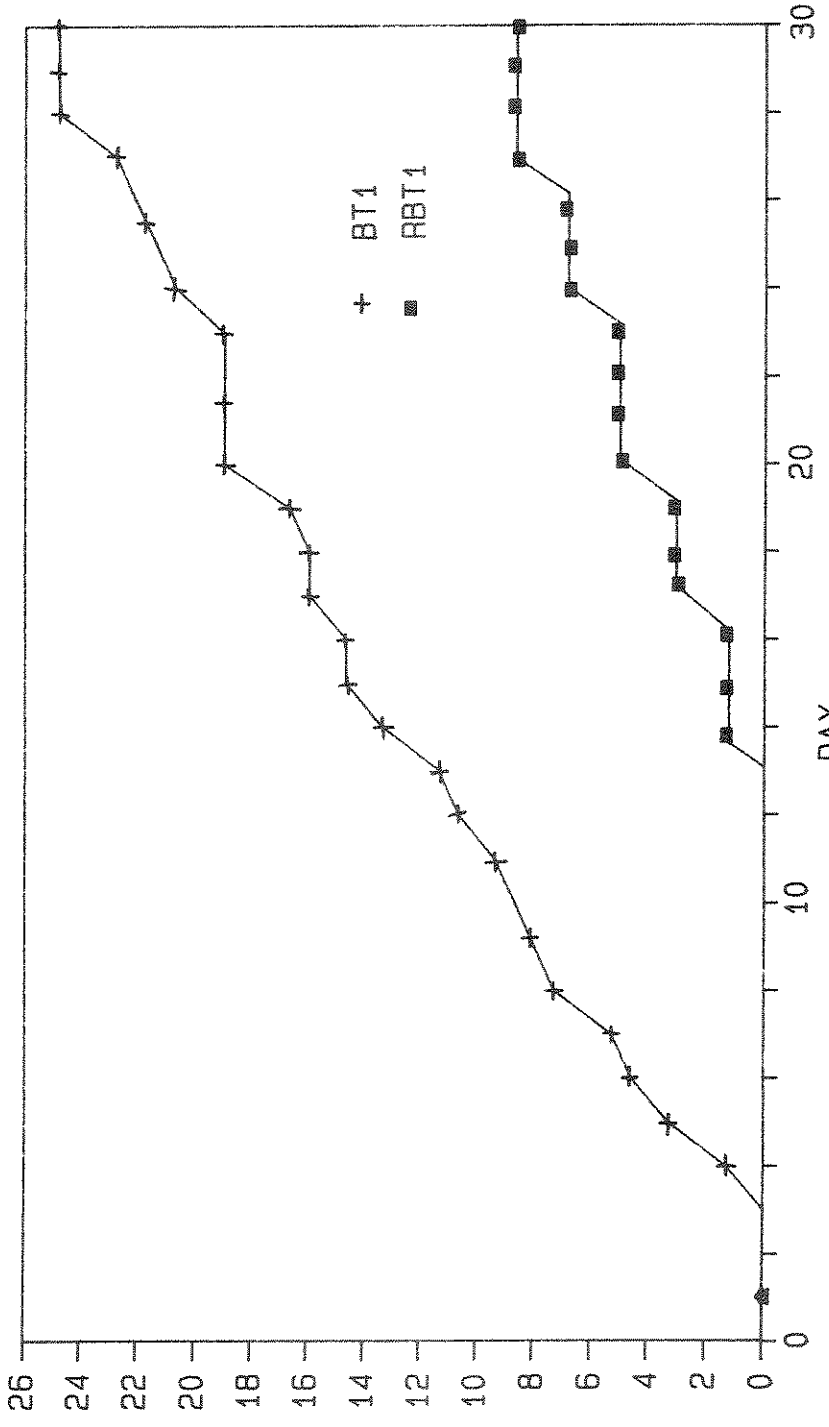

Figure 18. High treatment juvenile brown (mean beginning length and weight = 35 mm and 0.4 g) and rainbow trout (mean beginning length and weight = 34 mm and 0.4 g) percent cumulative mortality by day during test 1.

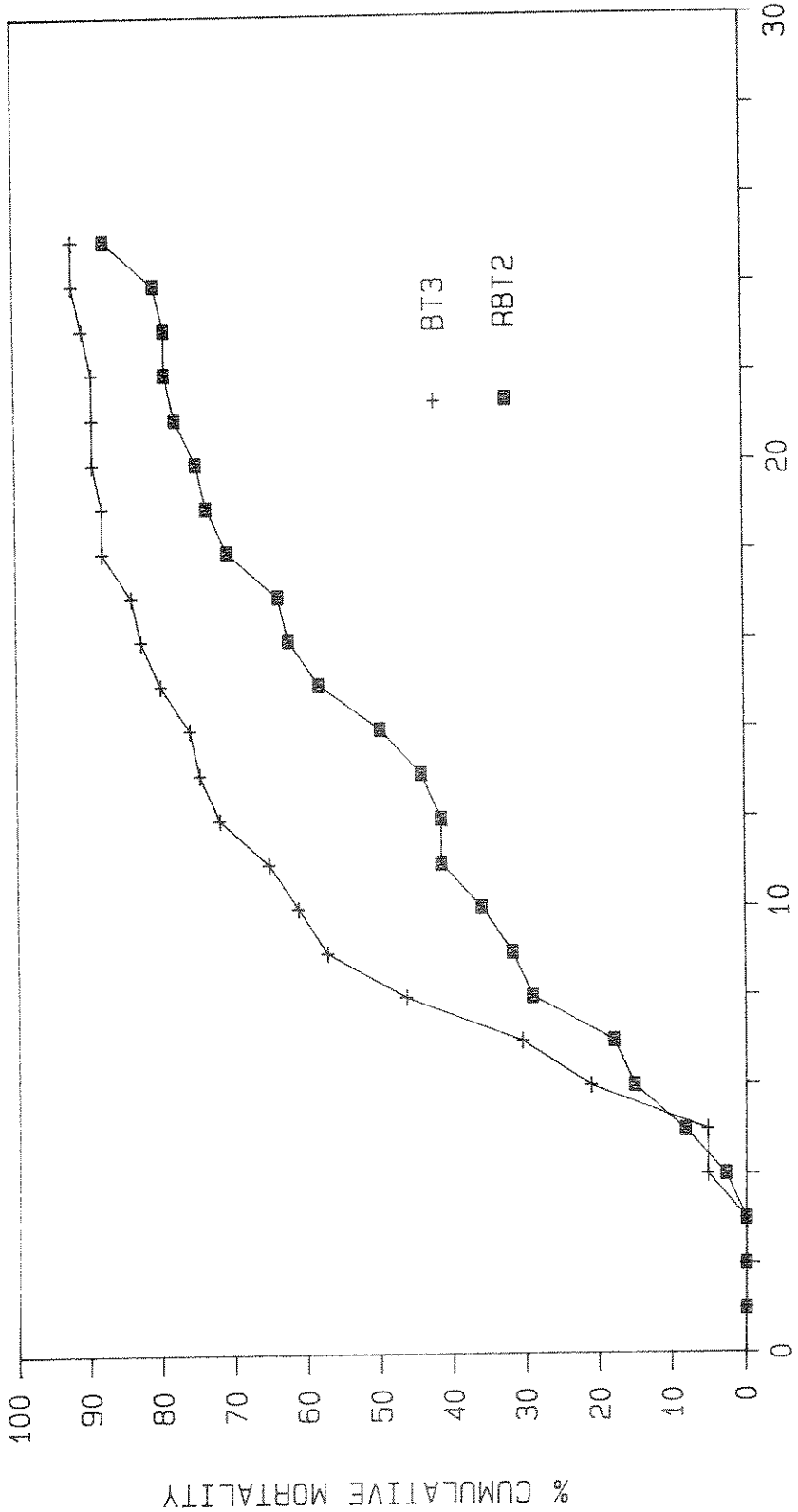

Figure 19. High treatment juvenile brown (mean beginning length and weight = 62 mm and 2.9 g) and rainbow trout (mean beginning length and weight = 71 mm and 3.4 g) percent cumulative mortality by day during tests 3 and 2, respectively.

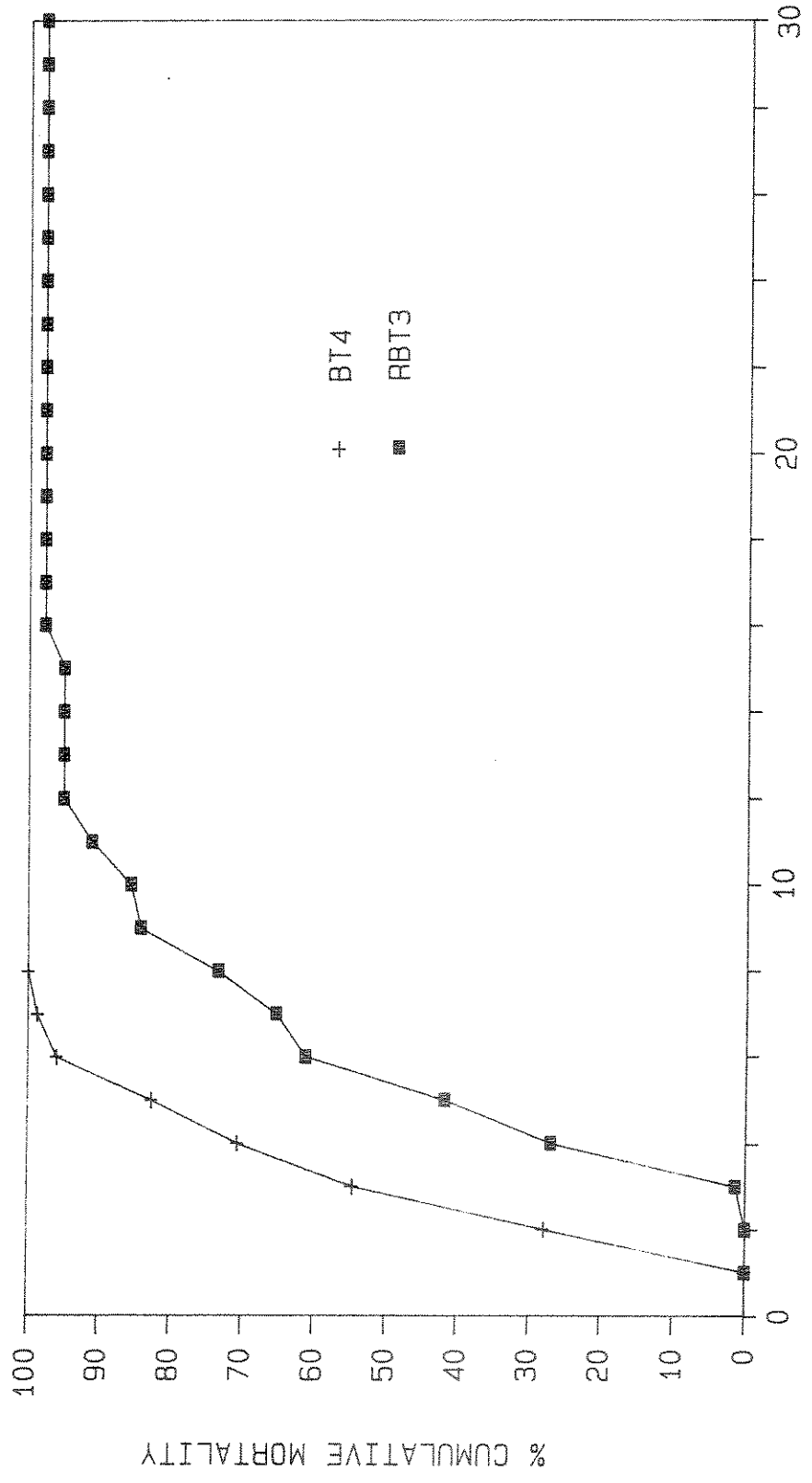

Figure 20. High treatment juvenile brown (mean beginning length and weight = 91 mm and 8.3 g) and rainbow trout (mean beginning length and weight = 90 mm and 8.9 g) percent cumulative mortality by day during tests 4 and 3, respectively.

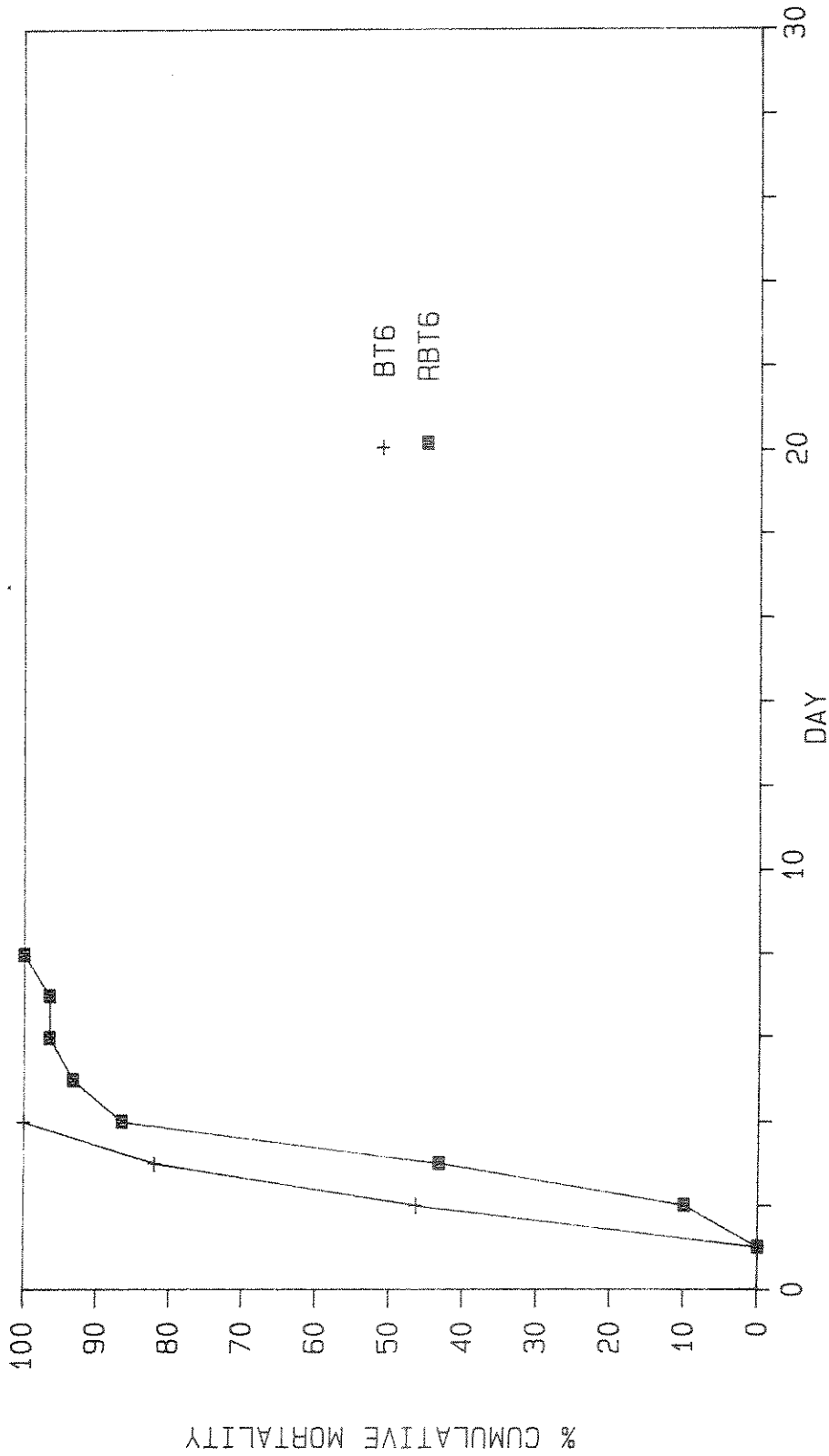

Figure 21. High treatment juvenile brown (mean beginning length and weight = 173 mm and 61.5 g) and rainbow trout (mean beginning length and weight = 179 mm and 60.5 g) percent cumulative mortality by day during test 6.

122% exposure test, while rainbow trout mortality was larger at the two higher gas levels tested. Mean time to 50% mortality at four lethal concentrations of gas supersaturated water was longer for speckled dace (Rhinicthys osculus) than cutthroat trout (Nebeker et al. 1980). Sea bass (Dicentrarchu labrax) and striped mullet (Mugil cephalus) had similar mortality responses when exposed to 127.4% and 129.6% gas supersaturated water (Gray et al. 1985). Differences in the anatomy of rainbow and brown trout may help provide an explanation for interspecific variation in mortality. According to Fidler (1985) emboli form faster in a fish with more rough areas in the walls of the vascular system. Perhaps, during the earliest life stages tested, brown trout vascular tissue was more irregular than that of rainbow trout. Irregularities in vascular tissue of rainbow trout may increase with fish size explaining the similarity in mortality of the species in later tests. Thorn et al. (1978) suggested that differences in interspecific mortality may be related to the capacity of the gas bladder. Increased capillary area on the bladder would facilitate passage of gas from the blood stream to the gas bladder, where it could be passed from the pneumatic duct. The vascular structure or functioning of the pneumatic duct may differ between brown and rainbow trout. A conclusive explanation for differences in interspecific mortality remains unknown.

Variation in occurrence of external symptoms (including exopthalmia) of GBT was noted between surviving brown and rainbow trout. Juvenile brown trout surviving exposure to 112% supersaturation exhibited more external symptoms than juvenile rainbow trout of similar size (Table 12).

Table 12. Frequency of occurrence of external symptoms of GBT on juvenile rainbow and brown trout survivors from the medium treatments (112% saturation) of tests 3, 4, 5, and 6 with associated p-values generated using Fisher's exact test.

Fish size (mm)		Rainbow trout survivors with signs of GBT		Brown trout survivors with signs of GBT		p-value
rainbow trout	brown trout	#	%	#	%	
100	94	4	6	12	23	0.003*
138	136	6	15	32	71	0.000*
182	177	4	13	14	47	0.006*

* Statistically different at alpha = 0.05

External symptoms of GBT form more readily on some species of fish than others. Ebel (1969) noted symptoms of GBT in 10 of 1,000 sockeye salmon (Oncorhynchus nerka) while no symptoms were observed in 1,762 Chinook salmon and steelhead trout (Salmo gairdneri) collected from the

Columbia River. Ebel concluded that juvenile chinook salmon and steelhead trout were either using depth to compensate for the 126% saturation or were less susceptible than sockeye salmon. Exopthalmia was more common in brown than in rainbow trout reared 36 weeks in identical supersaturations (Poston et al. 1973). Observations collected between 1968 and 1973 from the Columbia River revealed that 47% of adult sockeye salmon, 40% of steelhead trout, and 26% of American shad (Alosa sapidissima) possessed external symptoms of GBT; lampreys (Ichthyomyzon sp.) showed no symptoms (Bouck et al. 1976). Thorn et al. (1978) investigated the occurrence of eye damage found in rainbow, brook, and lake trout (Salvelinus namycush). Based on data from similar exposure periods and saturation levels these researchers showed that lake trout had the highest incidence of eye damage followed by rainbow, brook, then brown trout. Seventy-two percent of smallmouth bass (Micropterus dolomieui) and 84% of northern squawfish (Ptychocheilus oregonensis) collected simultaneously by anglers in the lower Snake and Columbia River drainages had external lesions associated with GBT (Montgomery and Becker 1980).

The difference in external symptom formation found between species in my tests appears to be related their physiology since fish were kept in identical environments. Emphysema formation may be the result of excessive trans-

port of gas from water into the epidermal cells of fish. The rate and amount of gas that passes through the skin is inversely proportional to epidermal thickness and directly proportional to capillary density beneath the epidermis (Hoar and Randall 1970). Brown and rainbow trout skin thickness or subcutaneous vascularization may differ.

Recovery from a Single Exposure to Gas Supersaturation

Juvenile brown and rainbow trout recovery (loss of external symptoms) from GBT varied with severity of external symptoms. Less severely affected rainbow trout recovered more often and faster (Table 13 and Figure 22). Five of six rainbow trout that did not recover within 30-d exhibited eye damage characterized by severe unilateral and bilateral exopthalmia, hemorrhaging, cloudiness, corneal cataracts, or bubbles in the eye orbit.

Juvenile brown trout with emphysema and mild exopthalmia recovered within 30-d. However brown trout with widespread emphysema and severe exopthalmia (GBT rating 3, Table 6) recovered less often (Table 14).

Exopthalmia developed during recovery in the two fish that did not recover from 17-d exposure to 111% gas supersaturated water (Table 14). Two brown trout rated 3 after exposure to 117% TGP for 5-d died rapidly after transfer to a recovery tank.

Table 14. Recovery of juvenile brown trout (133 ± 21 mm and 27.5 ± 9.5 g) by severity level, following exposure to various levels and durations of exposure to gas supersaturation, February 9, 1987.

GBT[a] rating	Beginning number	Exposure duration (days)	Gas level (%TGP)	Number dead after 30-d	Number retaining symptoms after 30-d
1	15	17	111	0	0
1	10	30	111	0	0
2	3	5	117	0	0
2	2	30	111	0	0
3	2	5	117	2	0
3	3	17	111	0	2

a = see Table 6 for rating description

Recovery from GBT has been documented for several species. Emphysema and exopthalmia regressed in scup (Stenotomus chrysops) within 24-h of removal from shallow tanks to tanks with pressure equal to 16 feet of water (Gorham 1901). Cutthroat, rainbow, and steelhead trout fingerlings raised in hatchery troughs at Puyallup Hatchery of the Washington State Department of Game showed high incidence of GBT symptoms. Transfer of fingerlings to an outside pond eliminated all external symptoms (Rucker and Hodgeboom 1953). Impaired swimming ability of juvenile chinook salmon exposed to 120% supersaturation was rectified within 2-h after removal to 100% saturated water (Schiewe 1974). Eratic swimming and yolk sac emboli

were alleviated in coho salmon sac fry using a recompression chamber (Adams 1974). Dawley et al. (1976) determined that 2 weeks was an adequate recovery period for juvenile chinook with GBT acquired from exposure to 110 - 120% supersaturation for 120-d. Ninety-one to 95% percent of juvenile chinook salmon surviving 10 - 20-d exposure in 1 - 4 m of water of 120 - 131% gas supersaturation lost all external symptoms of GBT after 20-d in an unsupersaturated environment (Weitkamp 1976). Apparently most fish with GBT will recover if moved to an unsaturated environment.

Eye damage in surviving salmonids may be the principal permanent effect of exposure to sublethal doses of gas supersaturated water. Dawley et al. (1976) removed chinook salmon with GBT to a non-supersaturated environment in which 95% of the fish recovered. The remaining 5% suffered hemorrhaging or bubbles in the orbit of the eye. The degree to which this condition could affect the well-being of the fish depends on the severity of the eye damage.

Physical degradation of the eye components from exopthalmia can be irreversible. Corneal lesion formation can be a precursor to loss of eye function (Hoffert and Fromm 1965). Machado et al. (1987) reported that juvenile rainbow trout exposed to 116.9 $\pm$ 0.7 percent nitrogen and 110.1 $\pm$ 4.6 percent oxygen supersaturation experienced

eye damage characterized by formation of space between the capillary layer of the choroid and the pigment epithelial layer of the retina, and degenerative vacuolization of the optic nerve, musculature, adjacent connective tissue, and glandular tissues of the fish. Two brown trout and one rainbow trout that eventually died were removed from gas supersaturated water during the "convulsive stage". This stage usually preceded death by a few minutes and is characterized by side-swimming, abnormal buoyancy, whirling movements accompanied by inactivity, and spasmodic convulsions (Machado et al. 1987). At this point emboli formation in the vascular system was probably at an irreversible stage.

The occasional formation of exopthalmia during recovery was perplexing. I originally attributed this to frequent handling of fish for rating, sharp blows against recovery tank walls, and exposure to the low level of supersaturation in the recovery tanks. However, post-exposure eye damage has been documented and interpreted differently. Bouck et al. (1976) reported perforation of the cornea, lens loss, and acute necrosis of the eyeball in adult sockeye salmon after removal from the supersaturated water of the Columbia River. The capture process and holding environment were dismissed as primary factors responsible for post-capture eye damage. The researchers hypothesized that the main cause of this problem was

previous exposure to gas supersaturated water, a theory they supported with data from other work completed on the Columbia River. A definitive explanation for post-exposure eye damage is still unavailable.

Effects of Repeated Exposure

Recovery and Sensitization

The recovery (loss of external symptoms) of juvenile brown trout from three 30-d exposures to 118% gas supersaturated water was influenced by severity of external symptoms. Most fish rated 1 (Table 6) after exposure recovered (Table 15). Two of 12 fish rated 1 after the first exposure did not totally recover due to eye damage incurred during the recovery period. Fifty percent of fish rated 2 after the first two exposures recovered. Fish that did not totally recover had permanent eye damage. Both fish rated 3 after initial exposure died during recovery (Table 15). One of these fish was in the "convulsive stage" when it was removed from gas supersaturated water. The second fish suffered from bilateral exopthalmia, impaired swimming, and severe emphysema. Permanent eye damage prohibited recovery of fish rated 3 after the second exposure. One of two fish that had no external symptoms after the second exposure was killed accidently when the recovery tank was cleaned. The third 30-d recovery period was affected when water temperature

increased to 23 ^{o}C for 24-h. Three of the six survivors died from a Pseudomonad sp. infection. One of the three surviving fish was rated 1 and totally recovered within 30-d, while one rated 2 and one rated 3 did not recover because of permanent eye damage (Table 15).

Overall, recovery of juvenile brown trout from GBT decreased with each exposure (Figure 23). Extreme eye damage (often occurring after previous less severe eye damage) and progression to the convulsive stage were primary reasons for lack of recovery.

Increased symptom severity in fish surviving an initial exposure to gas supersaturated water was noted by Gorham (1901). He subjected scup to a sequence of decompression/ recompression/ decompression three to four times for 30-min intervals. Eye bubbles from initial decompression grew during later decompression. Some juvenile brown trout in my study may also have sensitized to GBT.

Table 15. GBT rating,mortality and percent recovery of juvenile brown trout (fish size on day 1 of testing = 164 ± 17 mm and 43.2 ± 14.4 g) by GBT rating exposed to 118% gas supersaturated water for 120-h, three times and given 30-d to recover between exposures June 6 - September 18, 1987.

Rating	First exposure			Second exposure			Third exposure		
	# of fish	# dead by day 30	% recovery by day 30	# of fish	# dead by day 30	% recovery by day 30	# of fish	# dead by day 30	% recovery by day 30
0	0	0	---	2	1*	---	0	0	0
1	12	0	83	0	0	---	2	1*	100
2	6	0	50	4	0	50	3	2*	0
3	2	2	0	1	0	0	1	0	0

*Death not related to GBT

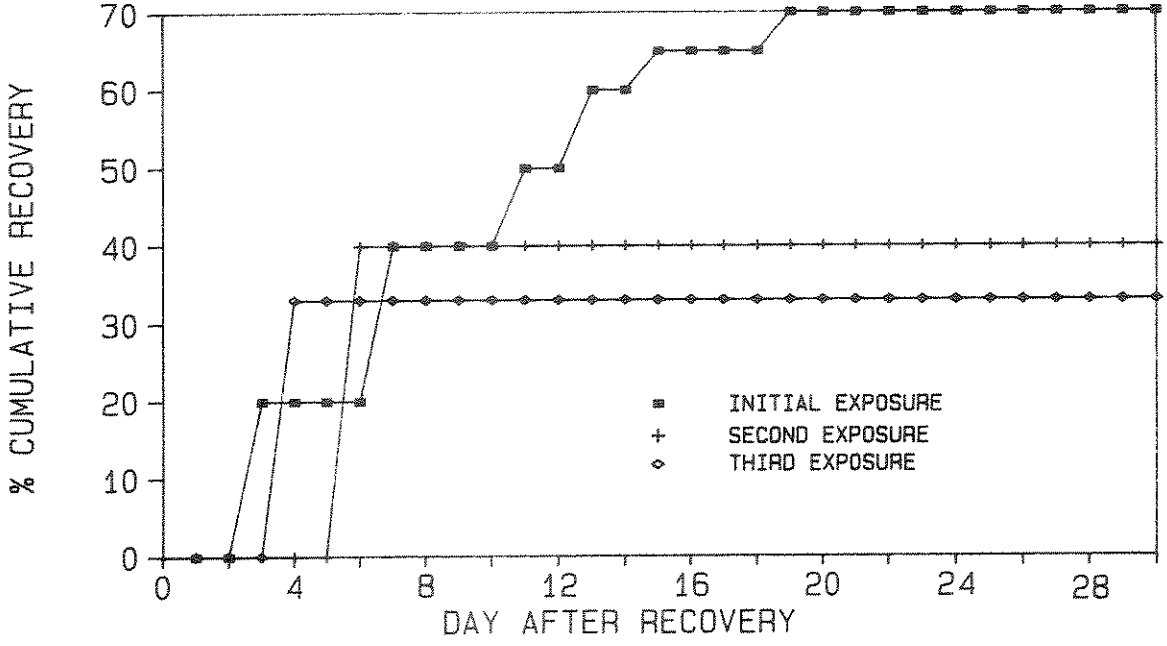

Figure 23. Percent cumulative recovery for juvenile brown trout (fish size on day 1 of testing = 164 ± 17 mm and 43.2 ± 14.4 g) from GBT calculated by combining GBT rating groups 1, 2, and 3 (see Table 6) from three 120-h exposure tests conducted June 6 - September 18, 1987.

Acclimization and Tolerance to Repeated Exposure

Different proportions of GBT rating were observed in the three progressive exposures. Mortality (rating 4;Table 6) of juvenile brown trout was significantly greater during the first and second exposure to gas supersaturated water than during the third exposure (Tables 17 and 18). A significantly greater proportion of fish were rated 1 after the first exposure than in subsequent exposures (Tables 16 and 17). The proportion of fish rated 2 increased significantly from exposure 1 to exposure 3 (Tables 16 and 17). The proportion of fish

rated 3 increased with each exposure, however the change was not significant (Tables 16 and 17).

Repeated exposure to gas supersaturated water may reveal juvenile salmonids are able to acclimate to gas supersaturation. Meekin and Turner (1974) attempted to acclimate juvenile summer chinook salmon to nitrogen supersaturation by conditioning them in progressively higher levels of water supersaturated with nitrogen for varying periods of time. Severe mortality occurred when nitrogen saturation was increased to 135%, regardless of conditioning; chinook salmon survived exposure to 135% TGP longer when removed to water of 100% saturation for 8-h/d than if exposed continuously. Weitkamp (1976) exposed juvenile chinook to 125% TGP for a sequence of three

Table 16. Gas bubble trauma rating for juvenile brown trout (fish size on day 1 of testing = 164 ± 17 mm and 43.2 ± 14.4 g) by number and percent composition after exposure to 118% gas supersaturated water for 120-h three times at 30-d intervals from June 6 - September 18, 1987.

GBT[a] rating	First exposure		Second exposure		Third exposure	
	#	%	#	%	#	%
0	0	0	2	12	0	0
1	12	24	0	0	2	33
2	6	12	4	23	3	50
3	2	4	1	6	1	17
4	30	60	10	59	0	0

a = see Table 6 for rating description

Table 17. Comparison of GBT severity in juvenile brown trout (fish size on day 1 of testing = 164 ± 17 mm and 43.2 ± 14.4 g) exposed to 118% gas supersaturated water three times at 30-d intervals June 6 - September 18, 1987 using Fisher's Exact Test.

Comparison GBT rating[a]	p - value
Exposure 1 Vs Exposure 2	
0 = 0	0.067
1 > 1	0.017*
2 = 2	0.921
3 = 3	0.831
4 = 4	0.581
Exposure 2 Vs Exposure 3	
0 = 0	0.554
1 = 1	0.059
2 = 2	0.239
3 = 3	0.941
4 > 4	0.013*
Exposure 3 Vs Exposure 1	
0 = 0	0.067
1 = 1	0.842
2 > 2	0.046*
3 = 3	0.972
4 < 4	0.007*

*Statistically significant at alpha = 0.05
a = see Table 6 for rating description

exposure/non-exposure periods. When the sequence was 16-h exposure and 8-h recovery, juvenile chinook developed external signs of GBT and were twice as likely to die as fish exposed for 8-h given 16-h to recover. Chinook

salmon, coho salmon, steelhead trout, rainbow trout, mountain whitefish, and largemouth bass (Micropterus salmoides) exposed to 110 - 130% nitrogen for 8-h given 16-h to recover lived longer than fish exposed constantly to similar nitrogen supersaturations (Blahm et al. 1976). Although these investigations show that relief from saturated conditions can extend the survival time of most fish, they do not document acclimation.

Juvenile brown trout in my study did not appear to acclimate to gas supersaturated water. External symptom severity did not decrease with repeated exposure (Table 17). However some brown trout were more tolerant of high dissolved gas levels. None of the six fish remaining after two exposures died when exposed a third time.

Tolerance to gas supersaturation was demonstrated by Thorn et al. (1978) who exposed bluegill sunfish to supersaturations ranging from 113 - 124%. Survivors of these tests were subjected to 124% then 127% gas supersaturated water, but showed no external GBT symptoms or mortality.

Tolerance is described in the literature as a heritable trait. Gray et al. (1982) showed that carp (Cyprinus carpio) and black bullhead (Ictulurus melas) of the Columbia River were more tolerant of gas supersaturation than those from Italy. The researchers suggested that this difference may be due to genetic adaptation traceable to historical supersaturation of Columbia River

water. Cramer and McIntyre (1975) hypothesized that a selection for fall chinook salmon with the phenotypes that promoted greatest resistance to GBT occurred in the Columbia River. A similar selection may be occurring in the Bighorn River below Yellowtail Afterbay Dam.

Sublethal Effects of Exposure to Gas Supersaturated Water

Growth

Exposure of juvenile brown and rainbow trout to 112% gas supersaturated water (medium treatment) did not affect growth. Beginning and final mean weights of medium treatment fish from tests 1, 3, 5, and 6 were not significantly different from control fish (Table 18). Growth data from medium treatment tests 2 and 4 were not used because mortality, which may have been related to fish size, occurred and could have biased results.

Some workers have shown that exposure to gas supersaturated water affects growth of surviving fish. Brown and rainbow trout reared in 120% gas supersaturated water grew more slowly than those reared in 108% gas supersaturated water for 36 weeks (Poston et al. 1973). Northern squawfish exposed to 120% gas supersaturated water for 12-d consumed an average of 12 g/d less food than did squawfish exposed to 100% saturation (Bentley et al. 1976). Yearling lake trout growth and food

Table 18. Effect of gas supersaturated water on the growth of control (104%) and medium treatment (112%) brown and rainbow trout from tests 1, 3, 5, and 6 and the associated p-values calculated using the Man-Whitney nonparametric rank test (alpha = 0.05).

Species	Beginning mean weight (g/fish)			p-value	Final mean weight (g/fish)			p-value
	control		medium treatment		control		medium treatment	
Brown	21.5	=	21.9	0.795	20.5	=	20.0	0.863
Rainbow	37.5	=	40.0	0.817	39.9	=	39.9	0.977

conversion was reduced during exposure to sublethal levels of gas supersaturation (Thorn et al. 1978). Stress experienced by fish during exposure to gas supersaturated water may be tied to decreased fish growth. Stress induced by unfavorable environmental conditions affects fish production traits (Piper et al. 1982). Exposure to sublethal levels of supersaturated water may cause improper functioning of the gastrointestinal tract. Stroud and Nebeker (1976) found that food was held in the stomach of juvenile steelhead trout up to 54-h at 120% TGP and 93-h at 115% TGP. They determined that this time period was longer than would be expected, possibly because of decreased peristalic movement of the gut. Poorer growth of fishes exposed to gas supersaturated water in these investigations may be due to inefficient food digestion attributable to gas supersaturated water.

Other researchers found that gas supersaturation does not affect fish growth. Juvenile steelhead trout surviving 40-d exposure to gas supersaturated water with delta-P from 150 - 180 mm showed no significant decrease in growth (Nebeker et al. 1978). Channel catfish (Ictalurus punctatus) surviving exposure to 104.7 - 115% TGP for 35-d showed no reduced growth or signs of GBT, yet some died (Colt et al. 1985). The response appeared to be all or nothing; fish either died or showed no growth effects.

During my tests, 112% TGP did not impair feeding

behavior, physiological growth factors, or cause significant mortality in juvenile brown or rainbow trout within the length and weight range tested. However, some mortality occurred in 30-d bioassays when percent TGP surpassed 112%. The "all or nothing response" identified by Colt et al. (1985) seems applicable to my study.

Predation Tests in Tanks

Tests run in circular tanks using the procedure of Bams (1967) showed no difference in predation vulnerability between juvenile brown and rainbow trout exposed to gas supersaturated water and non-exposed fish (Tables 19, 20, 21, 22 and 23). Problems with the large size of rainbow trout prey used during the 112%/30-d tests made it difficult for predators to consume a suitable number of prey before prey recovery from GBT (Table 19). Predator density was increased during the remaining tests (Tables 20, 21, 22, and 23) to avoid this problem.

Reduced fitness of prey can increase their vulnerability to predation . Kania and O'Hara (1974) found that the ability of misquito fish (Gambusia affinia) to avoid predation by largemouth bass was impaired after exposure to sublethal concentrations of mercury. Rainbow trout, chinook salmon, and sockeye salmon experienced elevated predation vulnerability after exposure to high water temperature (Coutant 1973, Sylvester 1972).

Table 19. Number of survivors (beginning number = 25), ratios of instantaneous predation rates (dp), and chi-square statistic corrected for continuity (X^2c) for predation tests with juvenile rainbow trout prey (77 ± 5 mm) after exposure for 30-d to 112% gas supersaturated water.

Test	Number surviving		Chi-square (1 df)	dp
	Treatment (expected)	Control (expected)		
1	21 (17.5)	14 (17.5)	1.4	0.301
2	14 (14)	14 (14)	0.0	1.000
Total of chi-squares(2df)			1.4	
Chi-square of totals (i.e, pooled chi-square 1 df)	35 (31.5)	28 (31.5)	0.777	
Heterogeneity chi-square (1df)			0.623 0.75 > p > 0.50	
Chi-square correction for continuity	$X^2c = \frac{([35-28]-1)}{73} = 0.493$		0.50 > p > 0.25	

Table 20. Number of survivors (beginning number = 25), ratios of instantaneous predation rates (dp), and chi-square statistic corrected for continuity (X^2c) for predation tests with juvenile rainbow trout prey (45 mm) after exposure for 30-d to 124% gas supersaturated water.

Test	Number surviving		Chi-square (1 df)	dp
	Treatment (expected)	Control (expected)		
1	10 (13.5)	17 (13.5)	1.815	2.376
2	16 (14.5)	13 (14.5)	0.301	0.682
3	12 (8.5)	5 (8.5)	2.882	0.456
Total of chi-squares(2df)			5.007	
Chi-square of totals (i.e, pooled chi-square 1 df)	38 (31.5)	35 (31.5)	0.123	
Heterogeneity chi-square (1df)			4.884 0.10 > p > 0.05	
Chi-square correction for continuity	$X^2c = \frac{([38-35]-1)}{63}$		= 0.0541 0.90 > p > 0.75	

Table 21. Number of survivors (beginning number = 25), ratios of instantaneous predation rates (dp), and chi-square statistic corrected for continuity (X^2c) for predation tests with juvenile brown trout prey (69 ± 7 mm) after exposure for 30-d to 110% gas supersaturated water.

Test	Number surviving		Chi-square (1 df)	dp
	Treatment (expected)	Control (expected)		
1	15 (17)	23 (17)	1.684	6.126
2	10 (14.5)	18 (14.5)	2.285	2.789
Total of chi-squares(2df)			3.969	
Chi-square of totals (i.e, pooled chi-square 1 df)	25 (34.5)	41 (34.5)	2.249	
Heterogeneity chi-square (1df)			1.520 0.25 > p > 0.10	
Chi-square correction for continuity	$X^2c = \frac{([25-41]-1)}{69}$		= 3.260 0.10 > p > 0.05	

Table 22. Number of survivors (beginning number = 25), ratios of instantaneous predation rates (dp), and chi-square statistic corrected for continuity (X^2c) for predation tests with juvenile brown trout prey (56 ± 7 mm) after exposure for 30-d to 112% gas supersaturated water.

Test	Number surviving		Chi-square (1 df)	dp
	Treatment (expected)	Control (expected)		
1	14 (14)	14 (14)	0.000	1.000
2	10 (11)	12 (11)	0.182	1.249
Total of chi-squares(2df)			0.182	
Chi-square of totals (i.e, pooled chi-square 1 df)	24 (25)	26 (25)	0.080	
Heterogeneity chi-square (1df)			0.102 p = 0.75	
Chi-square correction for continuity	$X^2c = \frac{([26-24]-1)}{50}$		= 0.020	
			0.90 > p > 0.75	

Table 23. Number of survivors (beginning number = 25), ratios of instantaneous predation rates (dp), and chi-square statistic corrected for continuity (X^2c) for predation tests with juvenile brown trout prey (56 ± 5 mm) after exposure for 13-h to 130% gas supersaturated water.

Test	Number surviving		Chi-square (1 df)	dp
	Treatment (expected)	Control (expected)		
1	12 (11.5)	11 (11.5)	0.043	0.894
2	14 (11)	8 (11)	1.636	0.509
3	13 (14)	15 (14)	0.143	1.280
4	9 (12.5)	16 (12.5)	1.960	2.289
Total of chi-squares(2df)			3.782	
Chi-square of totals (i.e, pooled chi-square 1 df)	48 (49)	50 (49)	0.041	
Heterogeneity chi-square (1df)			3.741	
			0.10 > p > 0.05	
Chi-square correction for continuity	$X^2c = \frac{([48-50]-1)}{98} = 0.010$			
			0.95 > p > 0.90	

Brook trout consumed more Atlantic salmon that had been exposed to insecticide than unexposed fish (Hatfield and Anderson 1972). Hertig (1967) concluded that bluegill and largemouth bass suffering from columnaris disease, parasitism, or starvation were more vulnerable to predation than healthy fish. Predators selected prey impaired because they were slow swimming and easy to catch. Hatchery reared sockeye salmon fry did not swim as well as wild fry, leading to increased vulnerability to predation (Bams 1967).

Gas Bubble Trauma affects fish swimming performance and vision. These impairments could make it easier for predators to discover and capture GBT inflicted fish. However, under laboratory conditions I did not observe increased vulnerabilty to predation.

Predation Tests in the Experimental Stream

Predation tests in the experimental stream showed that brown trout exposed to 118% gas supersaturated water for 5-d experienced significantly increased vulnerability to predation (Table 24). However, this is a tentative conclusion based on one replicate. There was no statistically significant selective predation on brown trout exposed for 4, 6, or 7 days (Table 24), nor on rainbow trout exposed for 11 to 12 days (Table 25).

Table 24. Ratio of instantaneous predation rates (dP) of juvenile brown trout exposed to 118% gas supersaturated water and non-exposed fish.

Duration of exposure (days)	Mean fish length (mm ± SD)	Number of replicates	Number Surviving		dp
			treatment	control	
4	46 ± 3	1	14	19	2.113
5	47 ± 4	1	13	21	3.751*
6	45 ± 3	1	13	15	1.280
7	46 ± 4	1	15	13	0.781

*Significantly different using Fisher's exact test at alpha = 0.05

Table 25. Ratio of instantaneous predation rates (dP) of juvenile rainbow trout exposed to 118% gas supersaturated water and non-exposed fish.

Duration of exposure (days)	Mean fish length (mm ± SD)	Number of replicates	Number Surviving		dp
			treatment	control	
11	58 ± 7	1	12	8	0.644
12	61 ± 5	1	7	6	0.892

*Significantly different using Fisher's exact test at alpha = 0.05

Juvenile brown trout exposed to 118% gas supersaturation for 5-d may have been most susceptible to predation because of the nature of fish response to

supersaturated water. Some fish die quickly, some acquire severe symptoms of GBT and die slowly and some are never affected. I observed that the number of brown trout with external symptoms of GBT peaked on day 5 of exposure. Fish removed from the exposure system for a given test were the first to display symptoms. Hence, remaining fish after each successive test were those which were most tolerant of supersaturation. The trend in instantaneous mortality rates supports this hypothesis (Figure 25) and may explain why predation was greatest on fish exposed to gas supersaturation for 5-d.

The ability of rainbow trout to rapidly recover from overinflation of the swimbladder may decrease their susceptibility to increased predation from exposure to gas supersaturated water. Schiewe (1974) reported that swimming impairment of juvenile chinook salmon caused by overinflation of the swimbladder was reduced within 2-h of removal from 120% gas supersaturated water to equilibrated water. During my second series of rainbow trout tests overinflation of the swimbladder was the only symptom in 18 of the 26 affected prey fish.

Tests to evaluate recovery time showed that rainbow trout released in a non-supersaturated pond lost all signs of excess buoyancy in 1 - 3 min. More than 3 min were required for the predators to discover the prey during stream predation tests. Rainbow trout prey may have

expelled excess gas via the pneumatic duct, thereby regaining equilibrium prior to predator feeding.

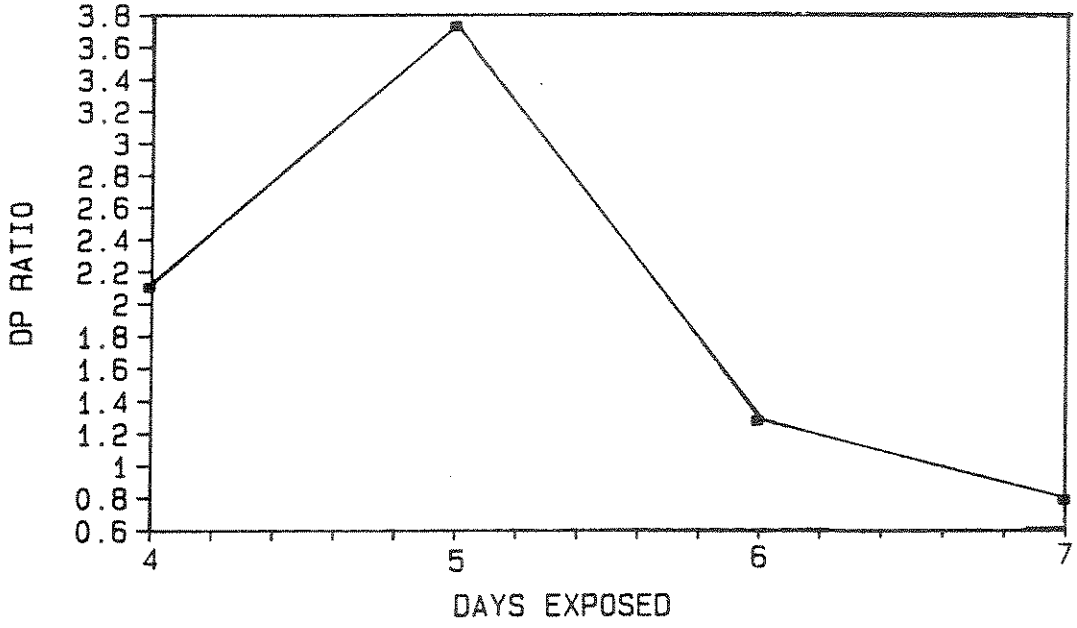

Figure 24. Ratio of instantaneous predation rates (dp) by rainbow trout predators on juvenile brown trout exposed 118% gas supersaturated water for 4 - 7-d and control fish (dp > 1 signifies greater predation on brown trout exposed to gas supersaturation).

Predators were not replaced between tests which also may have biased the study. Coutant (1973) suggested that reusing predator fish did not bias predator-prey study results. However, with time, predators may become more efficient at capturing prey. The four predators in the experimental stream were trained to feed on prey three to four times prior to testing and were used six times during testing. Werner et al. (1981) found that bluegill foraging efficiency increased four-fold over the course of six to eight foraging opportunities and Ware (1971) reported

that rainbow trout increased foraging efficiency six-fold in 8-d. My rainbow trout predators may have become too efficient at consuming prey, thereby biasing results.

Disease Resistance

Significantly more bacteria were identified in kidney samples of fish challenged with bacteria than in unchallenged fish (Table 26).

Table 26. Two sample t-test analysis of the number of A. hydrophila identified in kidney samples of juvenile brown trout challenged with bacteria and unchallenged fish.

Duration (min) challenged fish were treated w/bacteria	Comparison challenged fish (w/bacteria)		unchallenged fish (no bacteria)	p-value
10	no GBT	>	no GBT	0.0039*
20	no GBT	>	no GBT	0.0010*
30	no GBT	>	no GBT	0.0000*
40	no GBT	>	no GBT	0.0000*
10	GBT	>	GBT	0.0003*
20	GBT	>	GBT	0.0001*
30	GBT	>	GBT	0.0000*
40	GBT	>	GBT	0.0000*

*Significantly more A. hydrophila in juvenile brown trout challenged with bacteria than in unchallenged fish at alpha = 0.05

Juvenile brown trout with GBT from exposure to 118% gas supersaturated water challenged with bacteria for 20 - 40 min had more bacteria in kidney samples than fish similarly challenged but not exposed to gas supersaturated

water (Table 27). However, this difference was significant only for the 30-min challenge (Table 27). Results of the second replicate test were discarded because control fish died from a Pseudomonas sp. infection unrelated to experimental procedure.

Table 27. Two sample t-test analysis of the number of A. hydrophila identified in kidney samples of juvenile brown trout (196 ± 13 mm and 74.5 ± 17.3g) not exposed to gas supersaturated water (control) and fish (198 ± 12 mm and 75.4 ± 16.2 g) with GBT (test) from exposure to 118% supersaturated water for 120-h.

Challenge duration (min)	Mean number of bacterium/kidney		p-value
	control	test	
10	48 ± 9	53 ± 3	0.4234
20	54 ± 3	65 ± 4	0.0672
30	70 ± 3	79 ± 3	0.0145*
40	85 ± 4	92 ± 3	0.0535

*Significantly more A. hydrophila in juvenile brown with GBT at alpha = 0.05

Bacteria of the genus Aeromonas sp. are ubiquitous microbes of water and are commonly found in the alimentary tract of healthy salmonids (Trust and Sparrow 1974). However, under conditions of stress, A. hydrophila can become an opportunistic pathogen. Thorpe and Roberts (1972) described a septicemic outbreak in spawning brown trout of Loch Leven, Scotland. Grayish skin ulcerations,

abdominal distention, swelling and discoloration of internal organs, secretion of a yellow mucous in the intestine and subsequent swelling of the anal vent are common in infected fish during epizootics of motile aeromonad septicemia (Piper et al. 1986).

Trust (1986) speculated that bacterial infection may result from penetration of the thick mucosal layer of the skin. Post (1983) hypothesized that soon after a bacterium enters the tissue or blood stream of a fish, it is phagocytized and transported to the spleen or anterior kidney. Macrophages may break the bacterium into separate antigenic components. B-cells and plasma cells may produce antibodies specific to these antigens.

This information is complicated, but in general it appears that stress and increased systemic access can facilitate bacterial disease. During my tests, the stress caused by GBT may have impaired the defense (macrophages) and immune system (antibody production) functioning. The blistering of the epidermis common to emphysema may have provided bacteria open passage to capillary beds. Severe exopthalmia characterized by freely bleeding open eye wounds also may have provided bacteria direct access to the bloodstream of the fish.

SUMMARY

The critical threshold required to cause acute mortality in juvenile rainbow and brown trout (53.1 - 130.5 mm) appears to be between 113 - 117 percent TGP.

Length and weight of juvenile brown and rainbow trout had a significant influence on susceptibility to supersaturated water. During early tests intra-specific percent cumulative mortality for both species exposed to the high gas treatment was significantly less than during later tests when fish were larger.

The frequency of emphysema occurrence on certain regions of the body varied with fish size.

Daily mortality of brown trout exposed to high gas supersaturation levels (125%) was significantly greater than that of rainbow trout of similar size during all comparisons. The difference in susceptibility was most evident when fish were smallest (0.4 g). Differences in percent cumulative mortality between species were less in subsequent tests when fish were larger (8.3 - 60.5 g).

Juvenile brown trout surviving exposure to 112% supersaturation were more prone to external symptoms of GBT than juvenile rainbow trout of similar size.

Juvenile rainbow and brown trout recovery from GBT varied with external symptom severity. Fish of both species with less severe symptoms recovered more often than fish with more severe symptoms.

Non-recovering fish exhibited eye damage characterized by severe unilateral and bilateral exopthalmia, hemorrhaging, cloudiness or cataracts of the cornea, or bubbles in the orbit of the eye.

The condition of fish subjected to intermittent exposure worsened with each exposure leading to poorer recovery. The development of new symptoms with each exposure and recurrence and progression of prior symptoms was responsible for poorer recovery with each additional exposure.

Some fish may become sensitized to GBT, while other individuals may be tolerant to exposure to gas supersaturated water.

Exposure to 112% gas supersaturated water did not affect growth of juvenile brown and rainbow trout.

Predation tests in circular tanks showed no difference in predation vulnerability between juvenile brown and rainbow trout exposed and not exposed to gas super-saturated water.

Predation tests in an experimental stream suggested that brown trout exposed to 118% gas supersaturated water for 5-d were more vulnerable to predation. There was no statistically significant selective predation on brown trout exposed for 4, 6, or 7 days, nor on rainbow trout exposed for 11 to 12 days.

Bacterial challenges indicated that GBT may increase the susceptibility of brown trout to A. hydrophila infection.

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