ALLOWABLE GAS SUPERSATURATION FOR FISH PASSING HYDROELECTRIC DAMS

TASK 8
BUBBLE REABSORPTION IN A SIMULATED SMOLT BYPASS SYSTEM - CONCEPT ASSESSMENT

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EXECUTIVE SUMMARY

The monitoring program for examining the prevalence of gas bubble trauma (GBT) in Snake and Columbia river salmonids has been questioned. Dr. Larry Fidler in a letter to Mr. Ted Bottiger of the Pacific Northwest Power Planning Council suggests that the current monitoring program may be "invalid" because clinical signs of GBT may disappear during a fish's passage through the fish bypass system at dams. Only fish that pass through the bypass system are examined for GBT. Based largely on theoretical work, it appears that the high hydrostatic pressures experienced by the smolts as they pass through the smolt bypass system could quickly reabsorb any bubbles that had formed due to dissolved gas supersaturation.

Our objective for the tests recorded here was to examine the validity of Dr. Fidler's suggestion. We subjected fish (fingerling spring chinook salmon (Oncorhvnchus tshawvtscha)) to dissolved gas supersaturated water to develop signs of GBT and then exposed them to pressures that could occur during passage through a smolt bypass system. Separate groups of fish were examined for clinical signs of GBT prior to pressurization and after pressurization. Four pressurizations ranging from 5 to 120 minutes were tested. The test fish were spring chinook fingerlings (≈ 130 mm total length). During the pressurization and depressurization phase, the fish were held in normally saturated water so that bubble re-growth could not occur after depressurization.

A pressurization of 5 minutes to 100 feet of head resulted in a significant reduction in the clinical signs of GBT in the fins, lateral line, and gills. In terms of bubble reabsorption, the quickest loss of clinical signs of gas bubble disease were in the gills followed closely by the lateral line. The rate of bubble loss was significantly less for the fin bubbles. If the reabsorption potential of pressure-time history for salmonids is similar to the 5 minute pressurization treatment, the current smolt monitoring program may be under-estimating the prevalence of GBT in the Snake and Columbia Rivers.

While pressurization to 100 feet for 5 minutes resulted in significant reduction in the clinical signals of GBT, all bubbles in a fish will start to disappear if the fish is below the hydrostatic compensation depth. Therefore, there are an infinite number of depths and times that would result in the complete disappearance of bubbles. While this work does not prove that bubble reabsorption is actually occurring in in-river migrants, it does indicate that it may occur. In order to determine if bubble reabsorption is actually occurring in the smolt monitoring program, five research areas are suggested.

1.0 INTRODUCTION

The monitoring of smolts in the Columbia and Snake Rivers for signs of gas bubble disease is essentially limited to observations from fish collected from the smoh collection system. Because of the potential of bubble reabsorption during the fish passage through the smolt collection system, the overall validity of this sampling plan has been questioned (see Appendix A). Based largely on theoretical work, it appears that high hydrostatic pressures experienced by the smolts as they pass through the smolt bypass system (and turbines) could quickly reabsorb bubbles that had formed due to dissolved gas supersaturation. If significant bubble reabsorption occurs in the smolt bypass system, the current smolt monitoring program may be biased and may not provide accurate data on the prevalence of gas bubble trauma.

The overall purpose of this experimental work is to quickly assess the probability if bubble reabsorption occurs during a simulated bypass passage. If possible evidence of bubble reabsorption is found, additional experimental work will be needed to define the kinetics of bubble reabsorption and to define how the smolt monitoring program could be modified to better evaluate the prevalence of gas bubble trauma.

The experimental procedures and protocols used in this experiment were developed based on experience and professional judgment. The selected experimental design and potential protocols were discussed with Mr. Earl Dawley (National Marine Fisheries Service), Dr. Matt Mesa (National Biological Service), and Mr. Bill Maslen (Bonneville Power Administration) on March 30, 1995.

This test series was designed to assess whether gas bubble reabsorption occurs in fish collected and subsequently examined for the smolt monitoring program. To reduce the possibility of bubble regrowth (after depressurization and before examination), the fish were pressurized in normally saturated water (ΔPs c 20 mm Hg). The question of bubble regrowth is outside of the scope of this work. Pressurization times used in this work were based upon discussion with experts on fish passage at Columbia River dams. Although some data exists on how long it takes smolts to pass through the overall collection system, there is little quantitative data on their actual pressure-time history during passage through a dam.

2.0 FISH PASSAGE THROUGH SMOLT BYPASS SYSTEMS

The passage of a smolt through the smoh bypass system can be classified (somewhat arbitrarily) into 5 phases:

(1) Holding in the forebay of the dam at some depth Z_1 meters below the water surface for a period of H_1 hours,

(2) Movement from the forebay to submerged traveling screens and up into the gatewell (this may be relatively fast, measured in minutes),

(3) Holding in the gatewell at some depth Z_3 meters below the water surface for a period of H3 hours to days,

(4) Movement through the orifice and into the bypass channel or conduit (this is fast, measured in seconds), and

(5) Movement down the bypass channel or conduit to the smolt sampling facility or smolt holding system. The time in this phase will depend on whether the dam is a collector dam for transportation or releases the smolts back into the river. For the collector dams, the fish may be held up to 1-2 days before transport. For the non-collector dams, the time in this phase may be only hours.

At McNary Dam the time between the fishes' first encounter with the submerged traveling screens (STS) and movement to the collection or sampling system may range up to several days (personal communication, Mr. Brad Eby, October 10, 1994). Detailed information on the actual pressure-time history for a smolt is not available. The pressure-time histories used in this work (pressurization to 100 feet of head for 5 to 120 minutes) are possible pressure-time histories that fish may experience. There are an infinite number of other pressure-time histories that smolts could experience. There is no reason to expect that all smolt experience the same pressure-time history as they pass through a single dam or a series of dams.

3.0 METHODS AND MATERIALS

3.1 Location and Personnel

All experimental work was conducted by Dr. Ralph Elston, Dr. John Colt, and Mr. Scott Abemethy at the Pacific Northwest Laboratory (PNL) at Richland, Washington during the period from March 27 to April 7, 1995.

3.2 Exposure Equipment

The ΔP used to load the fish with dissolved gas supersaturation was generated with 4 - 1/2 inch diameter Membran® tube bundles. Compressed breathing air was used to pressurize the tube bundles. This gassing system had been previously used with the turbine system experiments (Montgomery Watson, 1995).

The fish were exposed to the dissolved gas supersaturation in a 10-gallon aquarium that had been partitioned into two compartments. The fish were exposed to a ΔP of 170 mm Hg (equivalent to 123% total gas pressure) for 16-20 hours.

Following gas loading, the fish were pressurized in the system that had been used previously for the bypass simulation experiments (Montgomery Watson, 1995). This system consists of two clear acrylic swim chambers (4 inches in diameter by 45 inches long). Screens at both ends of the swim chamber allowed water to flow through while keeping fish inside the chamber. The fish were added through a removable blind flange on the top of a 4-inch PVC cross. The fish were removed from the system through a threaded cap on the bottom of the 4-inch PVC cross.

The water supply line pressure to the simulated bypass system was approximately 60 psi. Water pressure in the system was controlled by adjusting the 4-inch gate valve on the discharge side of the system. The pressure was ramped up and down using the following schedule:

Time (s)	Pressurization (psig)	Depressurization (psig)
0	10	40
30	20	30
60	30	20
90	40	10
120	45	0

The water pressure was maintained at 45 psig for the duration of the pressurization phase, then ramped down to atmospheric pressure prior to removal of the treatment fish from the system. During the experimental work, the AP of Columbia River water ranged from 7 to 27 mm Hg.

3.3 Water Supply

Raw Columbia River water was used for all work. Over the period of the experiment, the temperature, DO, and ΔP were equal to:

Parameter	Mean $\pm$ SD
Temperature (^oC)	8.1 $\pm$ 0.1
DO (mg/L)	11.6 $\pm$ 0.4
ΔP (mm Hg)	17 $\pm$ 8

3.4 Experimental Design

The experimental design consisted of exposing groups of fish to a ΔP = 170 mm Hg for 16-20 minutes followed by pressurization treatments ranging from 5 to 120 minutes.

Test Number	Controls Examined at time = (minutes)	Treatment Examined at time = (minutes)
1	0	**60**
2	**0**	**120**
3	**0**	**30**
4	**0**	**5**

Each experimental run consisted of a paired control and treatment with 10 fish per group. Both the control and treatment were exposed to the dissolved gas supersaturation. The control fish were examined for signs of gas bubble trauma at time = zero minutes. The treatment fish were examined for signs of gas bubble after the pressurization phase. The experiments were not replicated.

The water flow through the test chamber during pressurization was 13 $\pm$ 3 gpm and the water velocity was equivalent to a swimming speed of 0.80 $\pm$ 0.20 body lengths/second, based on a total fish length of 130 mm.

The actual ΔP that a fish is exposed to is called the uncompensated ΔP or ΔP_{uncomp}:

$$\Delta P_{uncomp} = \Delta P - \rho g Z \qquad \text{Equation 1}$$

where

ΔP = Measured ΔP in the river (mm Hg)

ρg = Specific weight of water (mm Hg/m)

z = Depth in the water column (m)

At 15 °C, the specific weight of water is equal to 73.49 mm Hg/m (Colt, 1984). so for this temperature, Equation 1 can be rewritten as:

$$\Delta P_{uncomp} = \Delta P - 73Z \qquad \text{Equation 2}$$

The depth at which the $\Delta P_{uncomp} = 0$ is called the hydrostatic compensation depth and is equal to

$$Z_{comp} = \Delta P I73 \qquad \text{Equation 3}$$

The uncompensated pressure head (Z_{uncomp}) is equal to ΔP_{uncomp} expressed as static pressure:

$$Z_{uncomp} = \Delta P_{uncomp}/73 \qquad \text{Equation 4}$$

If a fish is above the hydrostatic compensation depth, bubbles may form. If the fish is below the hydrostatic compensation depth, bubbles will be reabsorbed. The potential for a bubble to be reabsorbed will be called the reabsorption potential and is proportional to the pressure gradient between the pressure inside the bubble and local ΔP. Assuming that the pressure inside a bubble at depth Z is equal to the hydrostatic pressure at depth Z and the ΔP in the water column is well-mixed, the reabsorption potential should be proportional to ΔP_{uncomp}. The reabsorption potential is assumed to be equal to the pressure gradient term x time. The pressure gradient can be characterized by the ΔP_{uncomp} (mm Hg) or the uncompensated pressure head (feet), so that the reabsorption potential can be computed in terms of ΔPcomp x Tie Exposure (mm Hg/day) or Uncompensated Pressure Head x Time Exposure (feet/day). The detailed computations are presented in Appendix B (ΔPxtime comps). The reabsorption potential included the contribution from the pressurization phase, constant pressure phase (100 ft), and the depressurization phase. The reabsorption potentials for the four pressure-time treatments are presented below:

Pressurization Period (minutes)	Reabsorption Potential (feet/day)	Reabsorption Potential (mm Hg/day)
5	-0.50	-12
30	-2.2	-52
60	-4.3	-100
120	-8.5	-196

A negative reabsorption potential indicates that gas will be transferred from a bubble into the water. The more negative the reabsorption potential, the greater possibility for gas to be transferred into the water. A positive reabsorption potential would indicate that gas would be transferred from the water into the fish, potentially resulting in bubble formation.

This previous discussion has ignored the contribution of other compensating pressures and the impact of surface tension on the pressure inside a bubble. Under certain conditions, these parameters may have a significant impact on the kinetics of gas transfer.

3.5 Experimental Fish

Fingerling spring chinook salmon (Oncorhvnchus tshawvtscha) were used in all experimental work. Spring chinook salmon were spawned in mid-August of 1993 at Leavenworth National Fish Hatchery in Leavenworth. Washington and the eggs were incubated at the PNL research hatchery. About 4,500 fry were maintained at the lab in 1994 for research purposes. Fish were held in a mixture of ambient Columbia River water and well water during the summer, then were placed in ambient river water during the winter. Rearing temperatures ranged from 3 to 18 "C. Mortality remained low throughout the rearing period.

3.6 Examination of Fish for Clinical Signs of Gas Bubble Trauma

Each control and treatment group of fish was sacrificed by placing in 4 liters of a lethal concentration (100 mg/L) of buffered MS-222 maintained at the experimental exposure temperature. During experiments 1 and 2, groups of 10 fish from controls and treatments were processed in batch. During experiment 3, two batches of five fish each from the controls and treatment were exposed and processed by batch. During experiment 4, pairs of control and treatment fish were processed alternately up to a total of 10 fish from each group. These changes were necessary because at the shorter pressurization time (5 and 30 minutes), it was not possible to process all the control fish before the treatment fish had to be examined. Processing began within 3 minutes of placement of fish in the anesthetic and time of processing for each fish was recorded. A sample standard data sheet is provided in Appendix B.

The caudal, anal, and dorsal fins were examined for the presence of gas bubbles within the tissues of the fins using a stereo microscope with transmitted illumination at a total magnification range of 7.5X to 75X. The percentage of each fin occupied by gas bubbles was estimated.

The left and right lateral lines were examined for the presence of gas bubbles by examining three 5-mm linear segments on each side at a magnification of 27X. The segments were located about 5 mm posterior to the operculum, ventral to the anterior insertion of the dorsal fin and ventral to the adipose tin.

Preliminary experiments showed that gill bubbles in affected fish could dissipate in less than two minutes from excised gill arches and in less than ten minutes in intact fish out of water. Therefore, we implemented two examinations for gill bubbles, For the first examination, the left operculum was removed from each fish and the presence of gill bubbles determined by examining at least 100 primary lamellae using a stereomicroscope. All fish were examined by this method prior to completing any other portion of the gill examination. Additionally, in order to maintain consistency with the monitoring program methods, the first left gill arch was excised from each fish and primary gill lamellae were excised and spread on a wet glass microscope slide and examined microscopically at up to 100X total magnification. The number of primary and secondary lamellae containing gas bubbles within vascular spaces was counted as well as the total number of primary lamellae examined.

Preliminary study showed that residual lesions could often be observed where bubbles had previously occurred in the fins. Therefore, the frequency of occurrence of residual lesions in individual fish was recorded and reported. These residual lesions consisted of hemostasis, extravasation of blood into connective tissue of the tins, and edema in the fins. A range of severity of these lesions existed and a conservative interpretation was adopted.

3.7 Data Analysis

The data for fin bubbles were expressed as the percent of fins affected by gas bubbles by averaging the percent of each of the three fins affected in each individual and subsequently calculating a group average. For the lateral line data, the six measurements for each fish (left and right anterior, mid, and posterior) were averaged to provide an individual average of the percentage of lateral line affected by bubbles. These individual values were then used to calculate a group average. The proportion of primary gill lamellae affected in each individual was expressed as a percentage and a group average calculated. Since the proportion of affected gill lamellae tended to be higher when using the intact stereoscopic examination method, the data from this method was used in the analysis. Both methods were compared numerically and the detailed results are contained in Appendix B.

4 .O RESULTS

The detailed data sheets for the four experiments are presented in Appendix B. Summary information for fin bubbles, lateral line, and gills are presented in Table 1.

4.1 Fins

The average bubble coverage in the tins ranged from 2.5 to 14.3 % in the control fish,

The variation in average bubble cover in the fins for the four pressurization times are presented in Figure 1. The time for 50 % of the bubble coverage to disappear was between 5 to 30 minutes of pressurization. After 120 minutes of pressurization, almost all clinical signs of fin bubbles had disappeared.

4.2 Lateral Line

The average bubble coverage in the lateral line ranged from 62.4 to 72.7% in the control fish.

The variation in average bubble coverage in the lateral line for the four pressurization times are presented in Figure 2. The time for 50 % of the bubble coverage to disappear was less than 5 minutes of pressurization. After 30 minutes of pressurization, almost all clinical signs of lateral line bubbles had disappeared.

4.3 Gills

Two methods were used to enumerate gill bubbles. In the first method, the gills were examined by excision of the arch and primary lamellae. This is comparable to the method used by National Biological Service (NBS). In the second methods, the operculum was cut and the gills were examined without excision. A comparison of the two results is presented below:

		Average Bubble Coverage of Gills (%)	
Pressurization (minutes)	Batch	In situ examination after excision of operculum	Excision of arch and primary lamellae (similar to NBS methods)
60	Control	6.2	5.0
60	Treatment	0.2	0.2
120	Control	16.4	14.4
120	Treatment	0	0.2
30	Control	0	0
30	Treatment	0	0
5	Control	22.9	19.0
5	Treatment	0	0

Table 1 Summary of experimental data from preliminary gas bubble reabsorption experiments

Fins

Pressurization (minutes)	Reabsorption Potential (feet/day)	Reabsorption Potential (mm Hg/day)	Average Bubble Coverage of Fins (%)		Reduction in Gas Bubble Coverage due to Pressurization (%)
			Treatment	Control	
5	-0.50	-12	6.5	14.3	55
30	-2.2	-52	1.7	2.5	32
60	-4.3	-100	1.7	7.3	77
120	-8.5	-196	0.1	5.5	98

Lateral Line

Pressurization (minutes)	Reabsorption Potential (feet/day)	Reabsorption Potential (mm Hg/day)	Average Bubble Coverage of Lateral Line (%)		Reduction in Gas Bubble Coverage due to Pressurization (%)
			Treatment	Control	
5	-0.50	-12	20.2	67.6	70
30	-2.2	-52	1.3	62.4	98
60	-4.3	-100	0.7	72.7	99
120	-8.5	-196	1.o	62.5	98

Gills

Pressurization (minutes)	Reabsorption Potential (feet/day)	Reabsorption Potential (mm Hg/day)	Average Bubble Coverage of Gills (%)		Reduction in Gas Bubble Coverage due to Pressurization (%)
			Treatment	Control	
5	-0.50	-12	0	22.9	100
30	-2.2	-52	0	0	0/0
60	-4.3	-100	0.2	6.2	97
120	-8.5	-196	0	16.4	100

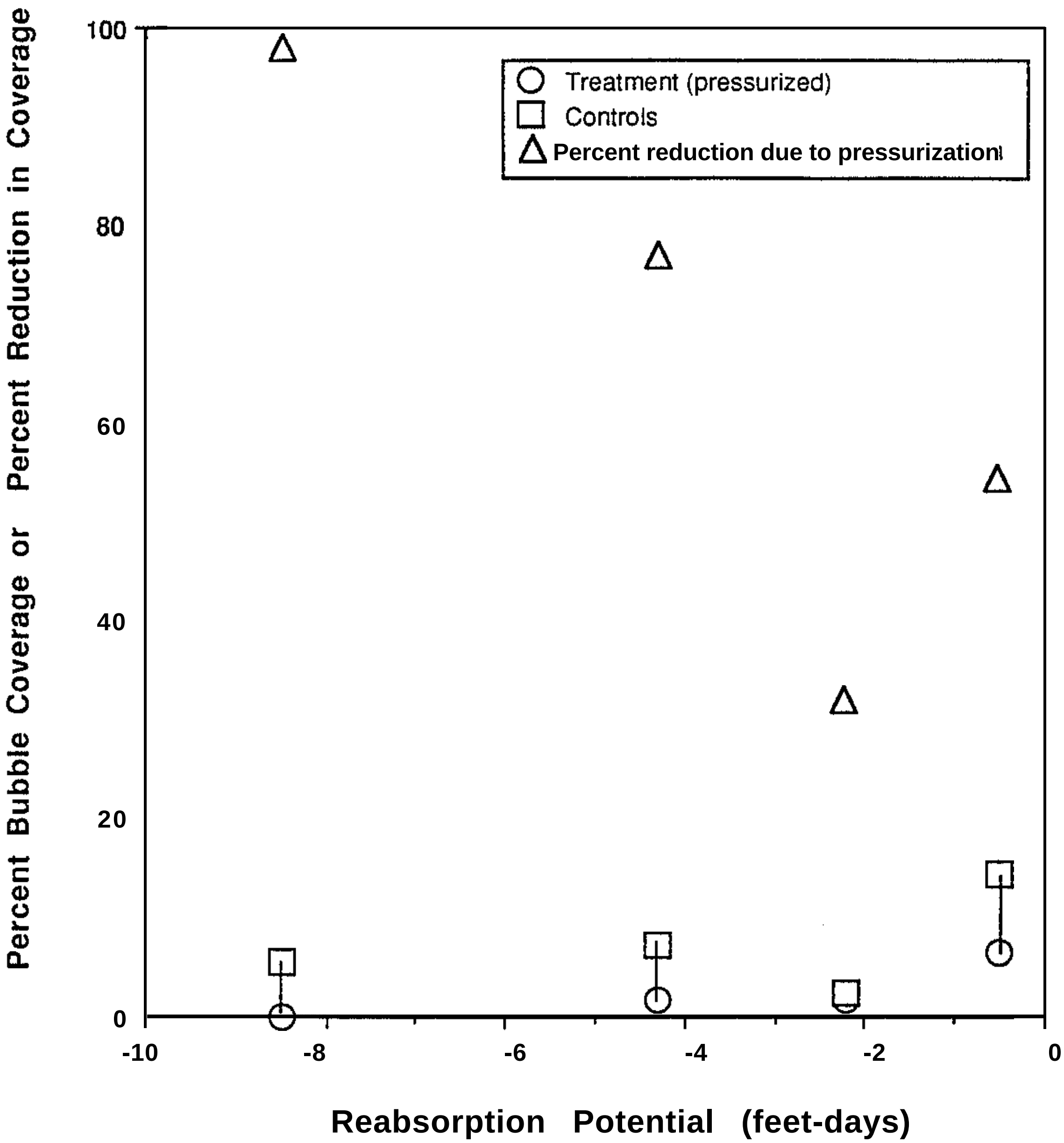

Figure 1. Change in percent gas bubble coverage in fins following pressurization. A more negative value of the reabsorption potential represents a greater potential for transfer of gas from a gas bubble into the water. The paired control and treatment observations for each reabsorption potential are connected by vertical lines.

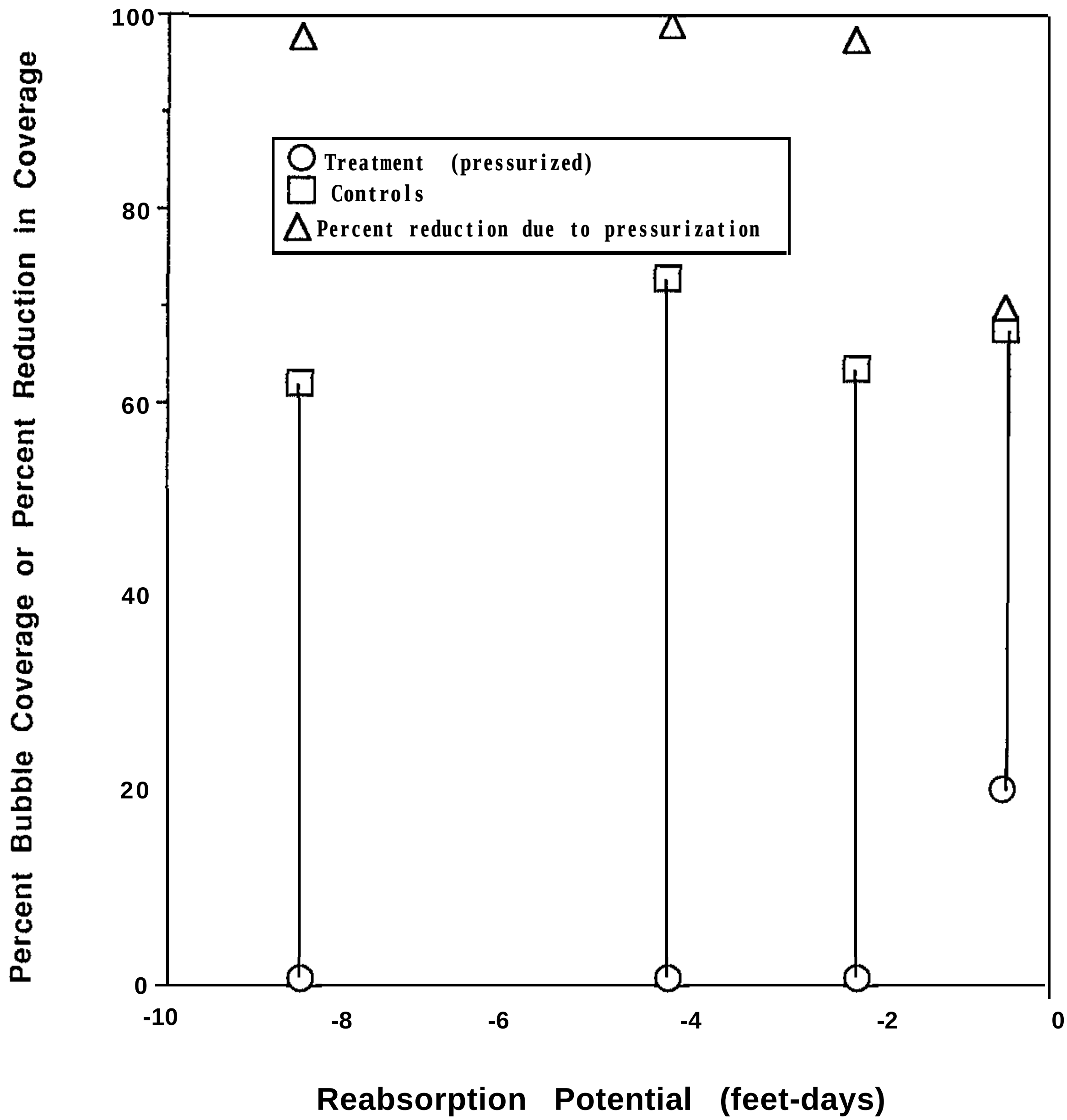

Figure 2. Change in percent gas bubble coverage in lateral line following pressurization. A more negative value of the reabsorption potential represents a greater potential for transfer of gas from a gas bubble into the water. The paired control and treatment observations for each reabsorption potential are connected by vertical lines.

The two methods are quite similar. Because processing time is shorter for the in situ method, all results and discussion of gill bubbles will be based on this method.

The average bubble coverage in the gills ranged from 0 to 22.9% in the control fish.

The variation in average bubble cover in the gills for the four pressurization times are presented in Figure 3. Gill bubbles disappeared very rapidly. Almost all clinical signs of gill bubbles had disappeared at the shortest pressurization time of 5 minutes.

4.4 Comparison of Signs of GBT in Fins, Lateral Line and Gills

The pressurization times required to reduce the average bubble coverage to 50% or 1-2% of the control levels are presented below:

Location	Time (minutes) required to reduce the average bubble coverage to 50% of the controls	Time (minutes) required to reduce the average bubble coverage to l-2 % of the controls
Fins	5-30	120
Lateral Lines	<5	30
Gills	<5	<5

The endpoints of time required to reduce the average bubble coverage to 50% or 2% of the controls were selected for monitoring considerations. The biological importance of these endpoints was not considered. The times presented in the above table were estimated from an inspection of the data presented in Table I. No detailed analysis of the data was attempted. In terms of gas bubble reabsorption, the quickest loss of signs of gas bubble disease were in the gills followed closely by the lateral line. The rate of bubble loss was significantly less for the fin bubbles.

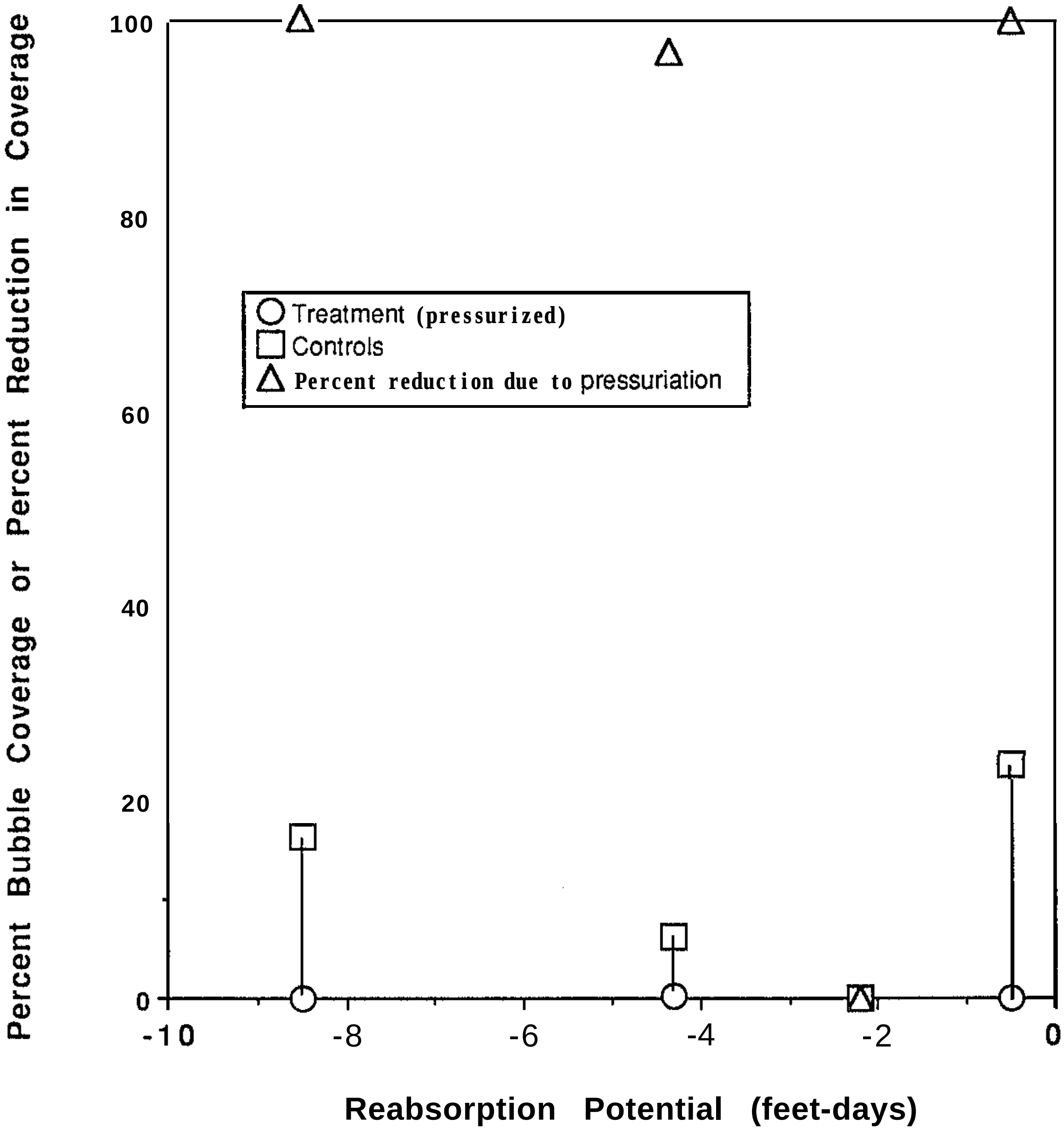

Figure 3. Change in percent gas bubble coverage in gills (based on in *situ* examination of gill without excision of the arch and primary lamellae) following pressurization. A more negative value of the reabsorption potential represents a greater potential for transfer of gas from a gas bubble into the water. The paired control and treatment observations for each reabsorption potential are connected by vertical lines.

5 .O DISCUSSION AND RECOMMENDATIONS

The examination of smolts at the Columbia and Snake River dams for signs of gas bubble trauma is used to manage the spill program and ensure that the smolts are not adversely impacted by the elevated levels of dissolved gas supersaturation produced by the spill. This is only one component of a larger smolt monitoring program. Only smolts that pass through the smolt bypass system are examined. Smolts that pass through the turbines or over the spillways are not sampled. There is limited sampling of smolts for examination for clinical signs of GBT from the reservoir, forebay, or tailrace.

A pressurization of 5 minutes (shortest time tested) to 100 feet of head resulted in a significant reduction in the signs of gas bubble trauma in the fins, lateral line, and gills. If the reabsorption potential of pressure-time history for smolts is similar to the 5-minute pressurization treatment, the current smolt monitoring program may be underestimating the prevalence of gas bubble trauma in the Columbia River.

At a total dissolved gas pressure of 125%, the hydrostatic compensation depth is approximately 8.2 feet. If the smolts remain below this depth, gas will be transferred from any existing bubbles into the water and the signs of gas bubble trauma will eventually disappear. Therefore, it is not necessary for smolts to remain at 100 feet for 5 minutes for significant reabsorption to occur.

The exposure of 5 minutes at 100 feet of head has a reabsorption potential of -0.50 feet/days. The required times for other depths are presented in Figure 4. In terms of the reabsorption potential, 62 minutes at 20 feet or 23 minutes at 40 feet are equivalent to the 5 minutes at 100 feet used in this experimental work. (This computation is based on the assumption of a river total gas pressure = 125% and ignores the contribution of the pressurization and depressuriziation phases in the computation of equivalent times.)

The overall purpose of this experimental work was to assess quickly the likelihood of gas bubble reabsorption in smolts during simulated bypass passage. Because of the preliminary nature of this research, the following additional research is recommended to resolve uncertainty with the smolt monitoring program:

(1) Development of a detailed understanding of the kinetics of gas bubble reabsorption and regrowth under laboratory conditions.

(2) Development and validation of protocols for the examination of smolts and adults for clinical signs of gas bubble trauma.

(3) Identification and assessment of the use of chronic lesions or biochemical indicators for quantification of exposure to dissolved gas supersaturation. (This should include both laboratory work followed by field verification).

(4) Development of accurate pressure-time histories for smolts passing down the Columbia and Snake Rivers (including both reservoir and dam passage).

(5) Comparison of the prevalence of signs of gas bubble trauma from smolts collected from the forebay and tailrace compared to smolts collected from the bypass system.

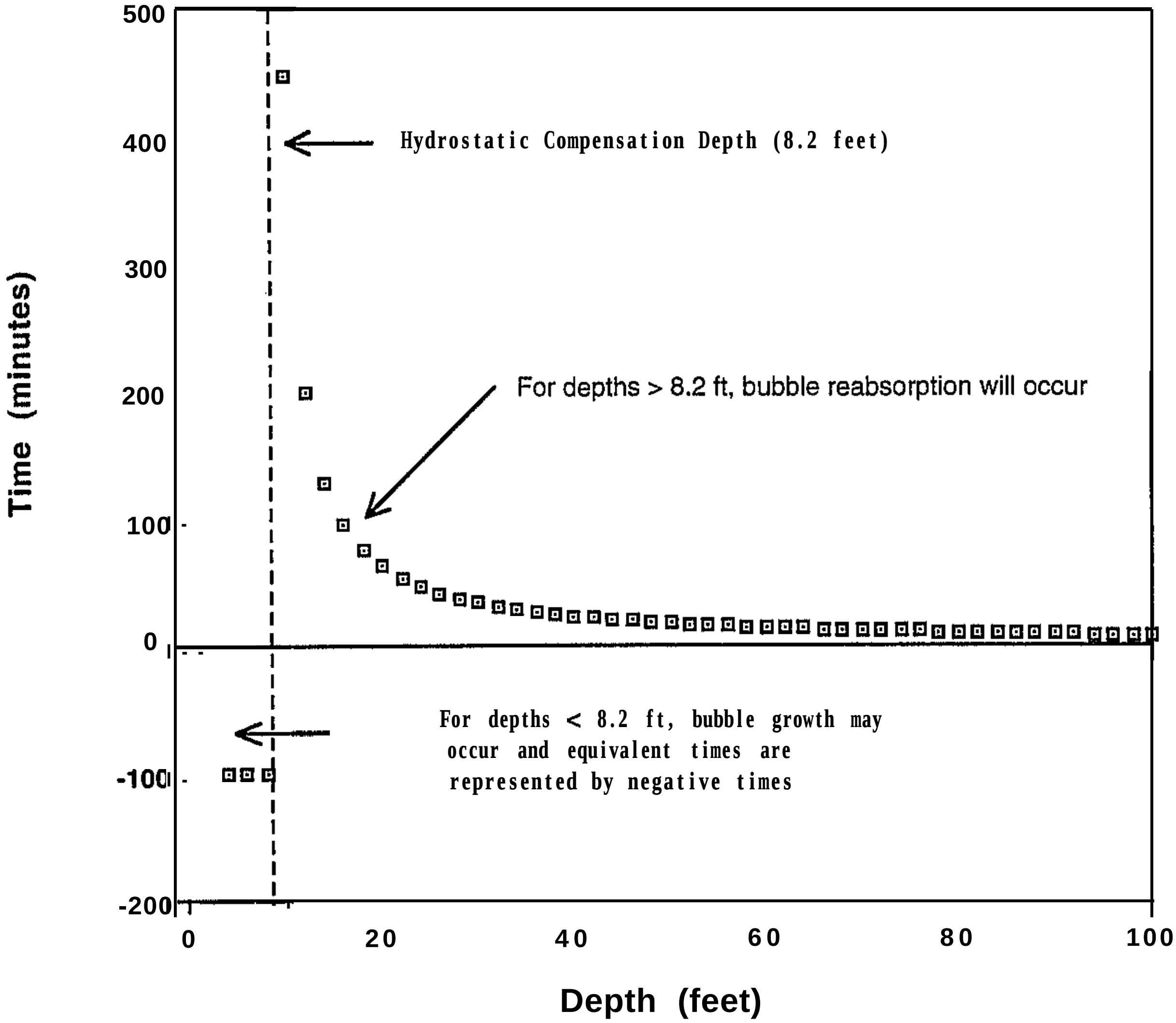

Figure 4. Times and depths equivalent to a reabsorption potential of -0.50 feet/days or 5 minutes at 100 feet (based on a TGP = 125%). The hydrostatic compensation depth is equal to be 8.2 ft. For depths less than 8.2 ft, bubble growth may occur and equivalent times are represented by negative times.

6.0 ADDITIONAL OBSERVATIONS

During the conduct of this experimental work, a number of other observations were made. These observations and comments are presented below:

- This study indicated that reduction and disappearance of gas bubbles from the lateral line and gills was extremely rapid in comparison to reduction and disappearance of gas bubbles from the fins. This is consistent with the structure and function of these organs and the in situ observation of gas dissipation from the gills made in this study.

- It was clear from the examination of many samples of fish exposed to hyperbaric conditions that the loss of gas bubbles from fins left residual inflammatory lesions where bubbles had been present. The presence of these lesions was thus recorded as a percentage of each fin covered. These lesions were characterized by a hemostasis of veins in the fin which were filled with fluid and cellular aggregates. In addition, surrounding tissues were edematous or contained cellular exudate.

- Observations in this study showed that gas bubbles in the lateral line could form in fish residing in vigorously aerated water at equilibrium saturation.

- The sequelae of residual lesions would be a wound healing process or infection and necrosis of the affected fins. In either case the result would be a chronic effect on the fish lasting up to several weeks, depending on temperature and other factors. The lesions in the fins are not characteristic of other known diseases of salmon in the river system and may be useful as indicators of prior exposure to dissolved gas supersaturation in the river.

- The short time in which gas bubbles can dissipate from the branchial vasculature confirms the necessity to analyze fish very quickly once they are captured. Small spherical bubbles from intact fish dissipated in less than two minutes while elongated bubbles dissipated in three to eight minutes. In some cases, residual lesions were observed in branchial arteries and veins after the disappearance of bubbles. Observations in this study were made using fiber optic incident illumination and remote transmitted illumination so that the stage of the microscope was not heated above ambient temperatures during examination. Heating of the specimen by other forms of illumination would greatly accelerate the dissipation of bubbles.

- Observation of bubbles in the intact gill arch, either in the fish or excised from the fish may offer a useful alternative method for determining the presence of gas bubbles in the gills. This method can be applied more quickly than methods requiring further dissection and appears to result in more sensitive detection of branchial gas bubbles.

- In this study, we determined that gas bubbles in the gills can be enumerated in live, intact, anesthetized fish. With some further development, this non-lethal gas bubble enumeration method may have application to gas bubble monitoring.

7.0 REFERENCES

Colt, J. 1984. *Computation of Dissolved Gas Concentrations in Water as Functions of Temperature, Salinity, and Pressure.* Special Publication No. 14, American Fisheries Society, Bethesda, Maryland.

Montgomery Watson. 1995. *Allowable Gas Supersaturation For Fish Passing Hydroelectric Dams.* Draft Report, Prepared for Bonneville Power Administration, Portland, Oregon, Project Number 93-8, Contract Number DE-AC79-93BP66208.

APPENDIX A LETTER FROM DR. LARRY FIDLER TO MR. TED BOTTIGER

Aspen Applied Sciences Ltd.

Environmental Scientists and Consultants

November 23, 1994

R. Ted Bottiger
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Dear Mr. Bottiger:

As you may recall, I appeared at your August council meeting to discuss some aspects of dissolved gas supersaturation (DGS) and gas bubble trauma (GBT) in fish. I also provided the council with a report describing research programs which I felt were essential to providing a more rational scientific basis for managing spill and DGS during the 1995 anadromous fish migration season.

Since then, I have had time to examine, in more detail, one of the concerns which I raised in the report. That concern was with the effect of high hydrostatic pressures associated with fish entering the smolt bypass system on signs of gas bubble trauma which may be observed at the various smolt monitoring sites. It is an area of concern which I raised in the Montgomery Watson 1994 smolt monitoring review report and in the NMFS DGS/GBT panel meeting of November 1-3, 1994. In this letter. I wish to advise you that I have additional evidence which suggests this effect may invalidate the entire smolt monitoring program as presently designed. In the following I will present some of the information which I believe supports this hypothesis.

In my work at the University of British Columbia, I derived equations which describe the growth of bubbles in the cardiovascular systems of fish. The equations and their solutions were compared with equations developed by other investigators for bubble growth in water (Pleset and Zwick 1954). I found that my solutions (when adjusted for water conditions) were identical to those of Pleset and Zwick (1954) for bubble growth in water. This helped validate the equations for use in predicting bubble growth in the vascular systems of fish. When I applied the equations to bubble growth in gill lamella, I found that at a water ΔP of 190 mmHg (sea level TGP = 125%) it took about five minutes for a nucleation site in the gill lamella (r_o = 12 μM) to increase its size to the point where it could block the afferent or efferent arteries of the primary gill lamella. In this case of bubble growth. it is important to consider that the ΔP driving bubble growth is 190 mmHg.(*i.e.*, water TGP = 950 mmHg while the initial gas pressure in the nucleation site is 760 mmHg).

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It should be noted that the time for a bubble to develop to this size is not the time to mortality for the fish. Mortality occurs only after many bubbles have formed and expanded into the afferent and efferent gill arteries to th e extent that a manor portion of the gill blood flow is blocked.

Now, consider a fish which has developed bubbles in the gill lamella or the lateral line and enters the smolt bypass system. To be intercepted by the traveling screens. the lish will have to descend in the water column to a depth of 15 to 20 m. At this depth. the hydrostatic pressure is 1 108 to 1478 mmHg (*i.e.*, 73.89 mmHg m). The total pressure of gases in gill or lateral line bubbles is now 1868 to 2238 mmHg [*i.e.*, 760 mmHg (sea level atmospheric pressure) + 1108 mmHg to 760 -- 1478 mmHg]. At the same time. the TGP of the water is still 950 mmHg (sea level TGP = 125%. ΔP = 190 mmHg). In this case. The ΔP gradient is reversed form that which initially caused the bubbles to grow. The ΔP which is now forcing gas our of these bubbles and causing their collapse is 918 mmHg (*i.e.*, 1868 - 350 mmHg) to 1288 mmHg (*i.e.*, 223X - 950 mmHg). Thus, the ΔP driving bubble collapse is much greater that the ΔP causing bubble growth (*i.e.*, 918 to 1288 **mmHg** compared to 130 **mmHg**). Given the fact that fish may be in the smolt bypass system for periods ranging from many minutes to days (personal communication form Brad Ebey - U.S. COE biologist. McNary Dam). It is likely that any pre-existing GBT bubbles. either in the gills or lateral line. will have collapsed before the fish are examined at the smolt GBT monitoring sites. The attached figure illustrates a hypothetical ΔP history that a salmon orr steelhead smolt might experience to the McNary Dam monitoring site.

In my involvement in the Montgomery Watson turbine passage experiment at Battlle Pacific Northwest Laboratories this past summer. I had an opportunity to see just how fast bubbles can collapse when subjected to elevated hydrostatic pressures. The Montgomery Watson experiments were designed to establish if passage through turbines incresed the impacts of dissolved gas supersaturation on fish. The experiments involved using clear acrylic hyperbaric chambers combined with a dissolved gas generation system to simulate turbine passage. Fish were placed in the chambers in dissolved gas supersaturated water and subjected to a hydrostatic pressure history which simulated passage through a turbine. During many of the experiments, bubbles were observed on the internal walls of the hyperbaric chambers. This was due to the presence of dissolved gas supersaturated water in the chamber - either from the gas exchange system or from the warming of the water in the chamber. When the portion of the pressure history corresponding to a fish descending into the tubine intake was applied to the chambers, all bubbles on the internal walls of the chambers collapsed in a matter of seconds. This portion of the pressure cycle took only 30 - 50 seconds. the first 35 - 40 seconds of which would be identical to that experienced by a fish entering the smolt bypass system. Furthermore, the bubbles which were initially observed in the chambers were much larger than those which would exist in the gill lamella or lateral lines of fish. Thus, it can be expected that gill and lateral line bubbles should collapse even more quickly. Based on this evidence, I again suggest that any bubbles which may exist in the gill filaments or lateral lines of fish in the reservoirs will have collapsed as a result of fish descending into the high hydrostatic pressures of the smolt bypass system and, therefore, will not be observed in the GRT monitoring program - even with the aid of microscopes.

There is the question as to whether the bubbles will redevelop in the gill filaments and lateral lines once fish have emerged from the high hydrostatic pressures of the smolt bypass system and before they are intercepted at the snolt monitoring stations. There are three reasons why this may not happen. First, at the high hydrostatic pressures of the snolt bypass systems. gill and lateral line bubbles may collapse into nucleation sites which are much smaller than the ones from which the original bubbles developed. In this case, the water TGP required to re-initiate bubble growth would have to be greater than that which initiated the original bubble growth. Next, the original bubbles may have formed as a result of elevated TGP levels at other locations higher in the river system (*e.g.*, at Wanapum or Dworshak in 1994). At the monitoring sites lower on the river, the TGP levels may not be high enough to re-initiate bubble growth once they have collapsed in the smolt bypass systems. Finally, through water turbulence, the smolt bypass systems at many of the dams rapidly dissipate dissolved gas supersaturation. Because dissolved gas levels are significantly reduced before the fish arrive at the smolt monitoring sites, the levels may not be adequate to re-initiate bubble growth.

If this hypothesis is correct, the entire smolt GBT monitoring program will have to be revised before spill programs are initiated in 1995. Otherwise, the spill operations will be done without any reliable feedback on the effects of dissolved gas supersaturation on smolts. Clearly, it is important that this hypothesis be examined as quickly as possible. Fortunately, this can be done with the hyperbaric chamber system at Battelle Pacific Northwest Laboratories. The existing pressure cycle which is programmed into the controls for the chambers will have to be modified slightly to simulate the range of hydrostatic pressure histories in the smolt bypass system. However, once that is done, it should be a straight forward matter of exposing juvenile rainbow trout to DGS in the hyperbaric chambers (one chamber with the simulated pressure histories and one chamber without) and observing the degree of bubble collapse. I estimate that the experimental portion of the work can be completed in three weeks or less with an additional week required to prepare a final report.

Thank you for taking the time to consider the information I have presented herein. I hope that it will be of help in planning for the 1995 spill and anadromous fish migration season. Should you have any questions regarding what I have presented, please do not hesitate to contact me.

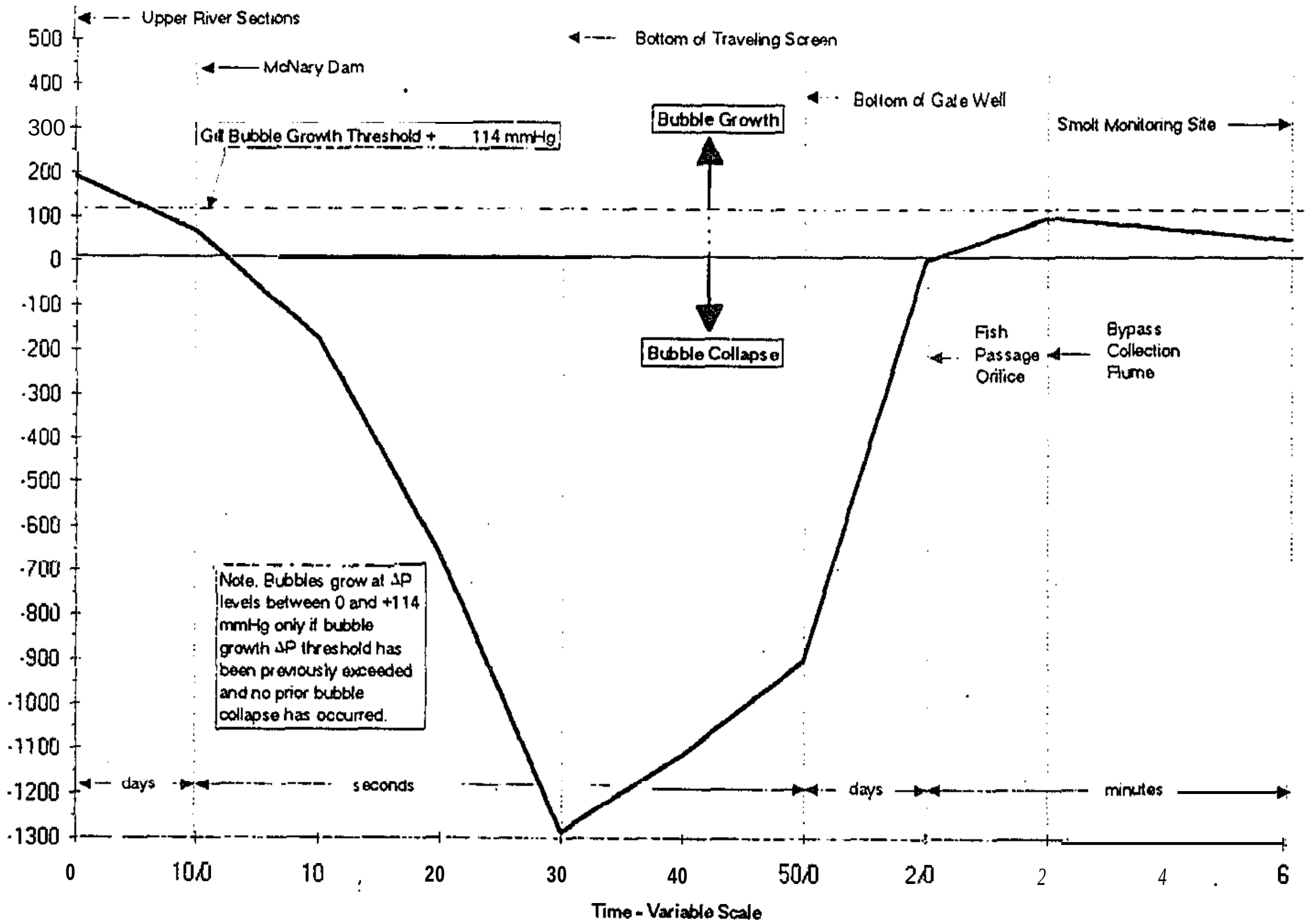
Sincerely

L. E. Fidler

Larry E. Fidler, Ph.D., R.P. Bio.
President

Copies: BP.4 - Bill Maslen
COE - Jim Athearn
Montgomery Watson - John Colt
NMFS - Earl Dawley
NMFS - Steve Grabowski
ORNL - Chuck Coutant

Hypothetical ΔP History for Salmon or Steelhead Smolts to McNary Dam Monitoring Site



APPENDIX B DATA SHEETS

GAS SUPERSATURATION EXPOSURES - EXPERIMENT DATA SUMMARY SHEET
Montgomery Watson - PNL 4-3-95 to 4-7-95
****

Date: April 4, 1994
Exper ID: 1. Gas loaded CONTROL fish for 1 hour pressure treated fish

INDIVID SAMPLE NO.	STD LENGTH	PRSCNCE RESIDUAL FIN LESN	% BUBBLE COVERAGE FINS: CAUDAL	DORSAL	ANAL	AVG % BUBBLE COVER FINS	% COVERAGE LATERAL LINE: LEFT ANT	LEFT MID	LEFT PST	% COVERAGE LATERAL LINE: RGHT ANT	RGHT MID	RGHT PST	AVG % COVER LAT LINE	GILLS: NUMB. 1°LAML AFFCTD	TOTAL NO. 1°LAML EXAMND	GILLS % 1°LAML AFFCTD
1	117	0	0	60	20	27%	60	100	20	100	80	90	75%	1	40	3%
2	156	0	0	0	5	2%	60	80	80	10	5	5	40%	23	40	58%
3	132	0	0	0	0	0%	100	60	50	70	40	90	68%	0	100	0%
4	119	0	0	0	0	0%	50	80	90	80	100	90	82%	1	100	1%
5	139	0	0	0	0	0%	30	50	50	20	60	90	50%	0	100	0%
6	132	0	20	0	0	7%	50	100	100	80	70	90	82%	0	100	0%
7	126	0	10	0	10	7%	10	80	90	100	80	80	73%	0	100	0%
8	146	0	0	0	10	3%	80	80	90	100	100	90	90%	0	100	0%
9	131	0	0	0	0	0%	90	70	90	100	90	100	90%	0	100	0%
10	125	0	30	0	0	10%	40	60	60	100	100	100	77%	0	100	0%
GROUP AVG\FREQ:	132	0/10	3/10	1/10	3/10	5.5%							72.7%	3/10		6.2%

Date: April 4, 1994
Exper ID: 1. Gas loaded TREATMENT Fish after one hour pressure treatment

INDIVID SAMPLE NO.	STD LENGTH	PRSCNCE RESIDUAL FIN LESN	% BUBBLE COVERAGE FINS: CAUDAL	DORSAL	ANAL	AVG % BUBBLE COVER FINS	% COVERAGE LATERAL LINE: LEFT ANT	LEFT MID	LEFT PST	% COVERAGE LATERAL LINE: RGHT ANT	RGHT MID	RGHT PST	AVG % COVER LAT LINE	GILLS: NUMB. 1°LAML AFFCTD	TOTAL NO. 1°LAML EXAMND	GILLS % 1°LAML AFFCTD
1	136	0	0	0	0	0%	0	10	0	0	10	0	3%	0	100	0%
2	144	0	0	0	0	0%	0	0	10	0	0	0	2%	0	100	0%
3	132	0	0	0	0	0%	0	0	0	0	0	0	0%	0	100	0%
4	125	1	0	0	0	0%	0	0	0	0	0	0	0%	0	100	0%
5	115	0	0	0	0	0%	0	0	0	0	0	0	0%	0	100	0%
6	125	1	0	0	0	0%	0	0	0	0	0	0	0%	0	100	0%
7		1	2	0	0	1%	0	0	0	0	0	0	0%	0	100	0%
8	117	1	0	0	0	0%	0	0	0	0	10	0	2%	2	100	2%
9	117	0	0	0	0	0%	0	0	0	0	0	0	0%	0	100	0%
10	128	1	0	0	0	0%	0	0	0	0	0	0	0%	0	100	0%
GROUP AVG/FREQ:	127	5/10	1/10	0/0	0/0	0.1%							0.7%	1/10		0.2%

GAS SUPERSATURATION EXPOSURES - EXPERIMENT DATA SUMMARY SHEET
Montgomery Watson - PNL 4-3-95 to 4-7-95
****

Date: 4-5-95

Exper ID: 2 Gas loaded CONTROL fish for 2 hour pressure treatment

INDIVID SAMPLE NO.	STD LENGTH	PRSCNCE RESIDUAL FIN LESN	% BUBBLE COVERAGE FINS: CAUDAL	DORSAL	ANAL	AVG % BUBBLE COVER FINS	% COVERAGE LATERAL LINE: LEFT ANT	LEFT MID	LEFT PST	% COVERAGE LATERAL LINE: RGHT ANT	RGHT MID	RGHT PST	AVG % COVER LAT LINE	GILLS: NUMB. 1°LAML AFFCTD	TOTAL NO. 1°LAML EXAMND	GILLS % 1°LAML AFFCTD
1	134	0	0	20	0	7%	30	60	90	60	20	100	60%	0	100	0%
2	139	0	50	0	0	17%	80	100	60	90	50	100	80%	48	100	48%
3	145	0	0	40	0	13%	30	100	70	90	100	10	67%	84	100	84%
4	147	0	0	0	0	0%	40	70	60	80	70	70	65%	0	100	0%
5	135	0	0	40	30	23%	90	80	80	80	50	100	80%	0	100	0%
6	156	0	0	0	0	0%	0	10	80	60	50	70	45%	0	100	0%
7	135	0	0	0	0	0%	10	100	30	30	40	80	48%	0	100	0%
8	122	0	0	0	0	0%	30	100	90	40	50	20	55%	0	100	0%
9	121	0	0	0	0	0%	90	10	30	40	70	90	55%	0	100	0%
10	134	0	0	40	0	13%	70	100	90	60	80	20	70%	32	100	32%
GROUP AVG/FREQ	137	0/10	1/10	4/10	3/10	7.3%							62.5%			16.4%

Date: 4-5-95

Exper ID: 2 Gas loaded TREATEMENT fish after 2 hour pressure treatment

Exper ID: SAMPLE NO.	STD LENGTH	PRSCNCE RESIDUAL FIN LESN	% BUBBLE COVERAGE FINS: CAUDAL	DORSAL	ANAL	AVG % BUBBLE COVER FINS	% COVERAGE LATERAL LINE: LEFT ANT	LEFT MID	LEFT PST	% COVERAGE LATERAL LINE: RGHT ANT	RGHT MID	RGHT PST	AVG % COVER LAT LINE	GILLS: NUMB. 1°LAML AFFCTD	TOTAL NO. 1°LAML EXAMND	GILLS % 1°LAML AFFCTD
1	123	1	5	0	0	2%	0	10	0	0	0	0	2%	0	100	0%
2	151	1	0	0	0	0%	0	0	0	0	0	0	0%	0	100	0%
3	134	1	10	0	0	3%	0	0	0	10	0	0	2%	0	100	0%
4	146	1	10	0	0	3%	0	10	0	10	0	0	3%	0	100	0%
5	133	1	0	0	0	0%	0	0	0	0	0	0	0%	0	100	0%
6	142	1	0	5	0	2%	0	0	0	0	0	0	0%	0	100	0%
7	141	1	0	0	0	0%	0	0	0	0	0	0	0%	0	100	0%
8	99	1	0	0	0	0%	0	0	0	0	0	0	0%	0	100	0%
9	149	1	10	0	0	3%	0	0	0	10	0	10	3%	0	100	0%
10	136	1	10	0	0	3%	0	0	0	0	0	0	0%	0	100	0%
GROUP AVG/FREQ	135	10/10	5/10	1/10	0/10	1.7%							1.0%			0.0%

GAS SUPERSATURATION EXPOSURES - EXPERIMENT DATA SUMMARY SHEET
Montgomery Watson - PNL 4-3-95 to 4-7-95
****

DATE: April 5, 1995
Exper ID: 3 Gas loaded CONTROL fish for 1/2 hour pressure treated fish

INDIVID SAMPLE NO.	STD LENGTH	PRSCNCE RESIDUAL FIN LESN	% BUBBLE COVERAGE FINS CAUDAL	DORSAL	ANAL	AVG % BUBBLE COVER FINS	% COVERAGE LATERAL LINE LEFT ANT	LEFT MID	LEFT PST	% COVERAGE LATERAL LINE RGHT ANT	RGHT MID	RGHT PST	AVG % COVER LAT LINE	GILLS NUMB. 1°LAML AFFCTD	GILLS TOTAL NO. 1°LAML EXAMND	GILLS % 1°LAML AFFCTD
1	141	0	0	0	0	0%	20	80	20	10	5	90	38%	0	100	0%
2	140	0	30	5	0	12%	40	90	30	5	15	100	47%	0	100	0%
3	132	0	10	30	0	13%	100	100	90	90	70	80	88%	0	100	0%
4	140	0	0	0	0	0%	90	100	100	30	20	100	73%	0	100	0%
5	129	0	0	0	0	0%	30	100	60	70	90	100	75%	0	100	0%
6	140	0	0	0	0	0%	40	80	50	70	50	70	60%	0	100	0%
7	148	0	0	0	0	0%	80	100	90	59	70	90	82%	0	100	0%
8	140	0	0	0	0	0%	70	60	90	10	40	50	53%	0	100	0%
9	135	0	0	0	0	0%	60	40	70	20	20	60	45%	0	100	0%
10	131	0	0	0	0	0%	60	90	100	20	40	70	63%	0	100	0%
GROUP AVG/FREQ	138	0/10	2/10	2/10	0/10	2.5%							62.4%			0.0%

DATE: April 5, 1995
Exper ID: 3 Gas loaded TREATMENT fish after 1/2 hour pressure treatment

INDIVID SAMPLE NO.	STD LENGTH	PRSCNCE RESIDUAL FIN LESN	% BUBBLE COVERAGE FINS CAUDAL	DORSAL	ANAL	AVG % BUBBLE COVER FINS	% COVERAGE LATERAL LINE LEFT ANT	LEFT MID	LEFT PST	% COVERAGE LATERAL LINE RGHT ANT	RGHT MID	RGHT PST	AVG % COVER LAT LINE	GILLS NUMB. 1°LAML AFFCTD	GILLS TOTAL NO. 1°LAML EXAMND	GILLS % 1°LAML AFFCTD
1	139	1	20	0	0	7%	5	10	0	5	0	0	3%	0	100	0%
2	148	1	10	0	0	3%	0	10	0	0	20	10	7%	0	100	0%
3	135	1	10	0	0	3%	0	10	5	5	0	0	3%	0	100	0%
4	144	1	10	0	0	3%	0	0	0	0	0	0	0%	0	100	0%
5	135	0	0	0	0	0%	0	0	0	0	0	0	0%	0	100	0%
6	120	0	0	0	0	0%	0	0	0	0	0	0	0%	0	100	0%
7	130	0	0	0	0	0%	0	0	0	0	0	0	0%	0	100	0%
8	142	0	0	0	0	0%	0	0	0	0	0	0	0%	0	100	0%
9	132	0	0	0	0	0%	0	0	0	0	0	0	0%	0	100	0%
10	127	1	0	0	0	0%	0	0	0	0	0	0	0%	0	100	0%
GROUP AVG/FREQ	135	5/10	4/10	0/10	0/10	1.7%							1.3%			0.0%

GAS SUPERSATURATION EXPOSURES - EXPERIMENT DATA SUMMARY SHEET
Montgomery Watson - PNL 4-3-95 to 4-7-95
****

DATE: April 6, 1995
EXPER ID: 4 Gas loaded CONTROL fish
for 5 minute depressurization treatment

INDIVID SAMPLE NO.	STD LENGTH	PRSCNCE RESIDUAL FIN LESN	% BUBBLE COVERAGE FINS: CAUDAL	DORSAL	ANAL	AVG % BUBBLE COVER FINS	% COVERAGE LATERAL LINE: LEFT ANT	LEFT MID	LEFT POST	% COVERAGE LATERAL LINE: RGHT ANT	RGHT MID	RGHT POST	AVG % COVER LAT LINE	GILLS: NUMB. 1 LAML AFFCTD	GILLS: TOTAL NO. 1 LAML EXAMND	GILLS % 1 LAML AFFCTD
1	124	0	0	0	0	0%	40	90	80	90	70	90	77%	0	100	0%
2	140	0	70	0	0	23%	10	90	100	20	50	40	52%	35	100	35%
3	125	0	60	0	0	20%	90	100	20	70	100	40	70%	14	100	14%
4	125	0	0	0	0	0%	80	100	100	60	20	100	77%	0	100	0%
6	129	0	10	50	0	20%	30	100	50	20	80	100	63%	6	100	6%
7	141	0	60	50	40	50%	40	90	100	90	90	100	85%	12	100	12%
7	144	0	0	0	0	0%	5	20	100	20	50	100	49%	26	100	26%
8	112	0	0	0	10	3%	30	80	100	20	70	70	62%	0	100	0%
9	130	0	0	30	30	20%	10	100	30	40	100	70	58%	70	100	70%
10	119	0	0	0	20	7%	90	90	100	70	90	60	83%	66	100	66%
GROUP AVG/ FREQ	129					14.3%							67.6%			22.9%

DATE: April 6, 1995
EXPER ID: 4 Gas loaded TREATMENT fish
after 5 minute pressure treatment

INDIVID SAMPLE NO.	STD LENGTH	PRSCNCE RESIDUAL FIN LESN	% BUBBLE COVERAGE FINS: CAUDAL	DORSAL	ANAL	AVG % BUBBLE COVER FINS	% COVERAGE LATERAL LINE: LEFT ANT	LEFT MID	LEFT POST	% COVERAGE LATERAL LINE: RGHT ANT	RGHT MID	RGHT POST	AVG % COVER LAT LINE	GILLS: NUMB. 1 LAML AFFCTD	GILLS: TOTAL NO. 1 LAML EXAMND	GILLS % 1 LAML AFFCTD
1	133	0	5	0	0	2%	5	10	20	50	0	10	16%	0	100	0%
2		0	0	40	0	13%	5	50	90	10	5	20	30%	0	100	0%
3		1	20	0	0	7%	0	20	30	10	10	30	17%	0	100	0%
4		1	0	0	0	0%	0	20	20	20	20	20	17%	0	100	0%
5	127	0	10	20	0	10%	30	0	20	80	20	30	30%	0	100	0%
6	134	0	0	0	0	0%	5	10	5	10	0	20	8%	0	100	0%
7	131	0	0	0	0	0%	10	40	0	5	0	60	19%	0	100	0%
8	135	0	20	0	0	7%	10	60	10	0	20	10	18%	0	100	0%
9	145	1	20	0	0	7%	10	0	20	10	20	20	13%	0	100	0%
10	140	0	10	40	10	20%	40	0	30	50	40	40	33%	0	100	0%
GROUP AVG/ FREQ	135					6.5%							20.2%			0.0%

Montgomery Watson - PNL gas bubble experiments April 3-6, *1995*

Comparison of two methods of determining gas bubbles in gills

		GILLS EXAMINED IN SITU			GILLS EXAMINED BY EXCISION OF ARCH AND PRIMARY LAMELLAE		
EXPER NUMBER	INDIVID SAMPLE NO.	NUMB 1 LAML AFFCTD	TOTAL 1 LAML EXAMND	% 1 LAML AFFCTD	NUMB 1 LAML AFFCTD	TOTAL 1 LAML EXAMND	% 1 LAML AFFCTD
	1	1	40	3%	4	40	10%
1 CNTRL	2	23	40	58%	16	40	40%
	3	0	100	0%	0	40	0%
	4	1	100	1%	0	120	0%
	5	0	100	0%	0	46	0%
	6	0	100	0%	0	95	0%
	7	0	100	0%	0	100	0%
	8	0	100	0%	0	65	0%
	9	0	100	0%	0	76	0%
	10	0	100	0%	0	60	0%
GRP AVG				6.2%			5.0%
	1	0	100	0%	0	65	0%
1 TRTMT	2	0	100	0%	0	100	0%
	3	0	100	0%	0	60	0%
	4	0	100	0%	0	60	0%
	5	0	100	0%	0	55	0%
	6	0	100	0%	0	50	0%
	7	0	100	0%	0	50	0%
	8	2	100	2%	2	100	2%
	9	0	100	0%	0	60	0%
	10	0	100	0%	0	60	0%
GRP AVG				0.2%			0.2%
	1	0	100	0%	0	100	0%
2 CNTRL	2	48	100	48%	72	100	72%
	3	84	100	84%	72	100	72%
	4	0	100	0%	0	70	0%
	5	0	100	0%	0	100	0%
	6	0	100	0%	0	100	0%
	7	0	100	0%	0	70	0%
	8	0	100	0%	0	100	0%
	9	0	100	0%	0	100	0%
	10	32	100	32%	0	40	0%
GRP AVG				16.4%			14.4%
2 TRTMT	1	0	100	0%	0	80	0%
	2	0	100	0%	0	100	0%
	3	0	100	0%			
	4	0	100	0%	0	100	0%
	5	0	100	0%	0	90	0%
	6	0	100	0%	0	100	0%
	7	0	100	0%	0	100	0%
	8	0	100	0%	2	100	2%
	9	0	100	0%	0	100	0%
	10	0	100	0%	0	100	0%
GRP AVG				0			0.2%

Comparison of two methods of determining gas bubbles in gills

		GILLS EXAMINED IN SITU			GILLS EXAMINED BY EXCISION OF ARCH AND PRIMARY LAMELLAE		
EXPER NUMBER	INDIVID SAMPLE ND.	NUMB 1 LAML AFFCTD	TOTAL 1 LAML EXAMND	% 1 LAML AFFCTD	NUMB 1 LAML AFFCTD	TOTAL 1 LAML EXAMND	% 1 LAML AFFCTD
******	******	******	******	******	******	******	******
3 CNTRL	1	0	100	0%	0	100	0%
	2	0	100	0%	0	100	0%
	3	0	100	0%	0	100	0%
	4	0	100	0%	0	85	0%
	5	0	100	0%	0	80	0%
	6	0	100	0%	0	100	0%
	7	0	100	0%	0	100	0%
	8	0	100	0%	0	100	0%
	9	0	100	0%	0	100	0%
	10	0	100	0%	0	100	0%
GRP AVG				0.0%			0.0%
3 TRTMT	1	0	100	0%	0	80	0%
	2	0	100	0%	0	80	0%
	3	0	100	0%	0	70	0%
	4	0	100	0%	0	70	0%
	5	0	100	0%	0	100	0%
	6	0	100	0%	0	100	0%
	7	0	100	0%	0	100	0%
	8	0	100	0%	0	100	0%
	9	0	100	0%	0	100	0%
	10	0	100	0%	0	100	0%
GRP AVG				0.0%			0.0%
4 CNTRL	1	0	100	0%	12	80	15%
	2	35	100	35%	35	80	44%
	3	14	100	14%	0	70	0%
	4	0	100	0%	0	70	0%
	5	6	100	6%	0	100	0%
	6	12	100	12%	0	100	0%
	7	26	100	26%	24	100	24%
	8	0	100	0%	0	100	0%
	9	70	100	70%	65	100	65%
	10	66	100	66%	42	100	42%
GRP AVG				22.9%			19.0%
4 TRTMT	1	0	100	0%	0	100	0%
	2	0	100	0%	0	100	0%
	3	0	100	0%	0	100	0%
	4	0	100	0%	0	100	0%
	5	0	100	0%	0	100	0%
	6	0	100	0%			
	7	0	100	0%	0	100	0%
	8	0	100	0%	0	100	0%
	9	0	100	0%	0	100	0%
	10	0	100	0%	0	100	0%
GRP AVG				0.0%			0.0%

ΔP =	15								
Pressurization/Depressurization									
Pressure	Head	Head	Uncomp ΔP	time	time	ΔPxtime	ΔPxtime	ΔPxtime	ΔPxtime
(psi)	(ft)	(mm Hg)	(mm Hg)	s	min	mm Hg x min	mm Hg x min	mm Hg x hr	mm Hg x days
10	22	517	-502	30	0.5	-251	(Total)	(Total)	(Total)
20	45	1034	-1019	30	0.5	-510			
30	67	1551	-1536	30	0.5	-768			
40	90	2068	-2053	30	0.5	-1027			
						-2555			
absorption Period									
45	101	2327	-2312		5	-11558	-16668	-278	-11.57
45	101	2327	-2312		30	-69346	-74456	-1241	-51.71
45	101	2327	-2312		60	-138692	-143802	-2397	-99.86
45	101	2327	-2312		120	-277384	-282494	-4708	-196.18
ΔP =	15	Compensation Depth (ft) =		0.66					
Pressurization/Depressurization									
Pressure	Head		Uncomp Head	time	time	Depth x time	Depth x time	Depth x time	Depth x time
(psi)	(ft)		(ft)	s	min	feet x min	feet x min	feet x hr	feet x days
10	22		-22	30	0.5	-11	(Total)	(Total)	(Total)
20	45		-44	30	0.5	-22			
30	67		-67	30	0.5	-33			
40	90		-89	30	0.5	-45			
						-111			
absorption Period									
45	101		-100		5	-502	-724	-12	-0.50
45	101		-100		30	-3011	-3233	-54	-2.24
45	101		-100		60	-6021	-6243	-104	-4.34
45	101		-100		120	-12043	-12266	-204	-6.62

Water Quality/reabsorption

length (inches)								
ID (inches)	3.875	Area (sf)	0.08189772					
	ΔP	DO	T	Flow				
	(mm Hg)	(mg/L)	(C)	(lpm)	gpm	cfs	inches/s	BL/s
	20	11.8	8	51.40	13.60	0.03029831	4.44	0.87
	11	11.9	8.1	54.50	14.42	0.03212564	4.71	0.92
	12	11.7	8.1	60.90	16.11	0.0358982	5.26	1.03
	8	11.1	8.2	53.80	16.06	0.03578031	5.24	1.02
	7	11.2	8.3	52.70	14.23	0.03171302	4.65	0.91
	12	11.6	8.1	52.70	13.94	0.03106461	4.55	0.89
	27	12.2	8.4	38.60	10.21	0.02275321	3.33	0.65
	23	11.8	8	27.00	7.14	0.01591546	2.33	0.46
	25	11.9	8					
	23	11.2	8					
mean	16.8	11.64	8.12	49.95	13.21			0.84
SD	7.54	0.36	0.14	11.56	3.06			0.20