THE EFFECTS OF SUPERSATURATION OF DISSOLVED GASES ON THE FISHERY OF THE BIGHORN RIVER DOWNSTREAM OF THE YELLOWTAIL AFTERBAY DAM.

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by:
Robert G. White, Principal Investigator
Glenn Phillips, Co-Principal Investigator
George Liknes, Project Biologist
Calvin Sprague, Research Aide
Jim Brammer, Graduate Assistant
William Connor, Graduate Assistant
Larry Fidler, Graduate Assistant
Tommy Williams, Graduate Assistant
W. Patrick Dwyer, Cooperator

Montana Cooperative Fishery Research Unit
Montana State University
Bozeman, MT
and
Montana Department of Fish, Wildlife & Parks
Helena, MT

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INTRODUCTION

Several excellent literature reviews describing problems associated with gas supersaturation in natural waters have recently been published (Bouck 1980; Weitkamp and Katz 1980); thus there is little need to review the gas bubble trauma literature here. Bouck (1980) describes gas bubble trauma (GBT) as a "physically induced process caused by uncompensated hyperbaric pressure of total dissolved gases." Gas bubble trauma has commonly been documented in fish living below dams where plunging water entraps atmospheric gases and forces them to a depth where they become supersaturated (Bouck 1980).

Symptoms of the trauma in fish includes formation of emboli in blood vessels and emphysema in soft tissues. Fish exposed to supersaturated gases commonly form bubbles in fins, on the opercles or in the oral cavity. In severe cases, fish may develop exophthalmia or pop-eye (Weitkamp and Katz 1980). All of these symptoms have been observed in fish from the Bighorn River downstream of the Yellowtail Afterbay Dam.

The Yellowtail Afterbay Dam serves as a reregulating facility below Yellowtail Dam and powerplant to provide uniform daily discharges into the Bighorn River. The presence of GBT in trout below the Afterbay Dam was first documented in 1973 (Swedberg 1973). The Bureau of Reclamation and Montana Department of Fish, Wildlife and Parks (MDFWP) subsequently concluded that gas entrainment occurs when water passes through gates in the Afterbay Dam, particularly the sluiceway gates (Bureau of Reclamation 1973). However, they surmised that no practical modifications in the Afterbay operation would preclude gas levels from exceeding 110% saturation, the present Environmental Protection Agency (EPA) criterion for total dissolved gases to protect freshwater aquatic life (EPA 1976).

A fish kill observed in 1979 was allegedly due to GBT (Porter and Viel 1980). Subsequent studies by the U. S. Fish and Wildlife Service demonstrated the geographic extent and biological severity of the problem. From February-August 1981, 33 and 11% of the brown and rainbow trout, respectively, exhibited external symptoms of GBT (Curry and Curry 1981). Disease incidence in brown trout during 1981 peaked in June when 72% of those captured were affected (Curry and Curry 1981). Also, most (90%) of the traumatized fish were found in the first 8 river kilometers (5 river miles) below Afterbay dam (Porter and Viel 1980).

In fall 1982, the Bureau of Reclamation attempted to solve the problem by installing deflector plates (flip lips) on the face of the dam. However, turbulence resulting from these structures caused rocks to be pulled into the afterbay stilling basin and threatened to erode the base of the dam; because of this they were removed in July 1983.

More recent monitoring by the MDFWP confirms that the problem persists and that large brown trout, those ≥ 356 mm (14.0 inches), are the most visibly affected segment of the population and that brown trout are more susceptible than rainbow trout. Additionally, the lack of successful rainbow trout reproduction in the mainstem Bighorn River may be due to the gas problem (Fredenberg 1985). It is clear from work conducted to date that we do not fully understand the relationships between reservoir operations, flows and other ambient conditions on gas supersaturation levels and the incidence of gas bubble trauma in fishes. Furthermore, we do not know why adult brown trout exhibit more severe external symptoms of gas bubble trauma than adult rainbow trout nor why rainbow trout reproduction appears suppressed. The Bighorn River, because of its relatively small size compared to other western hydroelectric projects where gas supersaturation problems exist (e.g., the Columbia River), presents an acceptable study site for increasing our understanding of the relationships between operation and design of dams and GBT. Accordingly, this study was undertaken to:

(1) relate fish population levels and seasonal mortality to gas bubble incidence and dissolved gas saturation regimes.

(2) determine survival rates and factors controlling survival of early life history stages of brown and rainbow trout in the Bighorn River.

(3) relate gas bubble trauma incidence to ambient conditions including gas saturation levels, flow rates and reservoir operation.

(4) determine if different species or life stages of trout partition themselves in the environment in such a way as to influence their susceptibility to gas supersaturation.

(5) determine the catchability of traumatized vs. healthy fish in order to assess the impact of gas bubble disease on the fishing public.

(6) in a more controlled setting, examine incidence of occurrence of gas bubble trauma for various life stages and species at varying water depths and for different river reaches.

(7) determine the ability of brown trout to recover from gas bubble trauma after acquiring symptoms of varying degrees of severity and to assess the influence of water temperature on rate of recovery.

(8) determine what mechanisms (physiological or otherwise) result in brown trout having a higher incidence of gas bubble trauma than rainbow trout in the Bighorn River and to determine what dissolved gas saturation levels are safe for each of these species.

(9) assess the impacts of gas supersaturation on invertebrates and forage fish in the Bighorn River.

Information presented in this report was collected between 1 January and 31 December, 1986. Work related to objectives 1-4 and 6-9 was conducted during the reporting period.

DESCRIPTION OF STUDY AREA

Yellowtail and Afterbay Dams are located in south-central Montana on the Bighorn River approximately 43 air miles southeast of Billings on the Crow Indian Reservation. The facility was constructed from 1963-1966 (WPRS 1980). The principal uses of Yellowtail include power generation, irrigation, flood control and fish and wildlife enhancement. A 250-MW peaking plant is located at the base of Yellowtail Dam. This thin-arch concrete structure is 160 m (525 ft) high and 442 m (1,450 ft) long at the crest. Afterbay Dam, which serves as a reregulating facility, is located 3.5 km (2.2 miles) downstream of the main dam; it is an earthfill embankment with concrete spillway, sluiceway and diversion works. The height of Afterbay Dam is 22 m (72 ft) and it has an overall crest length of 414.5 m (1,360 ft). The spillway has a discharge capacity of 566.4 m^3/s (20,000 cfs), and is 49.4 m (162 ft) wide. Flows are controlled by five, 9.1 m x 4.1 m (30x13.5 ft) radial gates and by the sluiceway, which is 10.4 m (34 ft) wide; its discharge is adjusted by three, 3x2.4 m (10x8 ft) slide gates. The height from the streambed to the maximum controlled water surface is 16.1 m (53 ft). The sluiceway gates can release water 6.9 m (22.5 ft) lower than the radial gates. Flow is continually adjusted to maintain a relatively uniform flow to the river.

The study area extends approximately 19.3 km (12 miles) downstream of Afterbay Dam to Bighorn Access (Figure 1). The gradient is approximately 1.9 m per river kilometer (6.3 feet/river mile) (Stevenson 1975) and the river channel braids in

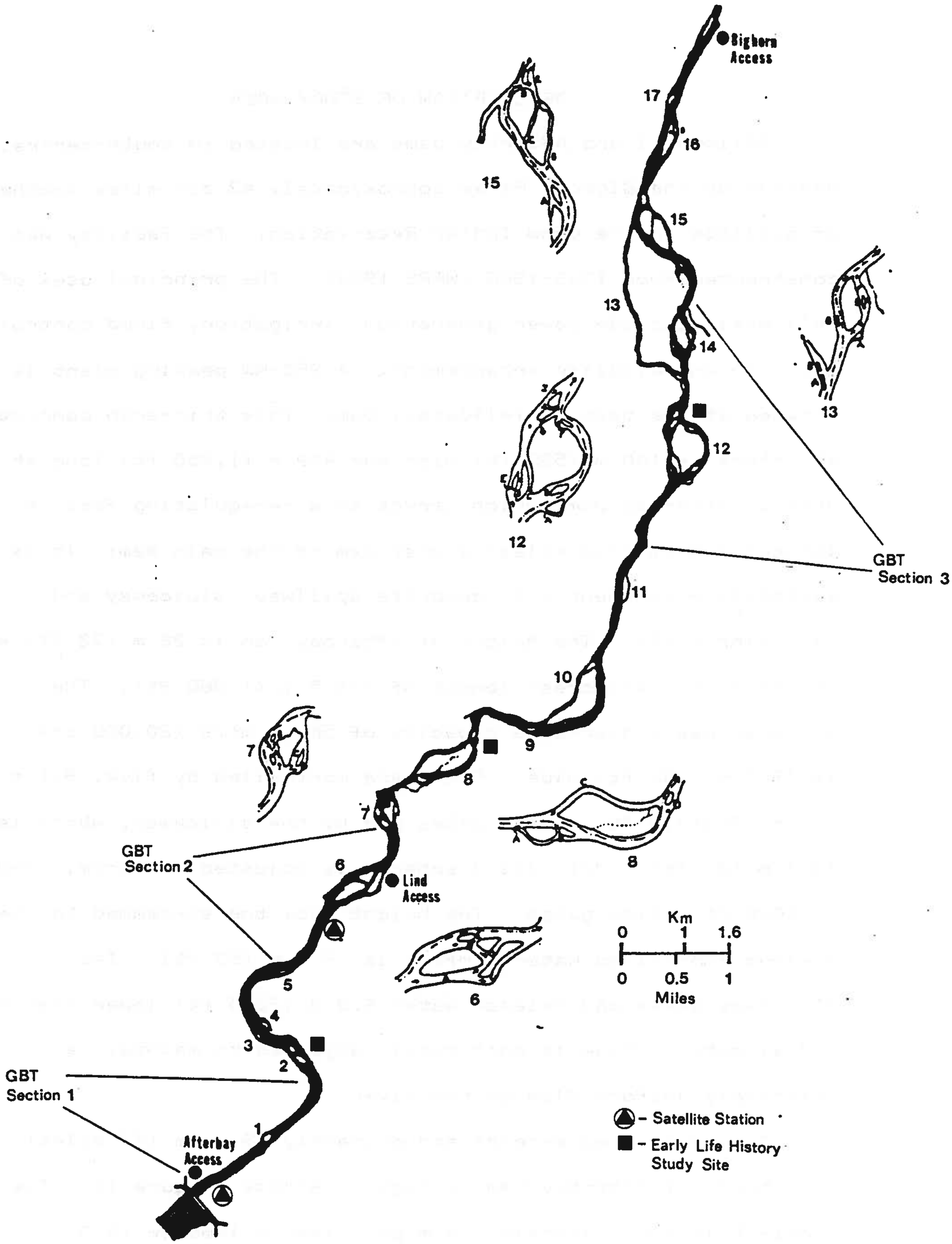

Figure 1. Map of the study area showing GBT electrofishing sections, early life history study sites, satellite relay stations, side-channel index and access sites.

several areas, forming numerous islands. The riverbottom is dominated throughout this length by cottonwoods. Public access sites are located immediately downstream of Afterbay Dam, at Rkm 5.4 (RM 3.4; Lind Access) and at Rkm 19.3 (RM 12.0; Bighorn Access). The largest tributary that enters the river within the study area is Soap Creek; its mouth is about 15.6 Rkm (9.7 RM) downstream of Afterbay Dam.

Electrofishing sections were established to obtain population estimates in the study area; one extends from the Afterbay to Rkm 3.9 (RM 2.4) and another from Rkm 3.9-15.4 (RM 2.4-9.6), the latter being one of MDFWP's established sections for fish population estimates. Additional subsections were established within the electrofishing sections at Rkm 0-1.9 (RM 0-1.2), Rkm 3.9-6.1 (RM 2.4-3.8) and Rkm 12.2-15.4 (RM 7.6-9.6) for monitoring incidence of GBT. Experiments to monitor early life history stages of trout were conducted at Rkm 2.4 (RM 1.5), Rkm 8.0 (RM 5.0) and Rkm 14.5 (RM 9.0).

WATER CHEMISTRY, TROUT POPULATIONS AND FIELD BIOASSAYS

Methods

Water Chemistry

Water samples for analysis of common ions and nutrients were collected at the Saint Xavier gagehouse 0.2 km downstream of Afterbay Dam every 2 to 4 weeks from 2 January - 1 December, 1986. Metals samples were obtained on three occasions during 1986. Nutrient and metal samples were preserved with sulfuric and nitric acid, respectively. Samples collected after 2 February, 1986 were delivered unfrozen to Northern Engineering and Testing, Inc. in Billings for analysis.

Gas Saturation Measurements and Formulas

During 1986 gas saturation levels were recorded two to three times per week at the gagehouse and each early life history study site (Figure 1). Measurements were made on a regular basis at the other satellite station (Rkm 4.8; RM 3.0) beginning on 12 April (Figure 1). Parameters measured included delta P (the difference between total gas pressure in water and in air), absolute barometric pressure, water temperature and dissolved oxygen concentration. Delta P was determined using a Bouck gasometer (Bouck 1982), which was allowed 30 minutes to reach equilibrium before each measurement. Barometric pressure was measured at the site with a Thommen 22000 pocket altimeter-barometer and in the office two to three times daily using a Princo Nova, fortin type mercurial barometer. Water temperature was measured with a mercury thermometer and dissolved oxygen was determined using liquid reagents and the azide modification of the Winkler method (APHA 1976). Formulas used to calculate gas levels are discussed thoroughly by Colt (1984) and include:

$$\% \text{ Total Saturation} = [(BP + \Delta P) / BP] * 100$$

$$\% \; O_2 \text{ Saturation} = ([O_2]/B\text{-}O_2 * 0.5318) / ((BP - P\text{-}H_2O) * 0.20946) * 100$$

$$\% \; N_2 + Ar \text{ Saturation} = [(BP + \Delta P - ([O_2]/B\text{-}O_2) * 0.5318) - P\text{-}H_2O) / (BP - P\text{-}H_2O) * 0.7902] * 100$$

$$\Delta P = TGP - BP$$

$$\Delta P\text{-}O_2 \text{ (mm Hg)} = [O_2]/B\text{-}O_2 * 0.5318 - 0.20946*(BP - P\text{-}H_2O)$$

$$\Delta P\text{-}N_2 + Ar \text{ (mm Hg)} = BP + \Delta P - [O_2]/B\text{-}O_2 * 0.5318 - P\text{-}H_2O - 0.7905*(BP - P\text{-}H_2O)$$

$$TGP = BP + \Delta P$$

$$P\text{-}O_2 \text{ (mm Hg)} = [O_2]/B\text{-}O_2 * 0.5318$$

$$P\text{-}N_2 + Ar \text{ (mm Hg)} = BP + \Delta P - [O_2] - P\text{-}H_2O$$

$$B\text{-}O_2 = e^{[A1 + A2*(100/T) + A3*\log_e (T/100)]}$$

$$P\text{-}H_2O = 760 \times e^{[24.4543 - 67.4509*(100/T) - 4.8489 * \log_e (T/100)]}$$

Where: BP = Barometric pressure (mm Hg)
ΔP = Delta P (mm Hg)
$[O_2]$ = Dissolved oxygen concentration (mg/l)
$B\text{-}O_2$ = Bunsen coefficient of oxygen (L/(L x atm))
$P\text{-}H_2O$ = Vapor pressure of water (mm Hg)
TGP = Total gas pressure (mm Hg)
$\Delta P\text{-}O_2$ = Oxygen Component of ΔP (mm Hg)
$\Delta P\text{-}N_2 + Ar$ = Nitrogen and Argon Component of ΔP (mm Hg)
$P\text{-}O_2$ = Oxygen Pressure (mm Hg)
$P\text{-}N_2 + Ar$ = Nitrogen and Argon Pressure (mm Hg)
T = Absolute water temperature (°C + 273.15)
$A1$ = -58.3877
$A2$ = 85.8079
$A3$ = 23.8439

Oxygen-nitrogen ratios were calculated using the O_2 and $N_2 + Ar$

components of delta P, not % saturation.

Continuous Monitoring Equipment

Continuous recording water quality monitoring equipment included two Common Sensing tensionometers (model TGO-F) and two Hydrolab's system 8000. Equipment was first installed at the gagehouse and Rkm 4.8 (RM 3.0) during spring 1985. The gagehouse tensionometer was replaced with a new model TBO-F, on 14 March, 1986. The tensionometers measure water temperature and total gas, oxygen, and nitrogen pressures. In addition to these parameters, model TBO-F also measures barometric pressure. The Hydrolab records water temperature, dissolved oxygen concentration, pH, conductivity and oxidation-reduction potential. The tensionometers and Hydrolab were interfaced with a Sutron satellite system which relays measurements taken every 30 minutes, 24 hours per day to an earth station in Boise, Idaho and a computer in Billings, Montana. Equipment was calibrated using the manufacturer's recommended procedures. Accuracy was assessed by comparing meter readings with water temperatures from a mercury thermometer, dissolved oxygen concentrations determined from a Winkler and total gas pressures from one or two Bouck Gasometers.

Temperatures were monitored at several points throughout the study area. A MDFWP Taylor 30 day thermograph was maintained at the Saint Xavier gagehouse below Afterbay Dam. Additional continuous recording temperature equipment was installed by the Bureau of Reclamation at the gagehouse and at Rkm 4.8 (RM 3.0).

Maximum/minimum thermometers were located at Rkm 2.4, 8.0, 14.5, (RM 1.5, 5.0 and 9.0).

GBT Incidence of Occurrence

Incidence of gas bubble trauma and progression and duration of symptoms were monitored using a boom-mounted electrofishing system equipped with either a 110 or 220 volt rectifying unit. Electrofishing runs were conducted at night if capture efficiencies were low during daylight hours or if recreational fishing pressure was high. Approximately 150 brown trout and as many rainbow trout as possible were collected biweekly or monthly and examined for external symptoms of gas bubble trauma. Lengths, weights, sex, missing fins and the presence of hooking scars were recorded. A rating system was used to categorize fish into one of four groups:

<u>Category</u>

0 - No visible external symptoms.

1 - Minor symptoms: Symptoms normally restricted to a few bubbles on fins or a bubble in one eye. Fish in this category do not appear seriously stressed.

2 - Serious symptoms: Numerous bubbles present in fins; bubbles may also be found in the skin, inside the the mouth, on the opercle, or in one eye. Fish in this category appear stressed.

3 - Severe symptoms: Large numbers of gas bubbles are present; hemmorhaging and/or fungal growth may also occur and one or both eyes may be blind. These fish are severely debilitated, lethargic and have a poor condition factor. Chances for survival appear questionable.

A rating system was also developed to describe the severity of gas bubble trauma on each fin or body part. Parts exhibiting

GBT symptoms were assigned one of the following codes:

- H - High numbers of bubbles present.
- M - Moderate numbers of bubbles present.
- L - Low numbers of bubbles present.
- R - Recovering but scars from previous bubbles apparent.

Finally, information for the entire fish was summarized to develop a total GBT rating.

Since the inception of the study, nearly 1600 trout in the upper 6.1 Rkm (3.8 miles) of the river have been marked with numbered Floy t-tags treated with algacide. Subsequent recaptures of these fish allowed us to evaluate the progression or remission of GBT symptoms.

Population Estimates

Boat mounted, fixed electrode electofishing systems were used by MCFRU and MDFWP personnel to conduct fish population estimates. Multiple mark and recapture trips were made during daylight hours to obtain adequate sample size; procedures were those of Vincent (1971 and 1974), adapted for computer analysis. Population estimates were made separately for various length interval groupings. At least four (but usually seven or more) recaptured trout were included within each 1.3 cm (0.5 inch) length interval. After reading scales, an estimate for each age was obtained by a summation of the proportions found in each size group. Population estimates were calculated using Chapman's modification of the Peterson formula (Ricker 1975) :

$$N = \frac{(M+1)\ (C+1)}{R+1}$$

where: N = Population estimate
M = Number of fish marked
C = Number of fish in the recapture sample
R = Number of marked fish in the recapture sample (C)

Embryo Survival

Brown and rainbow trout embryo survival from egg fertilization through hatching was tested using eggs and sperm obtained from Bighorn River spawning stock. Eggs from females and sperm from males were pooled to minimize handling or parental effects on egg survival. Eggs were incubated at various gas saturation levels including locations where saturation was high (Rkm 2.4; RM 1.5), medium (Rkm 8.0; RM 5.0), low (Rkm 14.5; RM 9.0), and in Afterbay Reservoir which served as a control (Figure 1).

On 9 December 1985, brown trout eggs were planted at each incubation site in eight fiberglass bags (100 eggs each), eight emergence traps (100 eggs each), and six egg incubation boxes (subsequently referred to as wedge boxes), each containing 200 eggs. Additionally, at least 1000 eggs were placed at each river site on Astroturf substrate in a modified Porter (1973) fry holding box. These eggs were to provide fry for rearing tests. The experimental design was repeated with rainbow eggs on 13 May 1986, except that only five wedgeboxes were installed at Rkm 8.0 and Rkm 14.5. No emergence traps containing rainbow eggs were installed. Four wedge boxes were installed in the Afterbay Reservoir to serve as controls. Brown trout eggs were again planted on 20 November 1986 at each early life history study site. Six wedge boxes were placed at each river site and in Afterbay Reservoir and a modified fry holding box was installed at each of the three river sites.

All egg bags and emergence traps were completely enclosed and presifted 1.3-2.5 cm (0.5-1 inch) gravel was utilized. Emergence traps were constructed of plywood or 20.3 cm (8 inch) sewer pipe with plywood tops and bottoms and had - 20.3 x 15.2 cm (8x6 inch) openings on each side (covered with 1.5 mm [0.0625 inch] mesh screen). Swim-up fry were captured in 3.8 cm (1.5 inch) PVC holding tubes on the downstream side of the trap.

Wedge boxes simulated "natural" conditions and eliminated some ambient variables that could influence egg mortality. The wedge-shaped plywood box rested on the river bottom and effectively shed drifting aquatic vegetation; its rectangular main compartment measured 25.4 x 20.3 cm (10 x 8 inch) on each side. Five of the sides had 15.2 x 15.2 cm holes (covered with screen) to allow water movement through the gravel. The presifted 1.3-2.5 cm gravel in the egg compartment eliminated substrate as a variable and enclosing the eggs prevented mortality due to disturbance by spawning fish or fishermen. Fry loss due to lateral movement was also prevented. Two boxes at each site, except in the Afterbay Reservoir, were equipped with holding tubes containing fykes for collecting emerging fry.

The number of brown trout eggs placed in each bag was determined by one of three methods: (1) visual counting, (2) paddle counter or (3) Von Bayer trough (Piper et al. 1982). The first two methods were exact while the Von Bayer troughs were slightly less accurate. Two subsamples of 10 troughs each showed the mean number (standard deviation in parentheses) of eggs to be 50($\pm$0.67) and 50($\pm$0.0). Rainbow eggs were counted exclusively with paddle counters.

Statistics

Statistical tests were performed using methods described by Snedecor and Cochran (1967). Significant differences were, unless otherwise stated, those with $P < 0.05$.

Results and Discussion

Water Chemistry

Water samples were collected from the Bighorn River at the gagehouse below Afterbay Dam on a regular basis throughout 1986. As seen in 1985, analysis of common ions showed that water discharged from Bighorn Lake is hard and has relatively high alkalinity, conductivity and total dissolved solid levels (Table 1). Cations found in the highest concentrations were sodium and calcium and while their levels were similar, magnesium concentrations were about two-thirds lower. Potassium values were low and usually close to detection limits. Principal anions were sulfate and bicarbonate, followed by chloride. As found in 1985, sulfate was present at higher concentrations than the 250 mg/l recommended for total dissolved solids in domestic water

Table 1. Mean and ranges of water quality parameters of 13 samples from the Bighorn River at the gagehouse below Afterbay Dam from 2 January - 1 December, 1986.

Parameter	Mean	Range
Total Hardness (mg/l as $CaCO_3$)	283	173-390
Total Alkalinity (mg/l as $CaCO_3$)	157	114-198
Bicarbonate Alkalinity (mg/l HCO_3)	192	139-242
Calcium (mg/l)	71	46-100
Magnesium (mg/l)	26	14-35
Sodium (mg/l)	75	37-95
Potassium (mg/l)	3	1-5
Chloride (mg/l)	10[a]	<1-14
Fluoride (mg/l)	0.5	0.3-0.6
Nitrate + Nitrite (mg/l)	0.61	0.45-0.71
Sulfate (mg/l)	265	114-357
Total Dissolved Solids (mg/l)	601	344-784
Conductivity (micromhos/cm)	827	475-1040
pH	8.0	7.7-8.1
Total Phosphorus as P (mg/l)	0.08	0.01-0.2
Ortho-Phosphate as P (mg/l)	0.05[a]	<0.01-0.12
Ammonia Nitrogen as N (mg/l)	0.2[a]	<0.2-0.31
Total Kjeldahl Nitrogen as N (mg/l)	0.44[a]	0.21-0.8[b]

a - values below detection limits considered to be equal to detection limit.
b - change in detection limits.

supplies (EPA 1976). However, the mean value dropped 30 mg/l from 1985 levels. The lab pH was alkaline and varied only 0.4 units. Mean total phosphorus and kjeldahl nitrogen concentrations were 0.08 and 0.44 mg/l, respectively. Total phosphorus levels were twice those found last year and exceeded Montana water quality criteria for both cold and warm water aquatic life on three sampling dates (DHES 1986). These phosphorus concentrations are higher than most locations in the upper Clark Fork River which is generally believed to have a problem with nutrients. It is not surprising that the Bighorn supports heavy growths of rooted macrophytes and cladaphora. Ortho-phosphate averaged 0.05 mg/l and was below detection limits (0.01 mg/l) only in the February samples. Mean ammonia concentration was 0.2 mg/l and unionized ammonia was always less than 0.02 mg/l. Concentrations of ortho-phosphate and ammonia were similar to those found during 1985 in this study and in 1968 and 1969 by Wright and Soltero (1973).

Concentrations of dissolved or total recoverable metals, with the exception of manganese, were lower than EPA guidelines (EPA 1976 and 1980) for protection of freshwater aquatic life on all three sampling dates (Table 2). However, maximum iron and manganese levels in 1986 were substantially higher than 1985 values. Manganese was above detection limits only on 5 April when concentrations were 80 ug/l. Wright and Soltero (1973) reported similarly low metal concentrations in 1968 and 1969, but their range of concentrations was much greater and detection

Table 2. Dissolved metal concentrations from the Bighorn River at the gagehouse below Afterbay Dam on three sampling dates (5 April, 2 May, and 1 August, 1986).

Parameter	Mean	Range
Dissolved Metals (ug/l)		
Cu	20[a]	-
Fe	70[a]	<50-90
Pb	20[a]	-
Mn	40[a]	<20-80
Zn	20[a]	-

a - values below detection limits considered to be equal to detection limit.

limits much lower. Metals analyses were discontinued after the August sampling date because of the low levels found and since previous researchers were unable to find any synergistic effect between supersaturation and copper and zinc (Garton and Nebeker 1977).

Mean daily conductivity measured by the gagehouse Hydrolab between 7 May - 31 December 1986 was 755 micromhos/cm, or 72 micromhos/cm lower than the mean value from biweekly or monthly samples. It peaked on 7 May (1003 micromhos/cm) and then steadily dropped to 508 micromhos/cm on 15 August (Figure 2). An increase occurred through mid-October but leveled out around 800 micromhos/cm for the remainder of the year. The small spike on 16 October, where the mean daily conductivity increased to 862 micromhos/cm, occurred during the annual drawdown at which time flow in the Bighorn River dropped to 5.32 m^3/s (188 cfs). Wright and Soltero (1973) found the same pattern for specific conductance in both 1968 and 1969, but the magnitude of change found in those years was lower than in 1986.

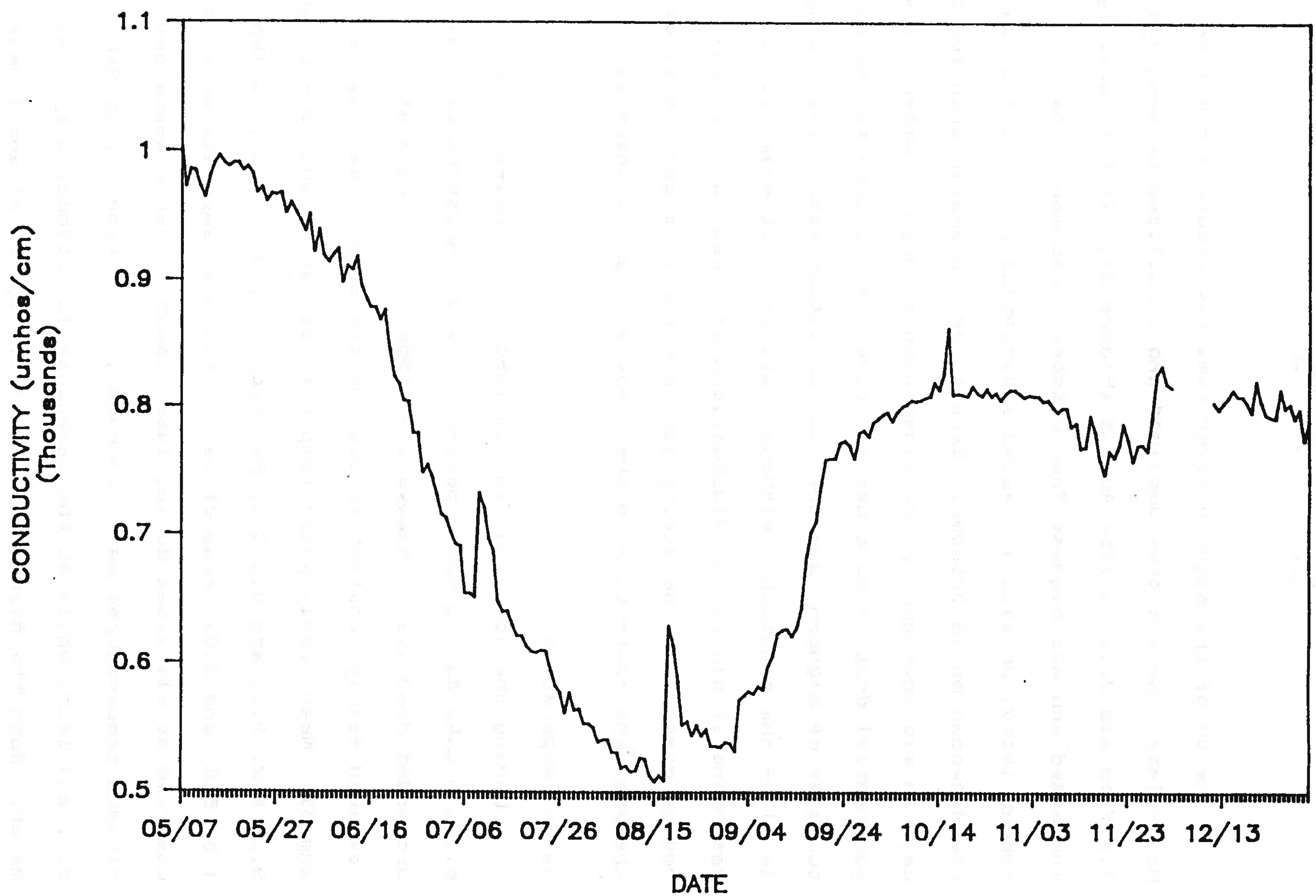

Figure 2. Mean daily specific conductance (micromhos/cm) from 7 May - 31 December, 1986 of the Bighorn River at the gagehouse below Afterbay Dam.

The pH of the Bighorn River remained around 7.9-8.1 from 7 May - late June and then declined and stabilized between 7.4 and 7.6 from mid-July to late August (Figure 3). It then quickly increased and was highest from October - December. As with conductivity, pH also increased substantially (0.3 units) during the drawdown on 16 October. Except for a dramatic drop for 5 days in mid November, pH remained high through December. The substantial drop in November may have been related to the fall turnover of Bighorn Lake when low pH bottom water moved up to the level of the penstock. Although data were not examined to determine if diurnal pH fluctuations occurred, photosynthesis may reduce carbon dioxide during daylight hours at certain times of the year and contribute to the seasonal changes observed.

Water Temperature

During the low flow year of 1985, water released from Bighorn Lake during May - September warmed substantially as it proceeded downriver. However, in 1986, water temperatures remained fairly constant throughout the study area (Figures 4 and 5). Mean weekly water temperatures varied only 0.4 C, being 9.5, 9.2, 9.6, and 9.5 C at Rkm 0.6, 2.4, 8.0, and 14.5 (RM 0.4, 1.5, 5.0, and 9.0), respectively. Maximum temperatures were observed at all sites during late August or early September. Minimum temperatures were observed in late February at Rkm 2.4, 8.0, and 14.5, while at the gagehouse it bottomed out in mid-March. Both the highest maximum (19.7 C; 67 F) and lowest minimum daily readings (0.6 C; 33 F) were recorded at Rkm 14.5.

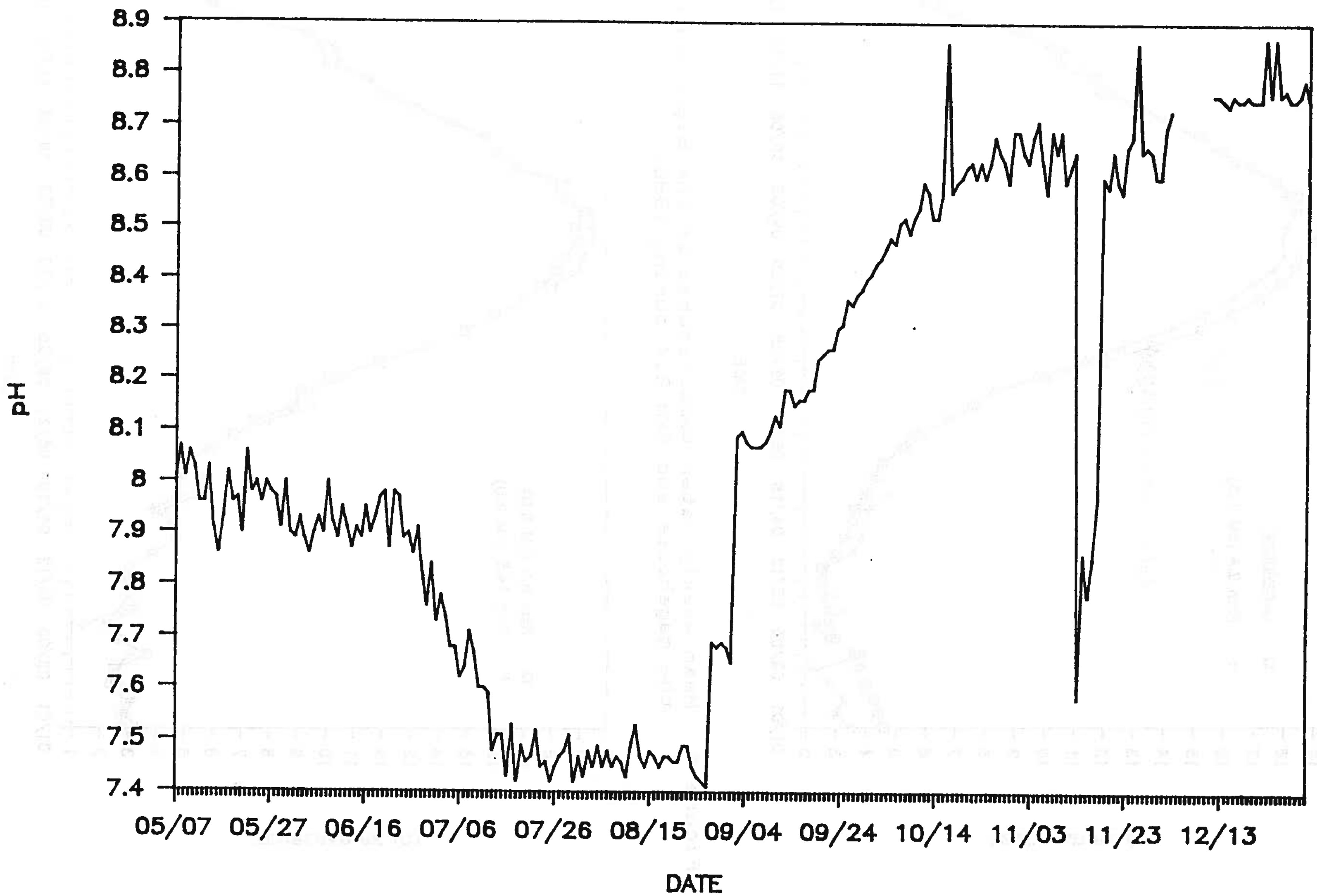

Figure 3. Mean daily pH of the Bighorn River at the gagehouse below Afterbay Dam from 7 May - 31 December, 1986.

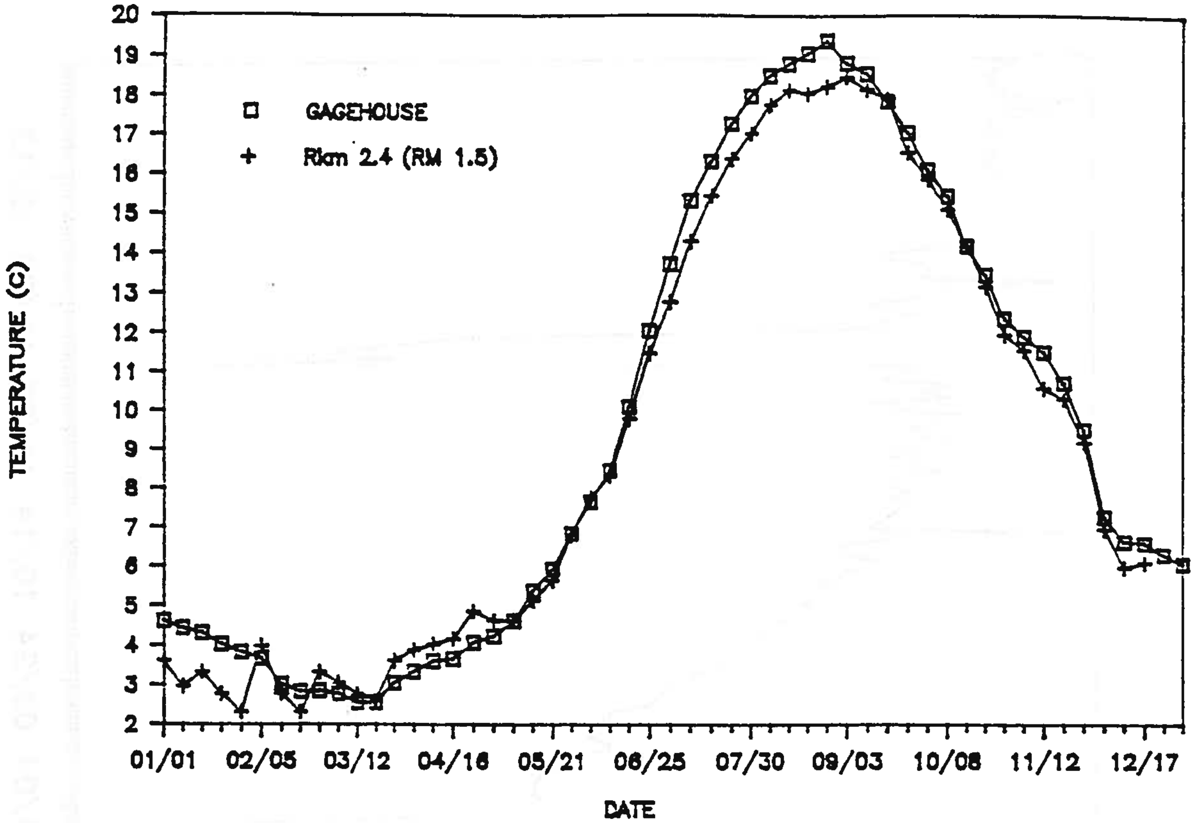

Figure 4. Mean weekly water temperatures of the Bighorn River at the gagehouse and Rkm 2.4 during 1986.

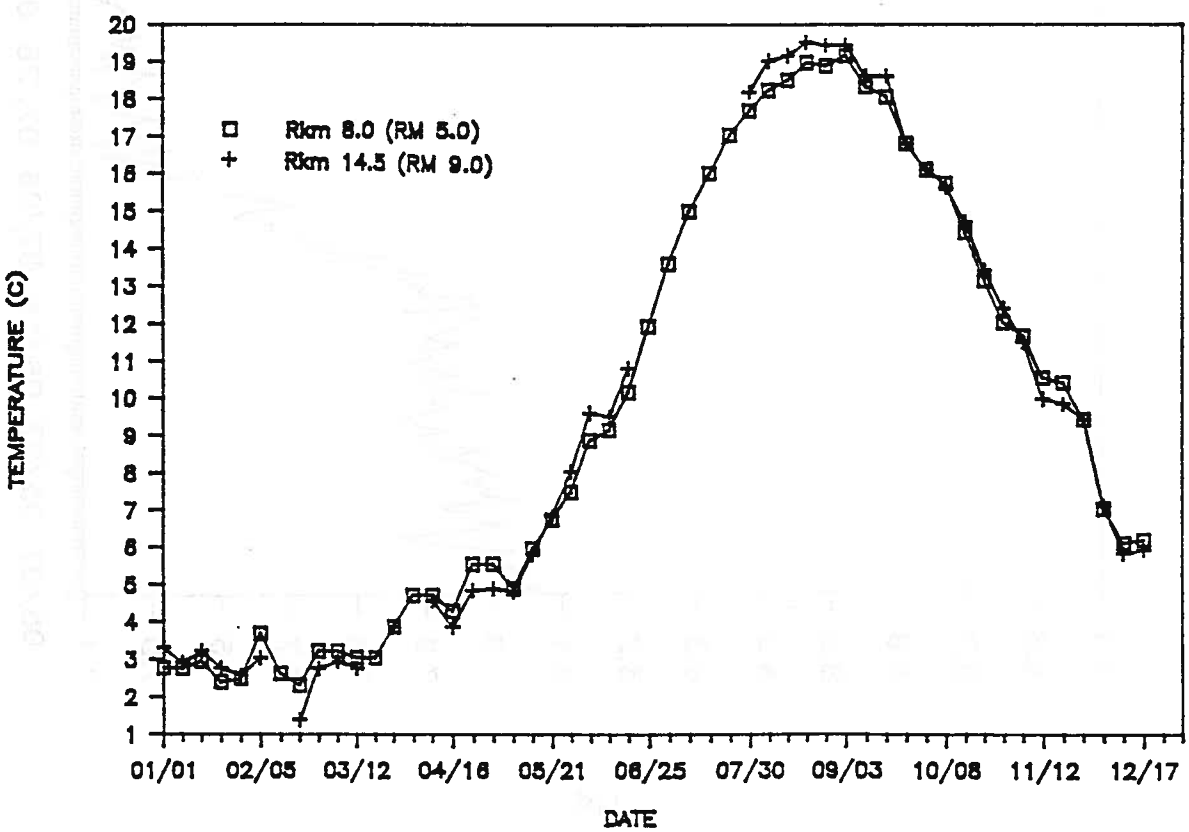

Figure 5. Mean weekly water temperatures of the Bighorn River at Rkm 8.0 and Rkm 14.5 during 1986.

Discharge

Mean daily discharge from Afterbay Dam remained quite constant (between 2400 and 2900 cfs; 67.97 - 82.13 m^3/s) during the first two months of 1986 (Figure 6). From March through early June, flows became increasingly variable, repeatedly flucuating over 1000 cfs (28.32 m^3/s). Then in June, flows increased to a peak level of 7431 cfs (210.45 m^3/s) and remained high until early August. After August, flow was fairly uniform and tended to increase through the fall with the exception of 14 October, when the mean daily flow dipped to 808 cfs (22.88 m^3/s).

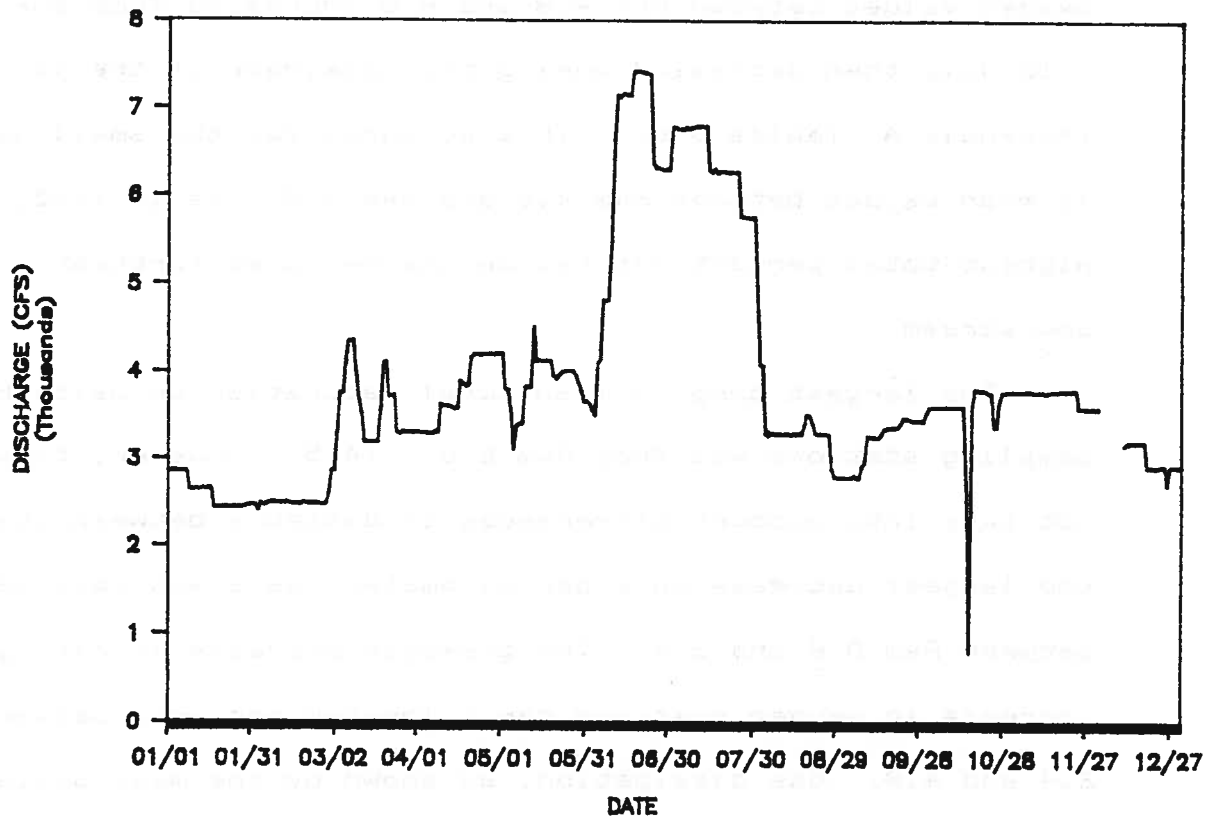

Figure 6. Mean daily discharge of the Bighorn River below Afterbay Dam during 1986.

Gas Monitoring

Mean values of gas levels measured with Bouck gasometers at five sites on the Bighorn River during 1986 show similar trends to those found in 1985 (Tables 3 and 4, Figures 7-11, and Appendix A Tables 1-8). Highest total gas and nitrogen levels occurred just below Afterbay Dam at Rkm 0.6; both parameters decreased with distance downstream. Mean nitrogen saturation dropped below 110% at Rkm 14.5 while mean total saturation did not decrease below 112%. Conversely, mean oxygen values were greatest at Rkm 14.5 and tended to decrease upstream. Mean oxygen values between Rkm 4.8 and 8.0 increased from the 16 April - 15 July then decreased during the remainder of the year (Appendix A Tables 3-8). This accounts for the small decrease in mean values between Rkm 4.8 and Rkm 8.0. As in 1985, the highest total percent saturation was measured furthest downstream.

The largest drop in mean total saturation or delta P between sampling stations was from Rkm 8.0 - 14.5. However, this does not take into account differences in distance between stations; the largest decrease on a per kilometer basis was observed between Rkm 0.6 and 2.4. The greatest decrease in nitrogen and increase in oxygen pressure per kilometer occurred between Rkm 2.4 and 4.8. Gas dissipation, as shown by the mean delta P of nitrogen and argon, occurs at a high rate from Afterbay Dam down to Rkm 4.8, decreases between Rkm 4.8 and 8.0 and then increases again between Rkm 8.0 and 14.5.

Table 3. Means and range of water temperature and dissolved gas levels at five sites on the Bighorn River below Afterbay Dam, 1 January - 31 December, 1986.

River km	Sample size	Temp. (C)	D.O. (mg/l)	% saturation Oxygen	N_2+Ar	Total
0.6	119	9.0 (2.2-18.9)	11.2 (7.7-13.7)	106.9 (89.3-115.5)	120.0 (111.4-126.9)	117.0 (108.9-122.3)
2.4	117	9.2 (2.2-18.9)	11.6 (8.2-14.1)	110.7 (94.5-120.6)	117.3 (105.0-122.5)	115.6 (105.0-121.2)
4.8	81[a]	12.3 (4.4-20.6)	11.8 (8.5-14.4)	122.6 (98.3-145.2)	112.9 (103.8-120.7)	114.7 (107.0-120.6)
8.0	117	9.7 (2.2-20.6)	12.5 (8.5-15.6)	121.7 (96.8-141.5)	111.8 (104.1-119.7)	113.6 (105.6-121.6)
14.5	114	10.3 (1.7-21.1)	13.1 (8.5-15.3)	129.2 (98.9-168.3)	108.0 (101.7-114.4)	112.2 (102.4-123.3)

a - data collection at Rkm initiated on 12 April, 1986.

Table 4. Means and ranges of barometric pressure and delta P's (mm Hg) at five sites on the Bighorn River below Afterbay Dam, 1 January - 31 December, 1986.

River km	B.P. (mm Hg)	ΔP (mm Hg)	ΔP-O_2 (mm Hg)	ΔP-N_2+Ar (mm Hg)
0.6	680 (664-697)	115 (61-152)	10 (-15-22)	106 (60-142)
2.4	680 (664-697)	106 (46-143)	15 (-8-29)	91 (26-119)
4.8	681 (664-690)	100 (48-140)	32 (-2-63)	68 (20-109)
8.0	680 (664-697)	93 (38-147)	30 (-4-58)	62 (22-104)
14.5	681 (663-697)	83 (16-159)	41 (-2-95)	42 (9-76)

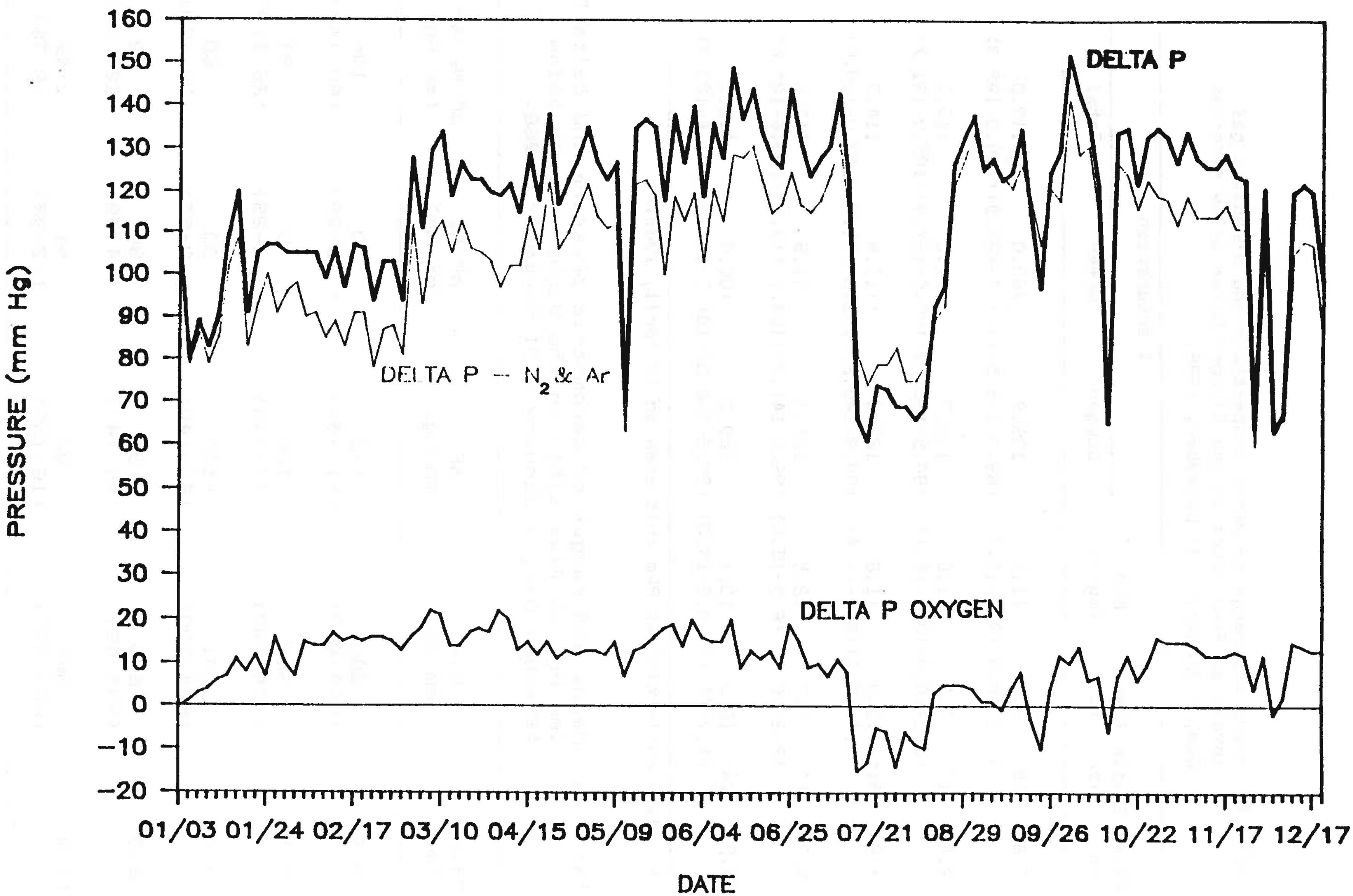

Figure 7. Delta P, P-O_2, and P-N_2+Ar at Rkm 0.6 (gagehouse) during 1986.

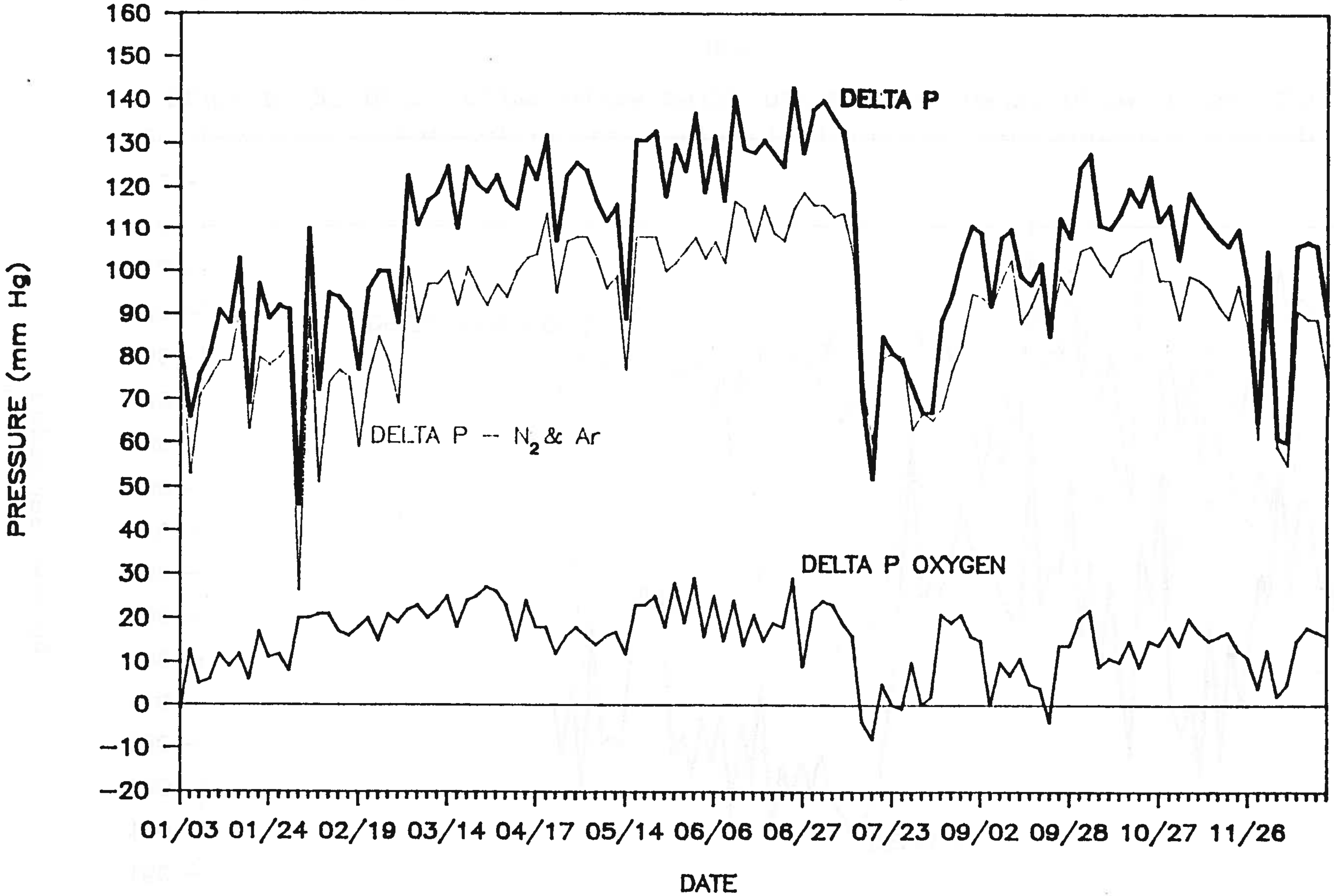

Figure 8. Delta P, P-O_2, and P-N_2+Ar at Rkm 2.4 (RM 1.5) during 1986.

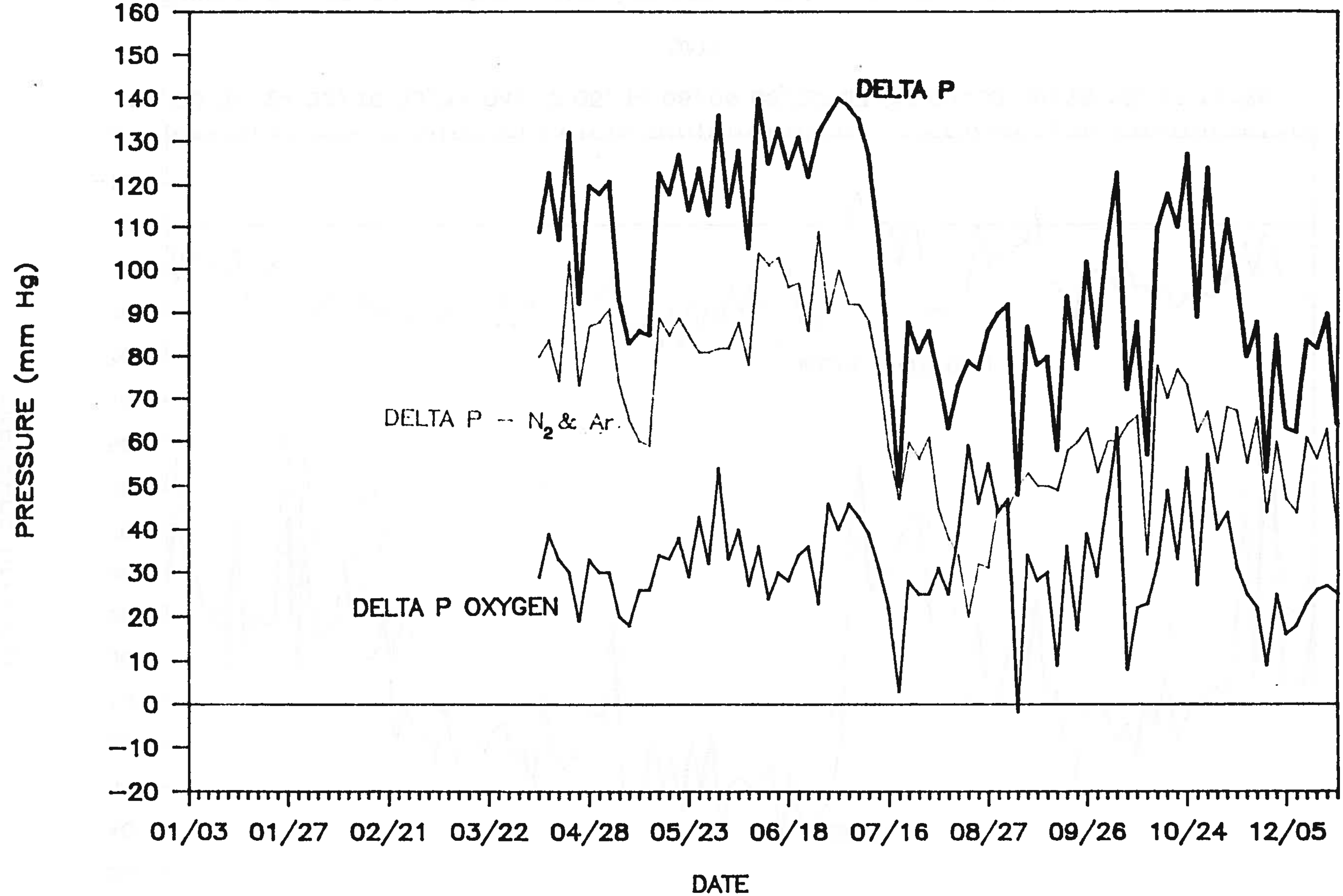

Figure 9. Delta P, P-O_2, and P-N_2+Ar at Rkm 4.8 (RM 3.0) during 1986.

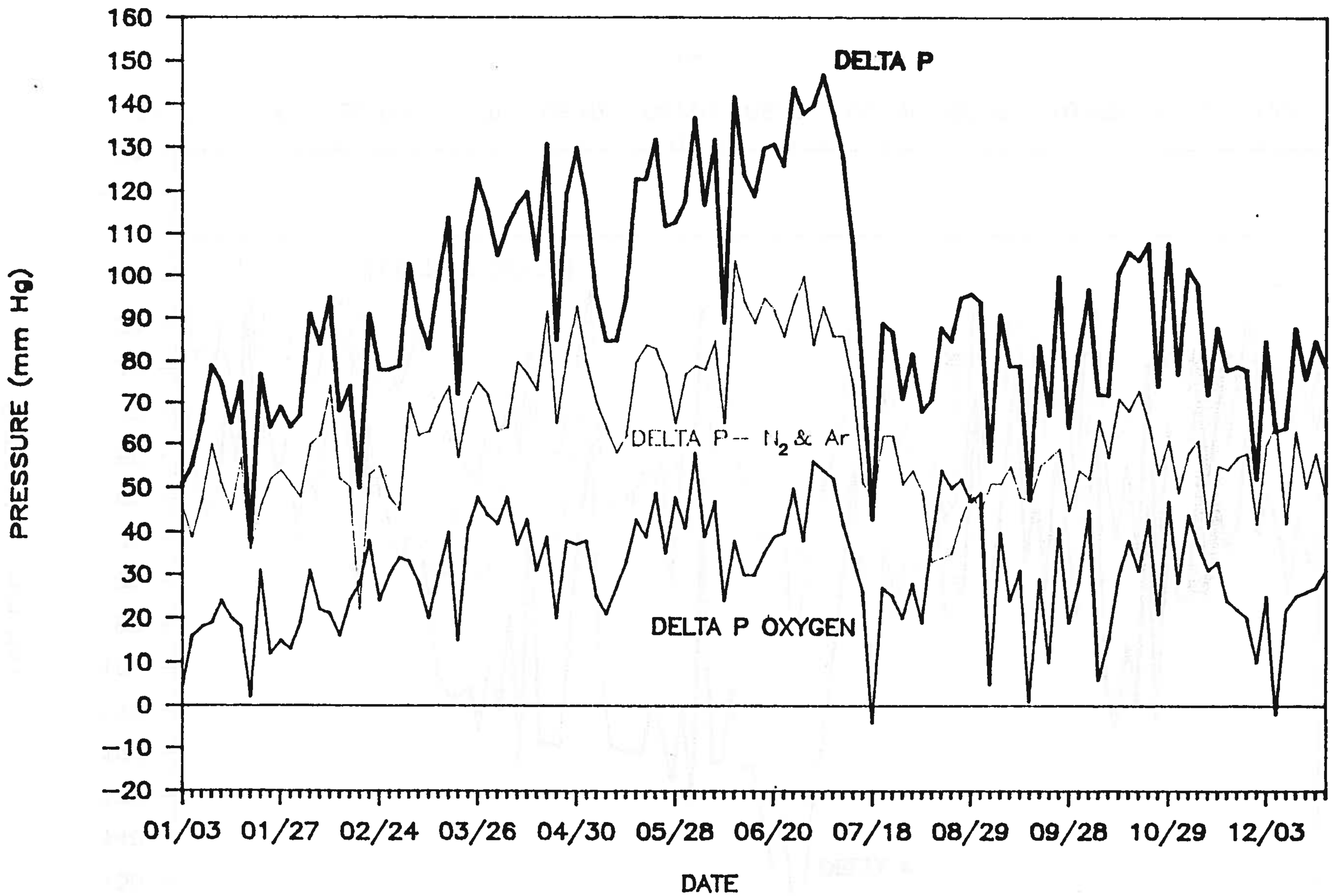

Figure 10. Delta P, P-O_2, and P-N_2+Ar at Rkm 8.0 (RM 5.0) during 1986.

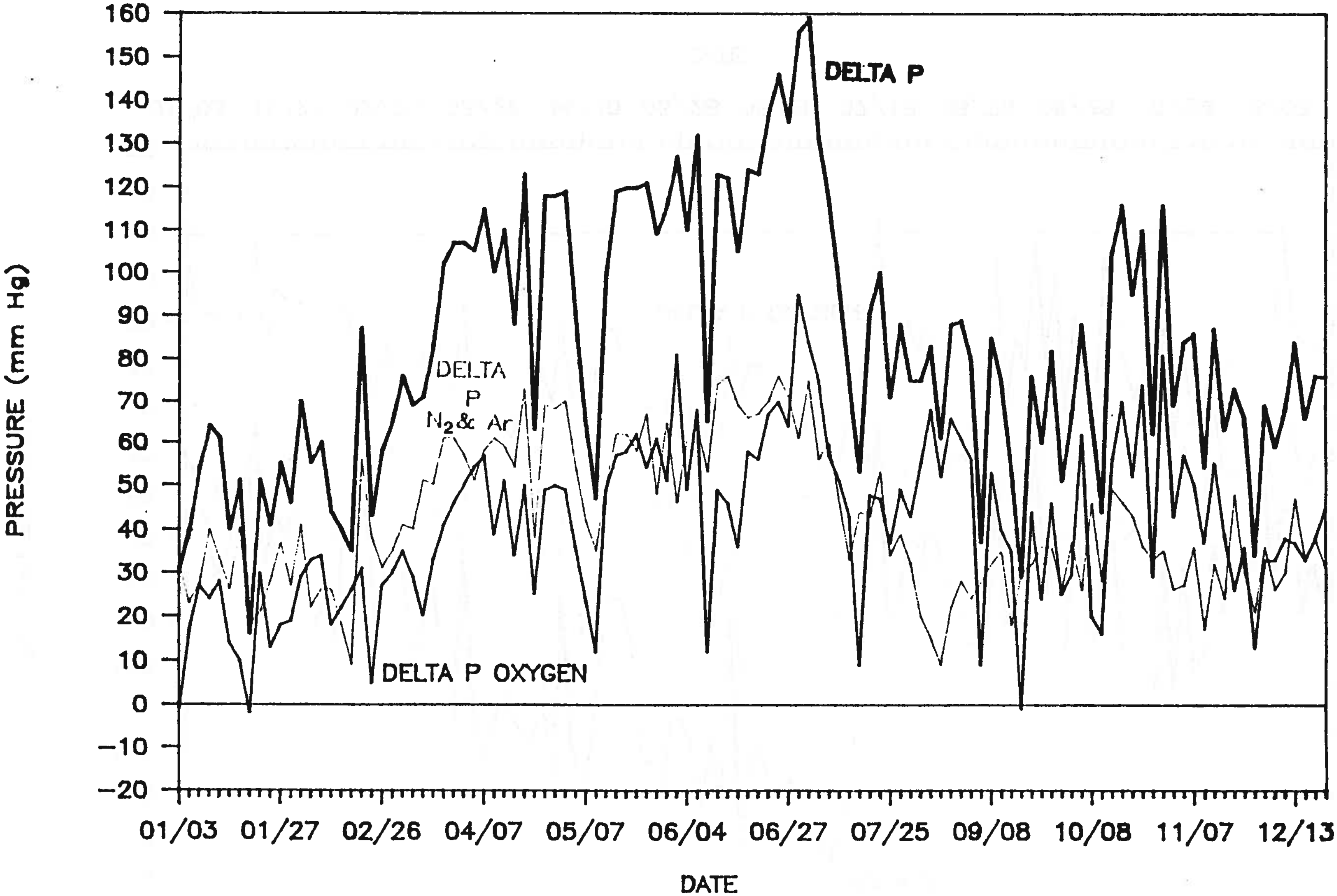

Figure 11. Delta P, P-O_2, and P-N_2+Ar at Rkm 14.5 (RM 9.0) during 1986.

Barometric pressures (Table 4) were similar at all stations. Mean values varied only 1 mm Hg between sites and ranges were nearly identical, except at Rkm 4.8, where measurements were not obtained on 12 November, when barometric pressure was higher than usual at 697 mm Hg.

Delta P measurements showed the lowest variability and the highest proportion of nitrogen at Rkm 0.6 (Figure 7). With distance downriver, delta P becomes much more variable, which may be partially due to changes in solar insolation and the increasing importance of oxygen. At the other downstream sites, total delta P increased from January - late June and July, then dropped in July, increased again through October, and then declined for the remainder of the year. Peak gas levels in late June and early July corresponded to periods of peak discharge (Figure 6). Increased discharge and accompanying increased stilling basin depth caused additional air entrainment, which elevated total gas pressure (TGP). However, other factors also affect saturation and could change this relationship.

During 1986 hyperbaric gas pressures dropped below 80 mm Hg on five occasions at the gagehouse. Four cases (14 May, 14 July-23 August, 14 October [annual drawdown], and 4-12 December) coincided with complete closure of the Afterbay Dam's sluiceway gates. On the other date (1 December), the sluicegates were not closed completely but were lower than typical operational levels. For the remainder of 1986, the gagehouse delta P varied from 80-152 mm Hg. The large decrease in delta P on 31 January at Rkm 2.4 (Figure 8) is an error that was not detected until after graphs were made; the delta P actually remained fairly stable and near 90 mm Hg.

Gas entrainment at Afterbay Dam affects the entire study area as shown by large drops in delta P at all monitoring sites when water is not routed through the sluiceway. However, the oxygen component of delta P becomes larger with distance downriver. Oxygen became the principal component of delta P for a short time during late summer and early fall at Rkm 4.8 (Figure 9) while at Rkm 14.5, it was the dominant component during the latter part of the summer and fall. Equations developed by Fidler (1985) describing bubble growth in arterial fish blood indicate that the threshold for bubble growth rises with the proportion of oxygen to total gas pressure. In other words, the consistently high nitrogen and TGP levels immediately below Afterbay Dam probably present a greater risk of bubble formation in the arterial side of the vascular system of large fish than supersaturation downstream where oxygen concentrations are higher. However, this would not be the case for small fish such as swim up fry, where the development of bubbles in the buccal cavity may result in mortality. Fidler (1985) showed that the relative composition of TGP was unimportant for bubble formation in the buccal cavity; the principal environmental factors that would affect bubble growth would be TGP, hydrostatic pressure and water surface tension. So, small fish would be susceptable in the Bighorn River where TGP is high and they remain at a position in the water column where compensatory pressures are not adequate to prevent bubble growth. Areas where fry may be exposed to high TGP may include a substantial portion of the study area during late spring and early summer.

Continuous Gas Monitoring

Gas tensions measured with the Common Sensing tensionometer at Rkm 0.6 (gagehouse) were not highly variable and all major changes in pressure noted by the tensionometer were also observed by the Bouck sampling (Figures 12 and 13). Mean daily values for parameters measured by the tensionometer from 7 May - 31 December (excluding 4-20 August) were closer to those obtained during point sampling than we expected. The tensionometer's mean barometric pressure was 2 mm Hg higher than the 681 mm Hg mean obtained from the Thommen hand-held barometer. Mean delta P, total gas, oxygen, and nitrogen pressures measured by the Common Sensing equipment were 115, 798, 150, and 649 mm Hg, respectively. This compares with values for delta P and TGP of 118 and 798 mm Hg obtained from a Bouck gasometer, a mean oxygen pressure of 150 mm Hg obtained from Winklers, and a nitrogen pressure of 648 mm Hg. Analysis of variance showed no significant difference between mean delta P values obtained from the two methods. If the same comparison was made at the downstream site, larger differences may be observed since gas pressures are more variable there. A comparison of Figure 7 with the delta P data derived from the tensionometer shows similar trends; the continuous data displays a more jagged graph with small peaks representing small changes in pressure missed by the

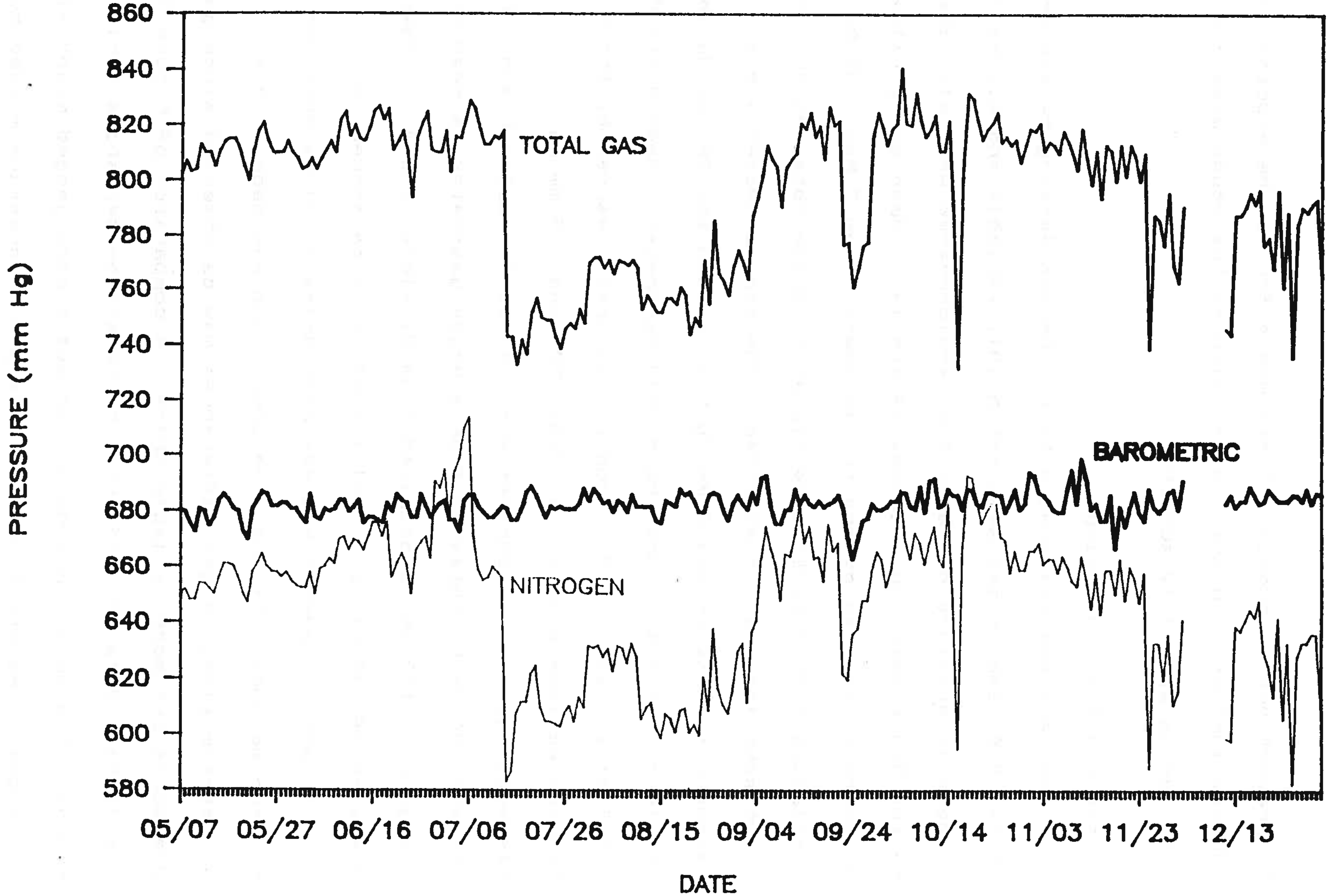

Figure 12. Uncorrected barometric, total, and nitrogen gas pressures (mm Hg) from the Common Sensing tensionometer at Rkm 0.6 (gagehouse), 7 May - 31 December, 1986.

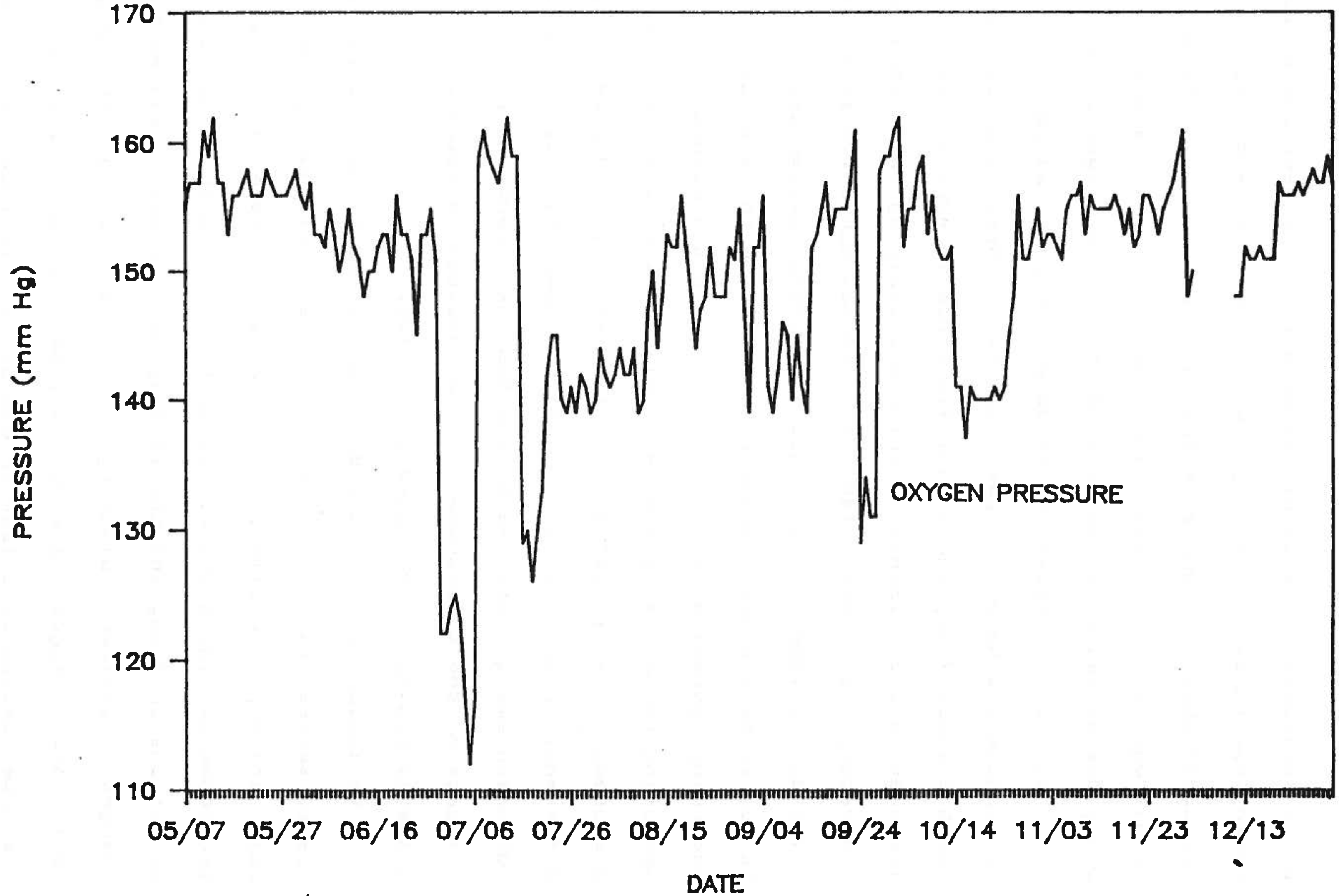

Figure 13. Uncorrected oxygen pressure (mm Hg) from the Common Sensing tensionometer at Rkm 0.6 (gagehouse), 7 May - 31 December, 1986.

point estimates. The satellite data have not been corrected for known departures from manual measurements (Table 5) and errors are observable in Figures 12 and 13. An example is the increase in nitrogen and a corresponding drop in oxygen pressure in early July, due at least partially to drift of the oxygen probe.

Calibration checks of the permanently installed tensionometers have been performed two - three times per week at Rkm 0.6 since 7 May and at Rkm 4.8 since 15 April, 1986. Temperature measurements at Rkm 0.6 closely agreed with a mercury laboratory thermometer (Table 5). The maximum difference was only 0.7 C during 1986. As previously reported, oxygen pressure tended to be the most variable parameter, with large deviations occurring sporadically. Results from the two methods of measuring barometric pressure consistently agreed. At times, TGP measurements varied between the two methods, with a 24 mm Hg difference observed on one occasion. However, most of the time, TGP measurements obtained by the two instruments were similar. Trimpots on the tensionometer were adjusted 12 times and the Silastic tubing was replaced only twice.

Variations between the Bouck and D'Aoust instruments may be due to several factors. Changes in gas levels during the calibration period affect the two meters differently because the tensionometer equilibrates sooner than the Bouck. The large deviations on 7 May and the 14 July were probably related to changes in sluiceway discharge during calibration. The large deviations in August were due to a malfunction in a Bouck gasometer caused by a leak in one of the fittings. This problem

Table 5. Differences between manual measurements and readings from the Common Sensing Tensionometer during calibration at the Saint Xavier gagehouse (Rkm 0.6), Bighorn River, 7 May - 18 July, 1986. Pressure values are in mm Hg. (An H after the date indicates that tensionometer readings were obtained via the Hydromet system.)

Date	Deviation from manual measurement: Temperature (C)	Total gas pressure (mm)	Oxygen pressure (mm)	Barometric pressure (mm)
5/7	+1.0 *	+31	0	
5/16	-0.2	-2	+3	
5/19	0.0	-3	+2	
5/21	-0.1	0	+2	
5/23	+0.1	+2	0	
5/28	-0.2	-4	-1	
5/30	+0.1	+2	+2	
6/2 - H	0.0	-9	-2	
6/4	0.0	-2	-2	
6/6 - H	-0.2	-4	-3	
6/9 - H	-0.3	+5	-4	
6/11	-0.1	0	+1	
6/13	-0.2	+5	+15	
6/16	+0.3	0	+22	
6/18	0.0	+10	0	
6/20	+0.1	+17 *	0	
6/23	+0.1	-5 *	-2	
6/25 - H	+0.4	+1	-2	
6/27 - H	+0.4	+7	-2	
6/30 - H	+0.2	+3	-35	
7/2 - H	+0.2	+2	-35	
7/7	0.0	+2	-43 *	
7/10 - H	+0.2	-3	0	
7/14 - H	+0.1	+14	+5	
7/16	+0.2	-2	+26 *	
7/18 - H	-0.6	+2	-3	
7/21	+0.1	+2	-11 *	0
7/23 - H	+0.3	+1	+6	+2
7/25 - H	+0.3	-3	+8	+2
7/28 - H	+0.2	+6	0	+2
7/30 - H	+0.4	+6	+4	+3
8/1 - H	+0.4	+3	+3	+2
8/4 - H	+0.5	+11	-2	+1
8/6	+0.0	+14	+9	0

* - asterisk indicates that a calibration adjustment was made.

Table 9. (continued).

Location[a] m(ft)	Time	Pressure (mm Hg)			Temperature (C)
		Total(ΔP)	O_2	N_2+Ar	
57.9(190)	18:11	769(85)	134	634	18.7
61.0(200)	18:18	770(86)	134	635	18.7
64.0(210)	18:24	766(82)	134	630	18.7

a - distance from right bank when facing downstream.

Table 10. Cross river transect of gas levels at Rkm 0.6 on 4 September, 1986. (Q = 2796 cfs; barometric pressure = 684 mm Hg from 10:13-11:37 and 683 mm Hg from 11:49-13:58, mean opening (range in parentheses) of sluiceway gates 1, 2 & 3 = 1.79 (1.46-2.10), 1.89 (1.61-2.19), and 2.18 (1.91-2.40), respectively; radial gates 1, 3, & 5 open 0.75 ft, radial gates 2 & 4 open 0.25 ft).

Location[a] m(ft)	Time	Pressure (mm Hg)			Temperature (C)
		Total(ΔP)	O_2	N_2+Ar	
1.5(5)	10:13	784(100)	150	633	18.7
4.6(15)	10:33	795(111)	145	648	18.7
7.6(25)	10:50	807(123)	145	660	18.7
10.7(35)[b]	11:01	811(127)	146	663	18.7
13.7(45)	11:13	808(124)	145	661	18.7
16.8(55)	11:24	807(123)	145	661	18.7
19.8(65)	11:37	802(118)	145	656	18.8
22.9(75)	11:49	800(117)	145	653	18.8
25.9(85)	12:01	793(110)	144	647	18.8
29.0(95)	12:13	785(102)	144	639	18.9
32.0(105)	12:25	779(96)	144	634	18.9
35.1(115)	12:37	777(94)	143	633	18.9
38.1(125)	12:49	774(91)	143	630	18.9
41.1(135)	13:00	772(89)	143	629	19.0
44.2(145)	13:14	770(87)	144	627	19.0
47.2(155)	13:25	771(88)	145	626	19.1
50.3(165)	13:35	772(89)	146	625	19.1
53.3(175)	13:47	773(90)	149	624	19.1
56.4(185)	13:58	779(96)	173	606	19.6

a - distance from right bank when facing downstream.
b - in line with continuous monitoring probe.

as we proceeded towards the right bank, dropping 39 mm Hg between 10.7 and 53.3 m. The minimum TGP, which was found at 44.2 m, was 40 mm Hg lower than the highest level. Oxygen pressure was constant across the river with the exception of measurements adjacent to the banks. In terms of saturation, TGP dropped 5.3% from 118-111.8% and nitrogen dropped 6.7% from 122.3-114.1% between 7.6 and 44.2 m. So, at times when the sluicegates are used, there is a considerable difference in gas regimes in as little as 33.5 m. During sluiceway use, fish may be able to reduce exposure to high gas levels by moving short distances longitudinally in the river in addition to sounding and taking advantage of the hydrostatic pressure if they are able to detect or differentiate pressure differences.

Incidence of Gas Bubble Trauma

Incidence of gas bubble trauma (GBT) in brown trout from the upper monitoring section continued to increase until reaching a peak in early June 1986 of 65% and 68% for all browns and those ≥ 356 mm, respectively (Table 12). In late June, incidence rates decreased by over 20% in both categories and by 20 July, affected individuals accounted for only 2-3% of the fish handled. During this period of low incidence, former bubble sites were visible as darkened, sloughing epithelial tissues. The incidence pattern among brown trout for the first half of 1986 in Rkm 0-1.9 was quite similar to that observed by the MDFWP in 1984 (Fredenberg 1985). Incidence for all browns increased in September to near 30% and remained there through early 1987. For the larger

Table 12. Incidence of gas bubble trauma (GBT) in brown trout from Rkm 0-1.9 (section 1) of the Bighorn River during 1986 (data are from right bank unless noted otherwise).

Date	Time	All brown trout		Brown trout ≥ 356 mm	
		No. caught	% w/GBT	No. caught	% w/GBT
1/7	day	156	17	95	25
1/28	"	163	20	95	24
2/25	"	183	18	116	22
3/18&20 (Right bank)	"	154	38	70	49
3/19 (Left bank)	"	171	12	65	20
3/18-20 (Total - both banks)	"	325	24	135	35
4/2&4 (Right bank)	"	358	34	107	56
4/1&3 (Left bank)	"	195	16	77	26
4/1-4 (Total - both banks)	"	553	28	184	43
4/29	"	192	63	91	66
6/3	"	105	65	65	68
6/24	night	175	41	123	47
7/20	"	180	2	114	3
8/17	"	157	3	92	3
9/15 (Right bank)	day	232	11	52	27
9/16 (Left bank)	"	424	2	92	3
9/15&16 (Total - both banks)	"	656	5	144	12
9/29 (Right bank)	day	160	28	46	46
9/30 (Left bank)	"	288	5	40	5
9/29&30	"				

(Total - both banks)		448	13	86	27

Table 12. (continued).

Date	Time	All brown trout		Brown trout ≥ 356 mm	
		No. caught	% w/GBT	No. caught	% w/GBT
11/14	day	174	30	57	37
12/16	"	192	32	36	50
2/4/87	"	168	30	44	55

browns, incidence increased 43% from mid-August to late September and tended to increase during the rest of the fall and winter.

Size distribution of fish with GBT appeared to follow the length-frequency of the catch for the four dates examined (2, 4, 29 April and 3 June). When GBT incidence increased during both the spring and fall, all size groups showed an increase. However, incidence among the larger size group (≥ 356 mm) always increased more quickly. In the upper section, incidence for all fish achieved levels similar to those of larger fish during the peak incidence period of early summer. But incidence among the larger fish remained much higher during fall. Perhaps the higher susceptability to GBT of older, larger fish could be expected if they have larger nucleation site radii than smaller fish. Fidler (1985) demonstrated an inverse relationship between the mean nucleation site radii and TGP thresholds.

Maximum incidence values in 1985 (28%) for all brown trout were substantially lower than for 1986, but similar for larger fish. However, average annual rate of incidence for browns ≥ 356 mm in 1986 (35.3%) was 19.9% greater than that found in 1985. The 1986 average incidence for all brown trout was 28.1% compared to only 10.6% in 1985.

In the upper study section during 1986, 91% of all browns that had GBT displayed minor symptoms while 87% of those ≥ 356 mm, had minor symptoms (Table 13). Serious symptoms were noted in 8% of all browns but this rose to 12% in the larger length group; severe symptoms were observed in 1% of each group. All fish with severe symptoms exceeded 356 mm total length. They were captured in late March, when GBT incidence was rapidly rising and again in early June when incidence peaked. Sampling in February 1987 showed that both incidence and severity of symptoms increased dramatically for the larger browns.

Peak incidence of GBT for rainbow trout in Section 1 occurred on 29 April (Table 14). This was before major increases in discharge and TGP but coincided with rainbow spawning activity. Incidence remained high through early June but dropped about 50% by late June and then to zero in late July. Incidence during early fall 1986 remained below 10% for both size categories, then increased to 17% for all rainbows and to 60% for larger rainbows in November. This large observed increase may have been biased due to small sample size. Incidence dropped to 10 and 13% for all rainbows and those ≥ 356 mm during December. Pattern of incidence was similar to that found in 1985, but incidence rates were higher. Overall, 13.7% and 18.4%

Table 13. Categorization of the severity of gas bubble trauma (GBT) in brown trout from Rkm 0-1.9 (section 1) of the Bighorn River during 1986. I = minor, II = serious and III = severe symptoms (MDFWP and MCFRU data).

Date	Time	All brown trout I No./%	All brown trout II No./%	All brown trout III No./%	Brown trout ≥ 356 mm I No./%	Brown trout ≥ 356 mm II No./%	Brown trout ≥ 356 mm III No./%
1/7	day	26/96	1/4		23/96	1/4	
1/28	"	29/91	3/9		21/91	2/9	
2/25	"	29/88	4/12		22/85	4/15	
3/18&20 (Right bank)	"	46/79	10/17	2/4	26/76	6/18	2/6
3/19 (Left bank)	"	21/100			13/100		
3/18-20 (Total-both banks)	"	67/85	10/13	2/2	39/83	6/13	2/4
4/2&4 (Right bank)	"	114/94	7/6		53/88	7/12	
4/1&3 (Left bank)	"	32/100			20/100		
4/1-4 (Total-both banks)	"	146/95	7/5		73/91	7/9	
4/29	"	111/92	10/8		51/85	9/15	
6/3	"	51/75	14/21	3/4	30/68	11/25	3/7
6/24	night	67/93	5/7		53/91	5/9	
7/20	"	4/100			3/100		
8/17	"	4/100			3/100		
9/15 (Right bank)	day	25/96	1/4		13/93	1/7	
9/16 (Left bank)	"	10/100			3/100		
9/15&16 (Total-both banks)	"	35/97	1/3		16/94	1/6	

Table 13. (continued).

Date	Time	All brown trout I No./%	All brown trout II No./%	All brown trout III No./%	Brown trout ≥ 356 mm I No./%	Brown trout ≥ 356 mm II No./%	Brown trout ≥ 356 mm III No./%
9/29 (Right bank)	day	41/93	3/7		20/95	1/5	
9/30 (Left bank)	"	15/100			2/100		
9/29&30 (Total-both banks)	"	56/95	3/5		22/96	1/4	
11/14	day	49/92	4/8		18/86	3/14	
12/16	"	61/100			18/100		
2/4/87	"	43/84	4/8	4/8	18/75	2/8	4/17
1986 TOTAL		657/91	62/8	5/1	354/87	50/12	5/1

Table 14. Incidence of gas bubble trauma (GBT) in rainbow trout from Rkm 0-1.9(section 1) of the Bighorn River during 1986 (data are from right bank unless noted otherwise).

Date	Time	All rainbow trout		Rainbow trout ≥ 356 mm	
		No. caught	% w/GBT	No. caught	% w/GBT
1/7	day	11	0	10	0
1/28	"	9	0	7	0
2/25	"	12	0	9	0
3/18&20 (Right bank)	"	23	17	16	25
3/19&21 (Left bank)	"	30	17	24	17
3/18-21 (Total - both banks)	"	53	17	40	20
4/2&4 (Right bank)	"	35	20	19	26
4/1&3 (Left bank)	"	27	11	19	16
4/1-4 (Total - both banks)	"	62	16	38	21
4/29	"	26	54	18	61
6/3	"	26	50	20	60
6/24	night	23	26	19	32
7/20	"	15	0	11	0
8/17	"	11	0	7	0
9/15&17 (Right bank)	day	111	8	59	8
9/16&18 (Left bank)	"	120	5	62	5
9/15-18 (Total - both banks)	"	231	6	121	7
9/29&10/2 (Right bank)	day	143	6	85	8
9/30 (Left bank)	"	126	5	69	6
9/29-10/2 (Total - both banks)	"	269	6	154	7

Table 14. (continued).

Date	Time	All rainbow trout		Rainbow trout $\geq$ 356 mm	
		No. caught	% w/GBT	No. caught	% w/GBT
11/14	day	18	17	5	60
12/16	"	41	10	8	13
2/4/87	"	26	8	2	0

of all rainbows and those $\geq$ 356 mm, respectively, displayed external signs of GBT in 1986, an increase of 9.5 and 13.2%, respectively, over 1985. Incidence for rainbows was approximately half of that found among brown trout, but the sample size was smaller (only 20-25% of that for browns). Seven and 9% of all rainbows and those $\geq$ 356 mm, respectively, were categorized as having serious external symptoms (Class II). All others were placed in Category I.

GBT incidence observed among brown trout on the right bank was consistently higher than on the left bank in March, April and September. Also, serious and severe symptoms were observed only on brown trout captured along the right bank. However, incidence rates among rainbows often did not vary between banks.

GBT incidence tends to increase as flow increases. However, this was not true in June when delta P's and discharge increased, yet incidence declined. Symptoms can occur only when total dissolved gas pressures (TGP) are greater than compensating pressures (P_{COMP}). Increased hydrostatic pressures (P_H) that accompany higher flows may help fish compensate for increased gas levels. The following example demonstrates how this could prove beneficial to fish health in the Bighorn River. The mean daily stage at the gagehouse for the period 29 April - 20 July increased from a low of about 963.4 m (3160.89 ft) on 3 June to 964.0 m (3162.85 ft) on 18 June and the mean barometric pressure for June was 681 mm. This increased height of the river provided an additional 41 mm Hg of P_H and P_{COMP}. Mean daily delta P values at the gagehouse (representative of actual gas levels on GBT incidence sampling dates) rose from 126 mm Hg on 4 June to 138 mm Hg on 25 June. A fish on 4 June in 1 m of water would have been subjected to a $\Delta P = \Delta P_{SURFACE} - P_H$ = 126 mm Hg - 68 mm Hg = 58 mm Hg. Assuming that fish took advantage of the additional depth, on 25 June exposure would have decreased to a ΔP = 138 mm Hg - 68 mm Hg - 41 mm Hg = 29 mm Hg, or 29 mm Hg less than the hyperbaric pressure on 4 June.

On 14 July the operational pattern of Afterbay Dam was modified so all discharge passed through the radial gates. This caused an immediate drop of delta P values to approximately one-half of their previous values. The low GBT incidence in July and August is attributed to this operational change. The decrease in discharge (14.2 m^3/s; 500 cfs) at 12:00 AM on 15 July did not substantially alter delta P levels. On 22 August the sluiceway gates were returned to service. Shortly after, incidence of GBT increased to levels near those observed in March and April. Flows were similar but water temperatures were higher. These

Flows were similar but water temperatures were higher. These higher water temperatures lowered gas solubility and increased delta P when tailwater elevation and entrainment were constant. Declining water temperature through the rest of the fall probably benefited the fish. Discharge from Afterbay Dam was fairly uniform but tended to increase slightly. The increase in P_W, if utilized, would also benefit the fish, but a flow increase would also increase the stilling basin depth and increase TGP. In addition, behavorial and habitat use changes among brown trout in the fall may have a bearing on incidence.

The pattern of GBT incidence for brown trout in Section 2, was similar to that in the upper section but rates were lower (Table 15). Excluding July and August, incidence ranged from 10-45% lower in section 2 than in the upper reach. Peak incidence was observed in late April when 33 and 38% of all browns and those $\geq$ 356 mm, respectively, had externally visible symptoms. Incidence rates then dropped to zero in August and remained near zero for the rest of the year except for a small rise in December. The 1986 annual average GBT incidence rate for the middle section was 7.5 and 10.7% for all browns and browns $\geq$ 356 mm, respectively. No brown trout sampled in section 2 during 1986 had severe symptoms of GBT (Table 16); 6-7% of both categories had serious, class II symptoms while the majority (93-94%) had minor symptoms.

What was thought to be an anomoly of sampling in 1985 was observed again during 1986. Incidence of GBT for all rainbows and those $\geq$ 356 mm from the middle section was 20 and 23.4%,

Table 15. Incidence of gas bubble trauma (GBT) in brown trout from Rkm 3.9-6.1 (section 2) on the Bighorn River during 1986 (data are from both banks unless noted otherwise).

Date	Time	All brown trout		Brown trout ≥ 356 mm	
		No. caught	% w/GBT	No. caught	% w/GBT
1/7	day	163	5	123	4
1/28	"	148	4	113	4
2/25	"	154	1	123	2
3/18&20 (Right bank)	"	74	20	57	23
3/19 (Left bank)	"	86	3	60	5
3/18-20 (Total - both banks)	"	160	11	117	14
4/2 (Right bank)	"	89	19	74	22
4/1 (Left bank)	"	67	9	49	8
4/1-2 (Total - both banks)	"	156	15	123	16
4/29	"	151	33	126	38
6/3-4	day & night	156	20	88	27
6/26 (Right bank)	night	184	14	117	18
7/23 (Right bank)	"	157	0.6	99	1
8/17	"	176	0	88	0
9/15 (Right bank)	day	160	1	45	2
9/16 (Left bank)	"	174	2	61	0
9/15&16 (Total - both banks)	"	334	1	106	1
10/1 (Total - both banks)	"	480	3	131	3

Table 15. (continued).

Date	Time	All brown trout		Brown trout ≥ 356 mm	
		No. caught	% w/GBT	No. caught	% w/GBT
11/14 (Right bank)	day	160	3	72	1
12/18 (Right bank)	day	207	11	50	22
2/3/87	"	157	6	52	10

Table 16. Categorization of the severity of gas bubble trauma (GBT) in brown trout from Rkm 3.9-6.1 (section 2) of the Bighorn River during 1986. I = minor, II = serious and III = severe symptoms (MDFWP and MCFRU data).

Date	Time	All brown trout I No./%	All brown trout II No./%	All brown trout III No./%	Brown trout ≥ 356 mm I No./%	Brown trout ≥ 356 mm II No./%	Brown trout ≥ 356 mm III No./%
1/7	day	8/100			5/100		
1/28	"	6/100			4/100		
2/25	"	2/100			2/100		
3/18&20 (Right bank)	"	13/87	2/13		12/92	1/8	
3/19 (Left bank)	"	2/67	1/33		2/67	1/33	
3/18-20 (Total-both banks)	"	15/83	3/17		14/87	2/13	
4/2 (Right bank)	"	17/100			16/100		
4/1 (Left bank)	"	6/100			4/100		
4/1-2 (Total-both banks)	"	23/100			20/100		
4/29	"	45/90	5/10		43/90	5/10	
6/3-4	day & night	28/90	3/10		21/88	3/12	
6/26 (Right bank)	night	25/100			21/100		
7/23 (Right bank)	"	1/100			1/100		
8/17	"	-			-		
9/15 (Right bank)	day	2/100			1/100		
9/16 (Left bank)	"	3/100			-		
9/15&16 (Total-both banks)	"	5/100			1/100		

Table 16. (continued).

Date	Time	All brown trout I No./%	All brown trout II No./%	All brown trout III No./%	Brown trout ≥ 356 mm I No./%	Brown trout ≥ 356 mm II No./%	Brown trout ≥ 356 mm III No./%
10/1 (Total-both banks)	"	14/93	1/7		3/75	1/25	
11/14 (Right bank)	day	4/100			1/100		
12/18 (Right bank)	day	22/100			11/100		
2/3/87	"	10/100			5/100		
1986 TOTAL		198/94	12/6		147/93	11/7	

respectively, substantially above that for browns in the section. Sample sizes were low in 1985, but were higher in 1986. No incidence of GBT was observed in late winter; incidence began increasing in March, and peaked at 83 and 91% in early June 1986 for all rainbows and for the larger fish, respectively (Table 17). Rates dropped to zero in July and remained low the rest of the year. Incidence among rainbows in the middle reach followed a pattern similar to browns in both sections 1 and 2, but the response was greater. Section 2 includes two rainbow spawning areas; perhaps rainbows spawning in this section inhabit shallow water and hence are more vulnerable to supersaturated conditions. About 10% of the rainbows in both size groups had serious symptoms in 1986; the remainder displayed minor symptoms.

Table 17. Incidence of gas bubble trauma (GBT) in rainbow trout from Rkm 3.9-6.1 (section 2) on the Bighorn River during 1986 (data are from both banks unless noted otherwise).

Date	Time	All rainbow trout		Rainbow trout ≥ 356 mm	
		No. caught	% w/GBT	No. caught	% w/GBT
1/7	day	10	0	10	0
1/28	"	19	0	19	0
2/25	"	16	0	13	0
3/18&20 (Right bank)	"	34	18	31	16
3/19&21 (Left bank)	"	28	0	25	0
3/18-21 (Total - both banks)	"	62	10	56	9
4/2&4 (Right bank)	"	89	25	86	26
4/1&3 (Left bank)	"	23	9	20	10
4/1-4 (Total - both banks)	"	112	21	106	23
4/29	"	59	53	56	54
6/3-4	day & night	42	83	34	91
6/26 (Right bank)	night	19	11	14	14
7/23 (Right bank)	"	7	0	7	0
8/17	"	2	0	2	0
9/15&17 (Right bank)	day	17	0	13	0
9/16&18 (Left bank)	"	33	0	24	0
9/15&18 (Total - both banks)	"	50	0	37	0
10/1-3 (Total - both banks)	"	59	0	23	0

Table 17. (continued).

Date	Time	All rainbow trout		Rainbow trout $\geq$ 356 mm	
		No. caught	% w/GBT	No. caught	% w/GBT
11/14 (Right bank)	day	28	4	13	0
12/18 (Right bank)	"	11	0	4	0
2/3/87 (Right bank)	"	18	11	6	33

Incidence of GBT in brown trout in Section 3 remained low, never exceeding 8% for all browns and 12% for the larger browns (Table 18). Incidence rates were highest in spring and summer and varied from 0-3% the rest of the year. The annual average incidence rates for section 3 were 2.8% and 4.5% for all browns and those $\geq$ 356 mm, respectively. All but one fish captured that had GBT displayed minor symptoms; the other fish had serious symptoms (Table 19). The number of affected rainbow was zero except on three dates (Table 20). GBT incidence peaked in May at 19-20% and was 2% in March and 3% in June. All rainbow trout in section 3 had only minor symptoms.

Since April 1986, mountain whitefish have also been collected during incidence of occurrence sampling (Table 21). Incidence in all three sections has never risen above 10% for all whitefish and 20% for those $\geq$ 356 mm; these levels were observed in the second section on 3 June. The number of whitefish caught

Table 18. Incidence of gas bubble trauma (GBT) in brown trout from Rkm 12.2-15.4 (section 3) on the Bighorn River during 1986 (data are from both banks unless noted otherwise).

Date	Time	All brown trout		Brown trout ≥ 356 mm	
		No. caught	% w/GBT	No. caught	% w/GBT
3/18	day	129	8	95	11
4/1&2	"	231	4	169	5
5/1	"	166	6	137	7
6/4 (Right Bank)	night	184	3	111	5
6/26 (Right bank)	"	166	7	90	12
7/23 (Right bank)	"	153	1.3	107	0.9
8/20 (Right Bank)	"	174	1.1	113	0.9
9/17 (Right bank)	day	181	0	65	0
10/1	day	190	0	71	0
11/18 (Right bank)	day	180	1.1	82	1.2
12/16 (Right bank)	"	180	1.7	73	2.7
2/5/87	"	165	1.2	94	1.1

Table 19. Categorization of the severity of gas bubble trauma (GBT) in brown trout from Rkm 12.2-15.4 (section 3) of the Bighorn River during 1986. I = minor, II = serious and III = severe symptoms (MDFWP and MCFRU data).

		All brown trout			Brown trout ≥ 356 mm		
		I	II	III	I	II	III
Date	Time	No./%	No./%	No./%	No./%	No./%	No./%
3/18	day	10/100			10/100		
4/1&2	"	7/87	1/13		7/87	1/13	
5/1	"	10/100			10/100		
6/4 (Right Bank)	night	6/100			6/100		
6/26 (Right bank)	"	12/100			11/100		
7/23 (Right bank)	"	2/100			1/100		
8/20 (Right Bank)	"	2/100			1/100		
9/17 (Right bank)	day	-			-		
10/1	day	-			-		
11/18 (Right bank)	day	2/100			1/100		
12/16 (Right bank)	"	3/100			2/100		
2/5/87	"	2/100			1/100		
1986 TOTAL		54/98	1/2		49/98	1/2	

Table 20. Incidence of gas bubble trauma (GBT) in rainbow trout from Rkm 12.2-15.4 (section 3) on the Bighorn River during 1986 (data is from both banks unless noted otherwise).

Date	Time	All rainbow trout		Rainbow trout ≥ 356 mm	
		No. caught	% w/GBT	No. caught	% w/GBT
3/18	day	47	2	43	2
4/1&2	"	27	0	27	0
5/1	"	16	19	15	20
6/4 (Right bank)	night	31	3	31	3
6/26 (Right bank)	"	24	0	22	0
7/23 (Right bank)	"	13	0	12	0
8/20 (Right Bank)	"	15	0	10	0
9/17 (Right bank)	day	36	0	23	0
10/1	day	42	0	26	0
11/18 (Right bank)	day	36	0	21	0
12/16 (Right bank)	"	32	0	9	0
2/5/87	"	34	0	9	0

Table 21. Incidence of gas bubble trauma (GBT) in mountain whitefish from the Bighorn River during 1986 (data are from right bank unless noted otherwise).

Date	Time	All mtn whitefish		Mtn whitefish ≥ 356 mm	
		No. caught	% w/GBT	No. caught	% w/GBT
		Rkm 0.0-1.9			
4/1&3 (Left bank)	day	41	5	39	5
4/2&4 (Right bank)	"	14	0	13	0
4/1-4 (Total both banks)	"	55	4	52	4
4/29	"	2	0	1	0
6/3	day	2	0	1	0
6/24	night	0	-	0	-
7/20	"	0	-	0	-
8/17	"	1	0	1	0
11/14	day	0	-	0	-
12/16	"	0	-	0	-
2/4	"	2	0	1	0
		Rkm 3.9-6.1			
4/1&3 (Left bank)	day	30	3	22	5
4/2&4 (Right bank)	"	41	0	20	0
4/1-4 (Total both banks)	"	71	1	42	2
4/29 (Both banks)	"	8	0	5	0
6/3	"	10	10	5	20
6/26	night	7	0	5	0
7/23	"	17	0	16	0

Table 21. (continued)

Date	Time	All mtn whitefish		Mtn whitefish ≥ 356 mm	
		No. caught	% w/GBT	No. caught	% w/GBT
			Rkm 3.9-6.1		
8/17	"	14	0	6	0
11/14	day	21	0	8	0
12/18	"	35	0	21	0
2/3	"	12	8	8	13
			Rkm 12.2-15.4		
5/1	day	3	0	3	0
6/4	night	18	0	15	0
6/26	"	9	0	5	0
7/23	"	13	0	7	0
8/20	"	0	-	0	-
11/18	day	18	0	3	0
12/16	"	29	0	8	0
2/5	"	6	0	4	0

on each electrofishing run has always been low, ranging from 0-35. Incidence for all of 1986 in the upper monitoring section averaged 2.6% for all fish and 3.4% for those ≥ 356 mm. This overall average dropped to 1.1 and 1.9% for all whitefish and large whitefish fish, respectively, in Section 2. No GBT was observed among the whitefish captured during 1986 in Section 3.

Our data seem to indicate that mountain whitefish are not sensitive to supersaturated conditions. However, Fickeisen and Montgomery (1975) found in bioassays that mountain whitefish were the most intolerant species to supersaturation of those tested. Other species tested and listed in increasing tolerance included the westslope cutthroat trout (*Salmo clarki lewisi*), largescale sucker (*Catostomus machrocheilus*), and the torrent sculpin (*Cottus rhotheus*). Supersaturation may limit the mountain whitefish population in the Bighorn River below Afterbay Dam.

The torrent sculpin appear to be more tolerant to supersaturation than we previously thought. Fickeisen and Montgomery (1975) found that the mortality curves for torrent sculpin were much flatter than for other species and no mortality was observed at 116% TGP. However, sculpins developed large external bubbles behind the pectoral fin insertion at lower TGP's, causing a loss of equilibrium and increased buoyancy. Even with this loss of equilibrium, sculpins were more tolerant than the other species. This information indicates that sculpin transplants could possibly be successful in the Bighorn River provided suitable habitat and adequate P_{comp} exists. If suitable P_{comp} did not exist, positively buoyant sculpin could be highly susceptable to predation. If the MDFWP determines that establishing a mottled sculpin (*Cottus bairdi*) population is desirable, the feasibility of this introduction should be explored.

Population Estimates

Brown and rainbow trout population estimates were obtained in two overlapping sections of the study area during March and September, 1986 (Tables 22 and 23). Brown trout estimates for the upper 6.1 Rkm were 22% greater for Age 2 and older fish in spring 1986 than in spring 1985. However, from Rkm 3.9-15.4 the estimated number of browns Age 2 and older during spring 1986 was 76% of that found in 1985. Most of the difference in population levels between the two sections during 1986 was in Age 2 fish; 184% more Age 2 browns were found in the upper reach.

Fall brown trout numbers in the upper section (Rkm 0-6.1) were 9% greater than found during fall 1985 (Table 23). Age 1 and 2 fish represented equal portions (41.6 and 41.0%, respectively) of the estimate while age 3 and older fish composed 17.4%. Preliminary analysis of age data suggests that growth in this portion of the river is slower for all age groups than in downstream sections. In the lower section, total brown trout numbers were 43.1% less than in the upper section but were 35.8% greater than in 1985 fall estimates. Larger fish represented a greater percentage of the population in this lower reach since the proportion in each size group was more uniform, with 28.3% in the largest size group and 34.6 and 37.1% in the middle and lower groups, respectively.

During March, rainbow trout and mountain whitefish numbers were 247 and 145/km, respectively, for the reach of river from Rkm 0-15.4 (Table 22). Although adequate numbers of recaptured rainbow trout were not obtained during spring sampling,

Table 22. Brown trout, rainbow trout and mountain whitefish population estimates from the upper Bighorn River below Afterbay Dam during March, 1986 (MCFRU and MDFWP data).

Section Rkm	Length interval (mm)	Number/km	80% Confidence intervals
	Brown trout		
0.0-6.1	152-290(Age 2)	870	633-1107
	291-429(" 3)	1641	1297-1985
	430-594(" 4+)	288	231-345
	Total	2799	2377-3221
3.9-15.4	152-290(Age 2)	306	209-4031
	291-429(" 3)	1527	1290-1764
	430-480(" 4+)	286	237-335
	481-607(" 4+)	136	105-167
	Total	2255	1993-2517
	Rainbow trout		
0.0-15.4	305-455	175	129-221
	456-620	72	53-91
	Total	247	197-297
	Mountain whitefish		
0.0-15.4	330-544(Age 3+)	145	107-183

Table 23. Brown and rainbow trout population estimates from the Bighorn River below Afterbay Dam during September, 1986 (MCFRU and MDFWP data).

Section Rkm	Length interval (mm)	Number/km	80% Confidence intervals
		Brown trout	
0.0-6.1	127-239(Age 1)	3238	2578-3898
	240-366(" 2)	3184	2893-3475
	367-594(" 3+)	1350	1172-1528
	Total	7762	7019-8505
3.9-15.4	127-239	1639	1426-1852
	240-366	1530	1368-1692
	367-594	1251	1104-1398
	Total	4420	4115-4725
		Rainbow trout	
0.0-6.1	229-404	185	134-236
	405-594	162	105-219
	Total	347	270-424
3.9-15.4	191-277	178	121-235
	278-404	59	33-85
	405-594	155	114-196
	Total	392	317-467

sufficient numbers were recaptured in September to estimate population abundance for both sections. Numbers and sizes appeared to be approximately equal in both sections. In the upper 6.1 km section of the river, a large portion of the rainbows captured appeared to be of hatchery origin.

Egg Incubation

Mean survival of brown trout embryos through the eyed stage in egg bags varied 11% between sites (Table 24); survival was lowest at the upstream location while it was highest and most variable at Rkm 8.0. Survival rates through eye-up in wedge boxes were similar for the control sample in Afterbay Reservoir and the most upstream site (Table 24); in the river, survival decreased in a downstream direction. Variation in survival between boxes at each site was low compared to that observed in egg bags. Between site variation in mean survival rates of brown trout eggs in bags at hatching was low (10%). Hatching was more successful at the upstream site (Rkm 2.4) and decreased with distance downstream. Hatching success from the wedge boxes was much more variable, but also decreased downriver. Hatching was 27% higher at Rkm 2.4 than in Afterbay Reservoir. Lower survival rate among the controls is probably due to movement of the wedge boxes while the eggs were in the sensitive stage between water-hardening and eye-up. As with the eye-up experiments, variation in embryo survival at each site was less in wedge boxes than in bags.

These data suggest that total dissolved gas and nitrogen levels encountered on the upper reaches of the study area during brown trout egg incubation and hatching tests did not increase mortality, since survival rates were similar or decreased downstream. Hyperbaric pressures were relatively low during brown trout egg incubation. Mean delta P's were 93, 75, and 58

Table 24. Brown trout egg survival from egg bags and wedge boxes planted 9 December, 1985 at the early life history sites on the Bighorn River and in Afterbay Reservoir. Survial ranges are in parentheses.

River km	% survival to eyed stage		% survival through hatching	
	Bags	Boxes	Bags	Boxes
Afterbay	----	69[a] (66-74)	----	48[b] (33-59)
2.4	38 (17-60)	68 (58-80)	27 (11-44)	75 (70-79)
8.0	49 (14-78)	44 (34-61)	23 (5-40)	51 (43-60)
14.5	43 (9-60)	37 (34-44)	17 (5-34)	28 (27-28)

a - Excludes one box with 28% survival.
b - Excludes one box with 11% survival.

mm Hg at Rkm 2.4, 8.0, and 14.5, respectively, from fertilization to eye-up (Table 25); levels through hatching increased 5-8 mm Hg. These mean delta P's are 23-50 and 15-44 mm Hg less than during 1985 rainbow egg incubation tests through eye-up and hatching, respectively. It appears that rainbow eggs hatch and fry emerge during periods of higher gas levels than encountered by brown trout eggs and fry. However, differing gas levels between sites does not appear to be an important factor in survival - a result similar to that found with rainbow trout eggs in "Astroturf" incubation boxes in 1985. Sediment appeared to be the most important factor affecting survival in the wedge boxes. The O_2/N_2 ratio increased in a downstream direction during brown

Table 25. Mean and range of delta P's (mm Hg) and the oxygen/nitrogen ratio during brown trout egg incubation tests at three sites on the Bighorn River from December, 1985 - April, 1986.

River km	Eyed stage Dates	Eyed stage ΔP	Eyed stage O_2/N_2	Through hatching Dates	Through hatching ΔP	Through hatching O_2/N_2
2.4	12/9-3/23	93 (66-125)	0.17	12/9-4/23	98 (66-132)	0.18
8.0	12/9-3/27	75 (38-123)	0.41	12/9-4/25	82 (38-131)	0.43
14.5	12/9-3/26	58 (16-107)	0.68	12/9-4/27	65 (16-123)	0.70

trout egg incubation tests and was substantially lower than ratios at each respective site during rainbow tests in 1985 (Table 25).

Uncompensated mean delta P's at all sites for brown trout in 1986 and rainbow trout in 1985 were above the "general" threshold where over-inflation of the swimbladder or bubble growth in the buccal cavity of small fish would occur (this is discussed in more detail in another section of this report). During 1986 brown trout embryo tests, only gas levels at Rkm 2.4 were greater than those required for bubble formation in the vascular system. Mean delta P's were greater than the arterial thresholds for all rainbow egg tests in 1985. However, these thresholds would probably be important only after hatching and swim up because internal pressures of salmonid eggs (which are 50-90 mm Hg near hatching; Alderice et al. 1984) and their location at the bottom of the water column would afford them protection. Additionally there is provisional evidence that these thresholds may be higher for resident Bighorn River species, possibly due to higher resistance of fish or the influence of high water hardness.

Additional brown and rainbow trout egg incubation tests were conducted but poor survival of eggs caused by high flow conditions limits the usefulness of these data. Also, due to the lack of adequate numbers of fry, field rearing experiments were cancelled.

Sluiceway Gates Discharge Equations

To determine the relationship between total gas pressure in Bighorn river water and the operation of Afterbay Dam, computation of that portion of the discharge routed through the sluicegates is necessary. Sluicegate openings usually vary throughout the day, since they automatically respond to changes in river elevation. The equations developed were based on Afterbay elevations between 3176.00 and 3189.00 ft (968.04-972.01 m); flows calculated from them deviated 0.01-2.96% from flows determined from Bureau of Reclamation discharge curves (Table 26). Since the formulas in Table 26 are applicable only if the gates are open exactly to the nearest foot, additional equations were developed to obtain coefficients in these equations so discharge for any gate opening can be determined.

Flow Test - Afterbay Operation Monitoring

As shown by limited data in 1985, the discharge from the sluice-way gates at Afterbay Dam show a stronger, linear

Table 26. Discharge equations for the sluiceway gates of Afterbay Dam on the Bighorn River.

Gate opening (feet)	Equation
1.0	$Q = (-0.91(E)^2 + 171.3(E) - 7137.6)/3$
2.0	$Q = (-1.43(E)^2 + 290.1(E) - 12794.5)/3$
3.0	$Q = (-3.12(E)^2 + 594.9(E) - 25631.7)/3$
4.0	$Q = (-2.86(E)^2 + 579.8(E) - 25448.1)/3$
5.0	$Q = (-2.15(E)^2 + 464.8(E) - 19812.1)/3$
6.0	$Q = (-2.54(E)^2 + 546.3(E) - 22953.1)/3$
7.0	$Q = (-2.99(E)^2 + 645.3(E) - 27046.1)/3$

where: Q = discharge from one 10 ft x 8 ft sluiceway gate (cfs)
E = Afterbay Elevation - 3100.00 ft

relationship to TGP at Rkm 0.2 than any other parameters considered (Table 27). In 1986 discharges from both the sluice and radial gates showed a stronger relationship to TGP than in 1985; however, radial gate discharge was negatively correlated to TGP while sluice gate discharge was positively correlated. Also as before, discharge from the two sets of Afterbay radial gates (1,3, & 5 and 2 & 4) showed a high degree of intercorrelation (r = 0.992-1.000) which is due to operational procedures; the amount that the three sluice gates was open was also more highly intercorrelated (r = 0.983-0.994) than in 1985. Other parameters, such as water temperature, discharge and river

Table 27. Pearson correlation coefficients between mean daily total gas pressure, water temperature (MDFWP thermograph), sluice and radial gate openings, afterbay level, river elevations, barometric pressure, and discharge for data collected at Rkm 0.6 from 7 May - 31 December, 1986.

	TGP (mm Hg)	Temp (C)	SG1	SG2	SG3	RG1	RG3	RG5	RG2	RG4	Afterbay level	River elev	B.P.	Q
TGP (mm Hg)	1.000													
Temp (C)	-0.259	1.000												
SG1	0.755	-0.294	1.000											
SG2	0.772	-0.287	0.983	1.000										
SG3	0.758	-0.287	0.994	0.983	1.000									
RG1	-0.603	0.450	-0.569	-0.574	-0.592	1.000								
RG3	-0.603	0.451	-0.569	-0.573	-0.591	1.000	1.000							
RG5	-0.611	0.446	-0.569	-0.574	-0.592	0.992	0.992	1.000						
RG2	0.061	-0.518	0.099	0.094	0.095	0.034	0.034	0.036	1.000					
RG4	0.062	-0.515	0.100	0.094	0.096	0.032	0.032	0.034	0.999	1.000				
Afterbay level	-0.163	0.063	-0.290	-0.258	-0.317	0.508	0.508	0.511	0.210	0.207	1.000			
River elev.	-0.017	-0.026	0.029	0.028	0.028	0.170	0.171	0.169	0.224	0.223	0.207	1.000		
B.P.	0.070	0.032	-0.163	-0.149	-0.144	-0.031	-0.031	-0.027	0.008	0.009	-0.040	-0.040	1.000	
Q	0.084	-0.037	0.254	0.256	0.220	0.480	0.480	0.475	0.515	0.512	0.627	0.335	-0.1864	1.000

SG# = Sluice gate number
RG# = Radial gate number
B.P. = Barometric Pressure (mm Hg)
Q = Discharge (cfs)

elevation, showed a weak linear relationship to TGP. However, these variables are important in affecting gas entrainment at Afterbay Dam; attempts will be made to determine the influence of these nonlinear relationships in the future.

We examined several models relating physical factors and the operation of Afterbay Dam to TGP measured on the right bank at Rkm 0.2 during 1986. The model that explained the most variation, 69.4%, included all parameters listed in Table 27. Data were not examined for violations of assumptions such as equality of variance, normality and independence of error. Utilizing these same methods with data transformations and entering variables in a stepwise manner, we will be able to obtain a model with the smallest set of significant variables and minimize standard error. Nonlinear regression analysis may also be used in the future in an attempt to predict gas levels below Afterbay Dam. Data related to Afterbay Dam operation collected during 1986 have been sent to Perry Johnson at the Bureau of Reclamation's Engineering and Research Center in Denver. Perry's expertise in gas transfer may provide a better understanding of how best to manage water releases from Afterbay Dam to maximize use of Afterbay Reservoir as a reregulation facility and to minimize gas levels in the river.

During a flow reduction from 3123-1500 cfs (88.4-42.5 m^3/s) on 15 October 1986, water (14.3 C) was discharged only from the sluiceway. Peak saturation levels on the right and left banks occurred at approximately 1870 cfs (53.0 m^3/s; Figure 14). This general pattern is comparable to that found on 19 October 1973 when water temperatures were 13.9 C (57 F) (Bureau of Reclamation 1973). Peak saturation levels were lower on the left bank, but only by 0.5% or 3 mm Hg TGP. As flows decreased below 1870 cfs (53.0 m^3/s), TGP dropped to about 116.3% on the left bank while pressures remained high on the right and reached a second peak at about 1660 cfs (47.0 m^3/s). Minimum gas values were found when the sluiceway discharge was above 2700 cfs (76.5 m^3/s). TGP near

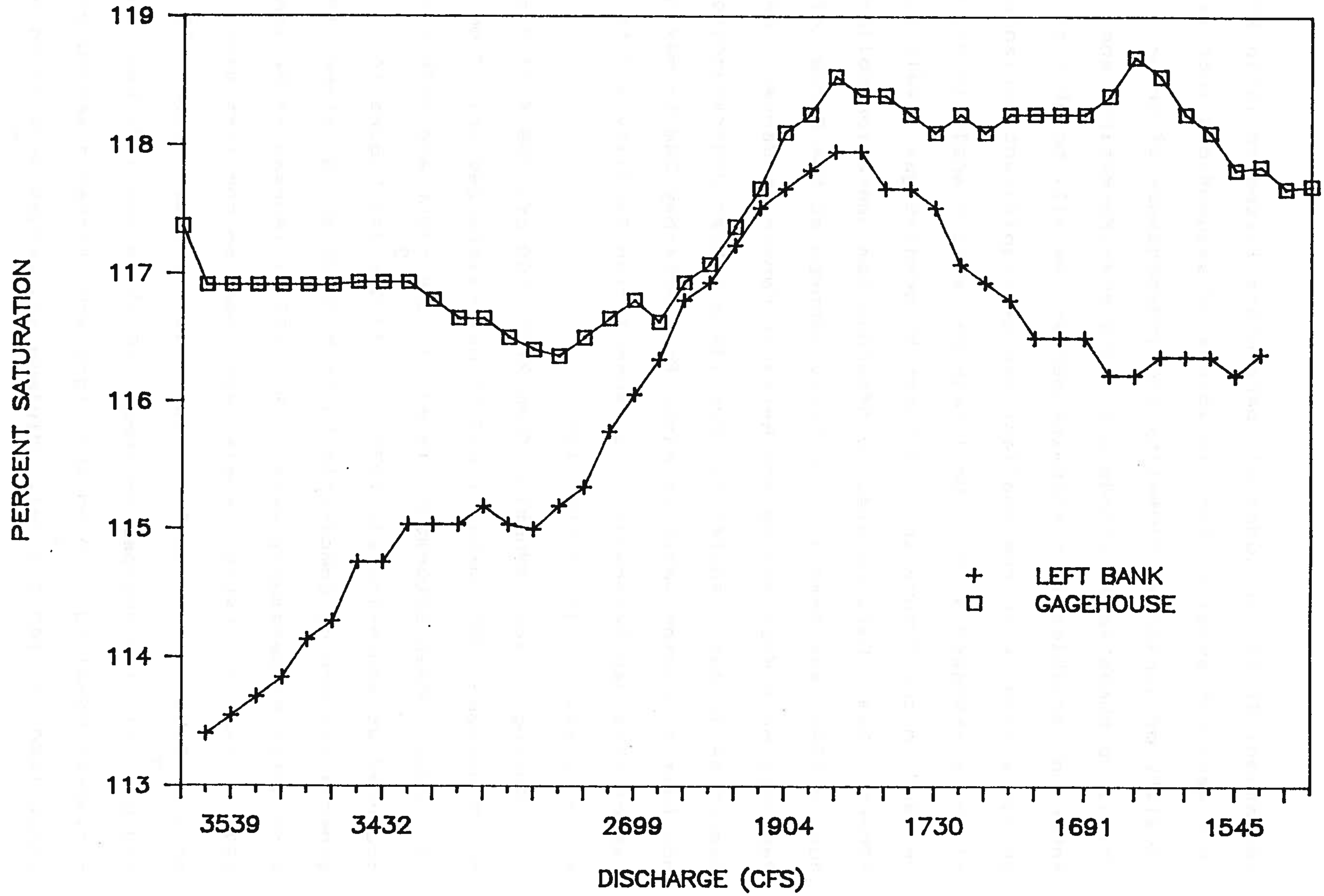

Figure 14. Gas saturation level versus discharge on 15 October, 1986. Water temperature = 14.3 C.

both banks (Figure 14) tends to be somewhat misleading at higher flows since radial gates were used to pass water until discharge was decreased to approximately 3125 cfs (88.5 m^3/s). Also, intervals between discharges in Figure 14 are not uniform.

The above data and those collected in 1973 suggest that maximum TGP production may be avoided by the development of general guidelines that would change seasonally according to expected discharge and water temperature. By varying the percentage of total discharge passed through the sluice gates, we may be able to prevent flows that produce the highest TGP and minimize wear on the sluice gates due to large, frequent movements which would occur if Afterbay operation maximized discharge through the radial gates and Afterbay elevations dropped below the radial gate crest elevation (969 m; 3179 feet). Maximizing discharge through the radial gates, however, would not necessitate frequent large adjustments of the sluice gates provided that flow conditions are similar to those of the last 2 years. The daily minimum Afterbay water elevation dropped below the spillway's crest elevation for only 29 and 8 days in 1985 and 1986, respectively; usually these were in groups of consecutive days. The fluctuation of Afterbay levels may not have been "typical" during 1985 because of a change in dispatching for the Yellowtail Project to Loveland and in both 1985 and 1986, generating units were off-line at times. The magnitude of the TGP reduction would depend primarily on the amount of sluiceway flow change and the nearness of the discharge to that which would produce maximum saturation for a given temperature. Data from October 1986 suggest that a flow increase from about 1870-3125 cfs (53.0-88.5 m^3/s) would reduce TGP approximately 12 mm Hg or almost 2% at a water temperature of 14.3 C. However, 1973 data show that at similar water temperature (13.9 C; 57 F), TGP could be reduced from 120% to about 110% by decreasing the sluiceway discharge from 1650-1000 cfs (46.7-28.3 m^3/s). Although reductions in TGP may be relatively small, they may be of significant benefit if levels drop below thresholds for bubble growth in fish. These reductions do not take into account benefits resulting from an increase of discharge from radial gates. During peak runoff when the Afterbay elevation remains near 971.7 m (3188 ft), it would be feasible to route more than half the discharge through radi gates.

Presently about half the water is routed through the radial gates and half through the sluice-way. Flows in the Bighorn River during the last 10 months of 1986, except during peak runoff in June and July, varied from about 2800-4400 cfs (79.3-124.6 m^3/s). During this entire time, discharge through the sluiceway would have been between 1400-2200 cfs (39.6-62.3 m^3/s) and most of the time it was >1500 cfs (42.5 m^3/s). These sluiceway discharges correspond to flows which create maximum TGP when water temperatures exceed 8.1 C (46.5 F). Altering the percentage of flow through the sluiceway from one-half to either one-fourth or three-fourths at this time would have reduced TGP in most cases.

Seasonal operational changes at Afterbay Dam should be considered if construction of a powerhouse does not appear likely in the near future. Before adopting permanent operational changes, the proposed guidelines should be tested by making empirical measurements while actually changing the proportion of total discharge passed through the sluice-way. This would ensure that a reduction of TGP has occurred and that sluice gates would still be able to automatically respond to river elevations changes.

Additional data were obtained during a flow test on 9 July, 1986 when gas levels were monitored at varying sluiceway discharge ratio. Also, gas tensions were monitored on 14 May and 16 July while gate openings were changed. These data will be discussed when data analysis is completed.

Current Data Analysis

In addition to data presented here, we are also in the process of tabulating data to determine if any relationship exists between condition factors and severity of GBT, the progression and remission of GBT on individual fish, fish movement, and the incidence of blindness, humpies, and hooking scars for various reaches of the river. Also, we plan to compare discharge from Afterbay with gas level data collected from 1984 to present.

EFFECTS OF GAS BUBBLE TRAUMA ON JUVENILE BROWN AND RAINBOW TROUT

There are number of factors that complicate our understanding of gas bubble trauma (GBT) in fishes. Variations in response have been noted for different species and sizes and information on recovery is relatively sparce.

Jensen et al. (1986) predicted that larger fish should be more sensitive to gas supersaturated waters than smaller fish. Fredenburg (1985) and White et al. (1986) found that large brown trout in the Bighorn River have more severe symptoms of GBT than smaller fish. Nevertheless the relationship between GBT and fish size has not been well defined.

Data from the Bighorn River (White et al. 1986) also indicated that brown trout are more susceptable to GBT than rainbow trout; an observation that has been noted by others.

Nebeker and Brett (1975) showed that coho salmon (<u>Oncorhynchus kisutch</u>) smolts were able to recover from GBT after transfer to unsaturated water. However, little information appears to be available for other species.

The objectives of our laboratory work conducted at the Bozeman Fish Technology were to:

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

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

(3) evaluate the ability of juvenile brown and rainbow trout to recover from GBT acquired at various levels of exposure.

Methods

Gas Supersaturated Water Production and Measurement

Gas supersaturated water was produced by using an air compressor to introduce air into a water pipeline. Two different levels of gas supersaturated water were produced by mixing artificially supersaturated water with 102-104% supersaturated spring water (Figure 15). Variations in the ΔP range were controlled by the installation of a constant differential pressure regulator. Six 30-day exposure tests were conducted in nine fiberglass tanks, each subdivided into two equal sections by a a wooden frame covered by plastic screening. An 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. Three tanks received 104% saturated water, three 112%, and three 124%. Daily monitoring of total gas pressure was accomplished using a Weis saturometer for the 104% treatment and Bouck gasometers for the two remaining treatments; these meters were calibrated weekly by taking measurements in a common gas supersaturated environment. Dissolved oxygen was measured using a YSI DO meter, standardized weekly by Winkler's procedure (APHA 1976). Temperature was measured using a mercury or digital thermometer. Total gas measurements were taken daily from one tank of each gas treatment. A rotation schedule was employed to ensure equal distribution of measurements. Daily temperature and dissolved oxygen measurements were taken on both sides of each tank. Barometric pressure was measured daily using a Princo Nova, fortin type mercury barometer.

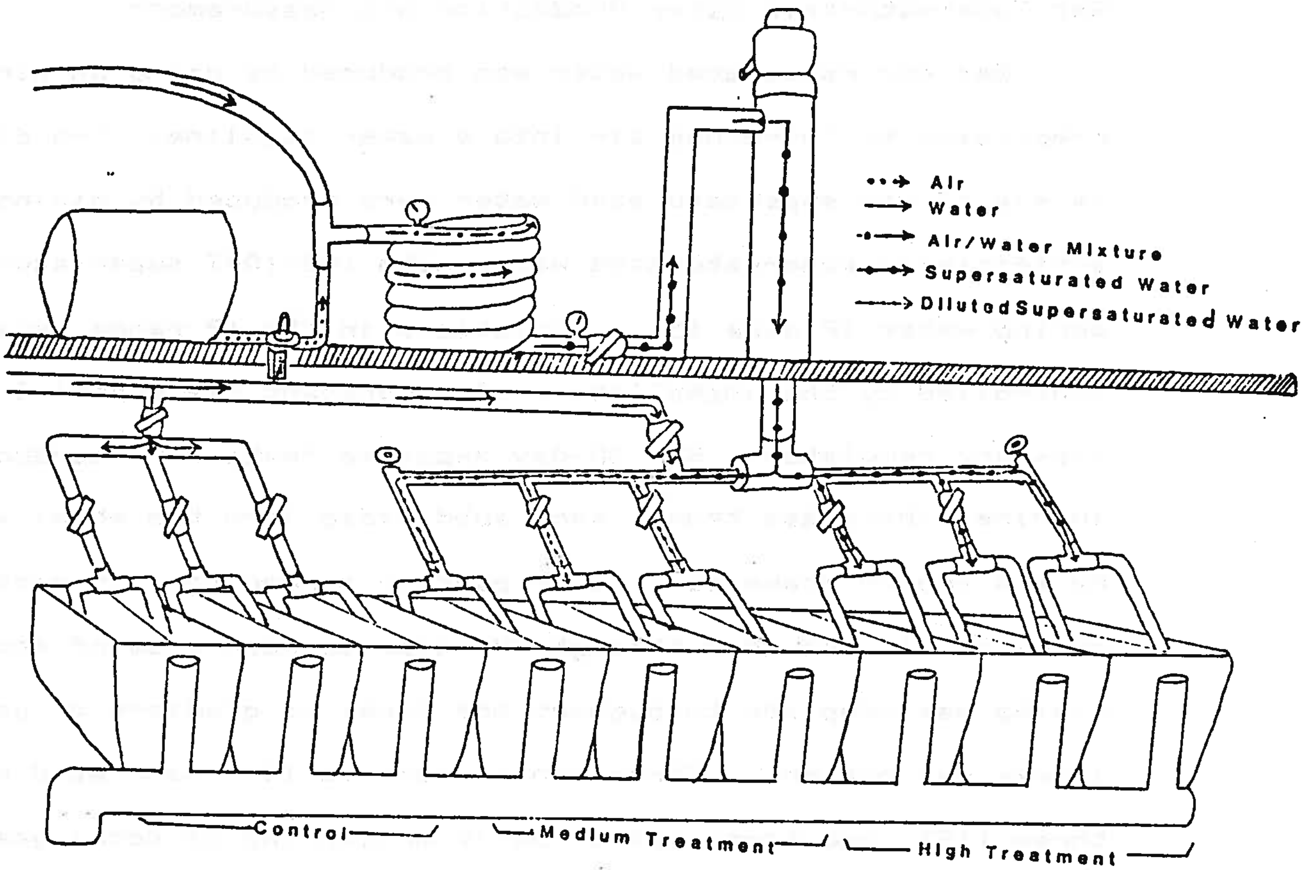

Figure 15. Laboratory apparatus used to test effects of three levels of gas supersaturation on juvenile brown and rainbow trout.

After each 30-day test, values for daily ΔP, DO, water temperature, and barometric pressure were used to calculate the mean 30-day gas levels employing the formulas of Colt (1984).

Fish Care

Brown trout eggs were obtained from wild stock from 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 Betadine and transported to the Bozeman Fish Technology Center where they were incubated in heath trays supplied with spring water (5 gpm at 10±1 C). Eggs were treated 5 days a week with a solution of 1:600 100% formalin. Eggs hatched in approximately 30 days. At the end of January fish were transfered to circular (D=4m) fiberglass tanks supplied with 11-13 gpm of 10±1 C spring water.

Feed rations were calculated on the first day of feeding and every 30 days thereafter using the formula of Piper et al. (1982). Fish were fed a diet of silver cup salmon (Murry Elevators Murry, UT) of appropriate size. Brown trout fry food size and quantity were varied to accommodate the less predictable needs of wild fish. Brown trout food requirements became more predictable as fish size increased. Growth rates of fish were controlled by varying the water temperature and fish were treated with hyamine and terramycin to prevent fin rot and mortality of smaller fish.

Fish Measurement Procedures

Before tests 1 and 2, 25 individuals from the rearing tanks were randomly selected, measured and returned. Fifty individuals of each species for test 1 (25 for test 2) were then randomly selected, weighed, and placed into each experimental tank. During tests 3, 4, and 5, size differences between the species made it necessary to select for specific size groups of fish for use in exposure tests so that fish of both species were approximately the same size. The number of fish used varied by test because as fish grew the number of fish each tank could support decreased. In test 3, 25 fish of each species were used, in test 4, 25 brown trout and 15 rainbow trout were used, and in test 5, 15 brown trout and 10 rainbow trout were used. Screen dividers in each tank prevented mixing of the two species. Each of the experimental groups was treated identically during the tests and mortality was assessed daily. At the completion of each test, surviving fish were measured and weighed. Length and weight data were then applied to the fulton-type condition factor (Nielsen and Johnson 1983) where:

$$\text{Condition} = \frac{\text{Weight}}{\text{Length}^3} \text{ (Arbitrary Scaling Constant)}$$

Data Analysis

The influence of fish length and weight on susceptibility to GBT of juvenile brown and rainbow trout and the comparison of relative sensitivity of juvenile brown and rainbow trout to gas supersaturated water were evaluated. Chi-square analysis (Weis & Hasset 1982) was used to delineate differences in percent cummulative mortality (Appendix B Tables 1 - 17).

Where: Percent Cummulative Mortality =

$$\frac{\text{Mortality at day } x + \text{Mortality at day } x+1 + \text{Mortality at day } x_n}{\text{Fish Number at Day 0}}$$

Sublethal Effects

Sublethal effects of GBT on juvenile brown and rainbow trout were evaluated using a predation susceptibility study similar to that used by Coutant (1973). The experimental procedure is shown in Figure 16. One hour prior to each test, eight predators (four during the first test) were transferred from a 1.8 m circular fiberglass tank (80 cm deep) to three replicate 1.2 m tanks (65 cm deep). Each tank was supplied with a constant flow of water which exited via a single stand-pipe. Predators used were Arlee hatchery rainbow trout ranging in size from 200-270 mm. Fish were trained prior to the tests to feed on salmonid fry and were allowed 1 hour condition time prior to the tests.

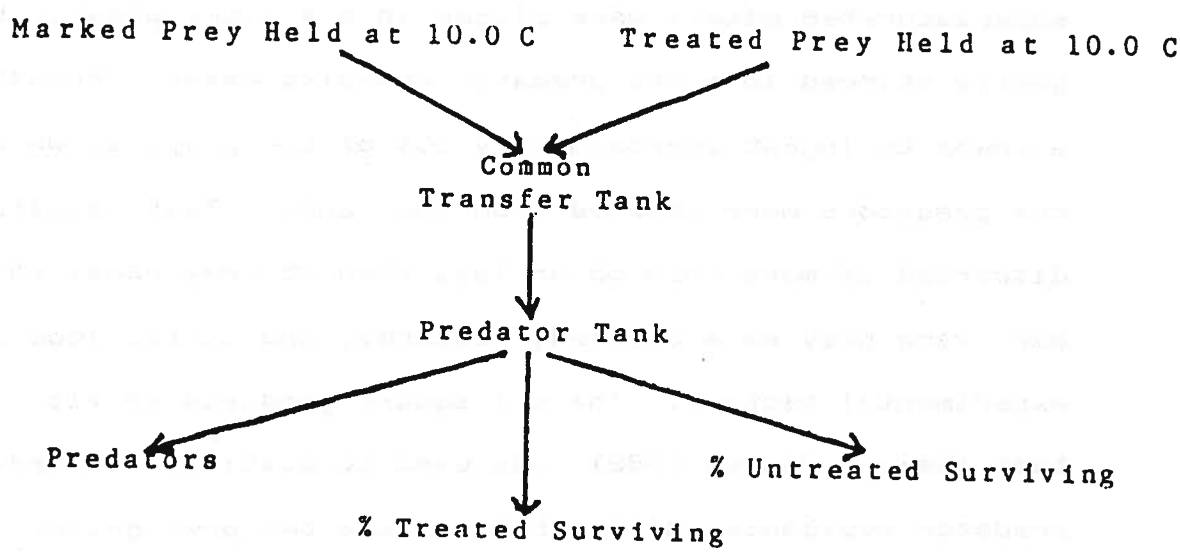

Figure 16. Flow diagram for conducting tests to determine relative vulnerability to predation between fish exposed to gas supersaturated water and unexposed fish.

Different prey fish were used during each test. Rainbow (mean length = 45.7 mm) and brown trout (mean length = 41.5 mm) that had survived exposure to 124% supersaturated water for 30 days were used during the first test. Larger rainbow (mean length = 76.5 mm) and brown trout (mean length = 55.5 mm) that had been exposed for 30 days to 112% supersaturated water or for 13 hours to 130% supersaturated water were used during the second and third tests.

Three days prior to predator avoidance tests, numbers of control fish equal to numbers of experimental fish were adipose clipped. Control fish were from the same cohort, fed the same food, and were reared at the same water temperature, but had not been exposed to supersaturated water.

Prey (25 control and 25 having received previous exposure to supersaturated water) were placed in a 4 litre plastic bowl and gently stirred into the predator occupied water. Predators were allowed to ingest approximately 50% of the prey, at which time the predators were removed from the tanks. Test results were discarded if more than 35 or less than 15 prey remained. Surviving prey were counted, measured, and sorted according to experimental history. The chi-square goodness of fit test (Weis & Hasset 1982), was used to distinguish difference in predator avoidance ability between the two prey groups.

Recovery Tests

A different gas supersaturating system was employed during the recovery studies (Figure 17). A predetermined number of both rainbow and brown trout were placed into two circular tanks and exposed to 115% supersaturated water until signs of GBT appeared. This gas level was chosen because higher levels quickly caused mortality and lower levels induced little visible evidence of GBT. Fish exhibiting signs of GBT (i.e. exopthalmia, loss of equilibrium, etc.) were removed from the treatment tanks, symptoms were recorded, and when possible photographs were taken. Fish were then transferred to 104% supersaturated water and recovery was monitored daily. Results obtained using this procedure and from direct observation of fish behavior during exposure to supersaturated water were used to design a GBT classification system.

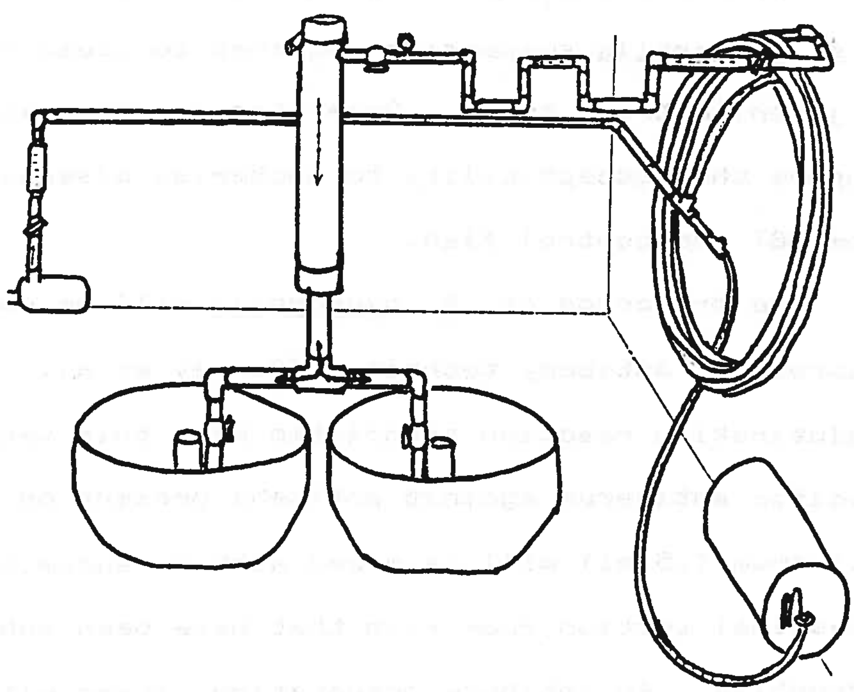

Figure 17. Laboratory appartus used to produce gas bubble trauma in juvenile brown and rainbow trout for recovery tests.

Disease Resistance

An additional experiment is being designed to test effects of GBT on juvenile rainbow and brown trout susceptibility to the pathogen _Aeromonas hydrophila_. _A. hydrophila_ CDC Strain III A-20 was obtained from Wyoming Department of Game and Fish, Laramie, WY. Pathogenicity was increased by passing the bacteria through fish. This was accomplished by injecting 0.5 ml of a saline solution containing at least 1.0×10^5 cells/ml into two 300 mm rainbow trout. After the fish died, kidney and fecal streaks were made on TSA agar and sent to Fort Morgan Fish Disease Control Center to be characterized. Duplicate cultures were streaked on TSA slants, incubated at 28 C until growth occurred, and incubated at 10 C.

Tests are presently being completed to determine the dose of an _A. hydrophila_ suspension required to cause 50% mortality in juvenile brown trout. Once this is determined, we will compare the susceptibility to bacterial disease of fish suffering from GBT and control fish.

The prescence of _A. hydrophila_ will be confirmed using the fluorescent antibody technique (Garvey et al. 1977). The agglutination reaction associated with this technique requires specific antiserum against antigens present on _A. hydrophila_. Antiserum (.5 ml) will be mixed with a random kidney or intestinal section from fish that have been subjected to _A. hydrophila_. An antibody preparation marked with a flourescent tag specific for the _A. hydrophila_ antibody in rabbit antiserum will be applied to this mixture. A flourescent antibody technique microscope will be used to detect tagged bacteria. Results from this test will be evaluated for fish with GBT and control fish.

Preparation of the antigen was completed using the method of Garvey et al. (1977). Five hundred milliliters of tripticate soy broth was inoculated with broth culture of A. hydrophila that had incubated at 28 C for 18 hours. At the end of the incubation period, an equal volume of 0.6% formalinized saline was added. This preparation was incubated at room temperature for several days. Sterility of the bacterial suspension was checked by inoculating a plate of Triptic Soy Agar, incubating for 48 hours at 28 C, and examining the plate for growth.

The bacteria were collected using centrifugation at approximately 3000 rpm for 1/2 hour. The supernatant fluid was discarded and the bacteria resuspended in 100 ml of 0.85 saline. At 4-day intervals, a 2.5 kg rabbit was intravenously injected with 0.5, 1.0, 2.0, and 3.0 ml of of the A. hydrophila antigen preparation diluted 50% with physiolgical saline. The rabbit was rested for 6 days after the last injection.
A small serum sample was obtained by venous puncture and its titer was tested by the agglutination reaction. Specificity of the antiserum was confirmed by testing it against other ubiquitous aquatic microbes. The rabbit is currently being bled +at 3 week intervals and the antiserum is being preserved with 0.2% sodium azide and refrigerated.

Results and Discussion

Saturation Levels

Gas levels fluctuated during tests 1, 2, and 3 (Figures 18, 19, and 20), but mean saturation was maintained near the desired levels. Greater fluctuation in ΔP values exposure test 2 and 3 (Figures 19 and 20) were due to a variety of problems includi[ng] difficulties with the metering device and with the measuring instruments. These problems were eventually overcome through modifications of the equipment. More stable gas levels were achieved by installing a a constant differential pressure regulator on the air compressor. Fluctuations of ΔP for test 4 were much reduced (Figure 21).

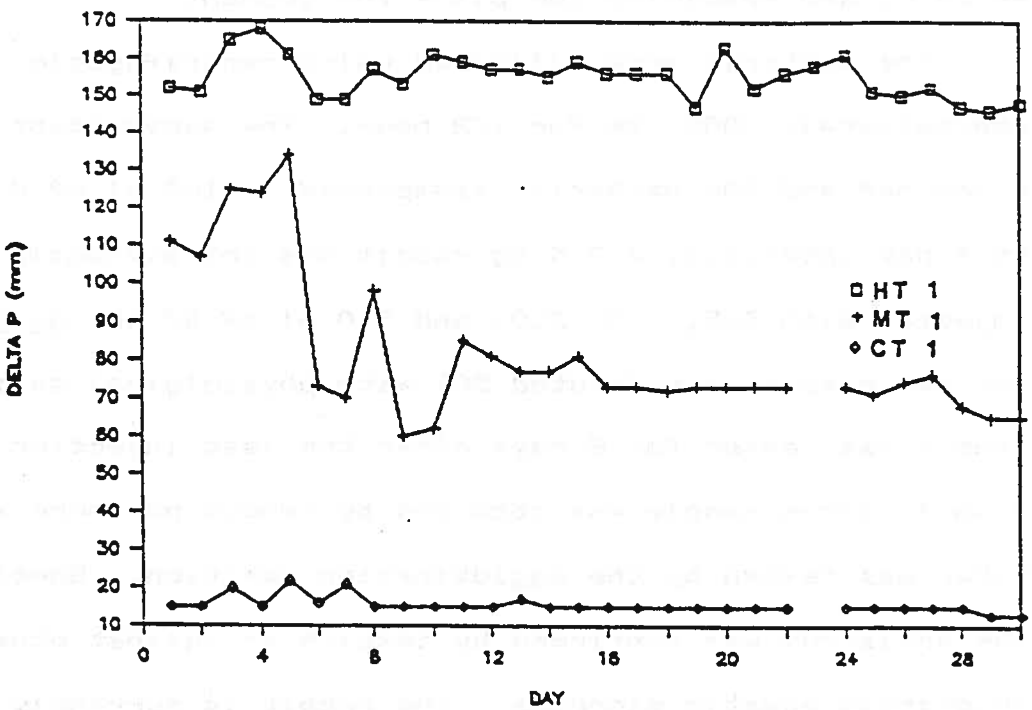

Figure 18. Delta P of water for the high (HT1; $\bar{X}$ ΔP = 155.0 mm and $\bar{X}$% Total Saturation = 124.3), medium (MT1; $\bar{X}$ ΔP = 81.6 mm and $\bar{X}$% Total Saturation = 112.8), and control treatments (CT1; $\bar{X}$ ΔP = 15.6 mm and X % Total Saturation = 102.5) test 1 March 15 - April 15, 1986.

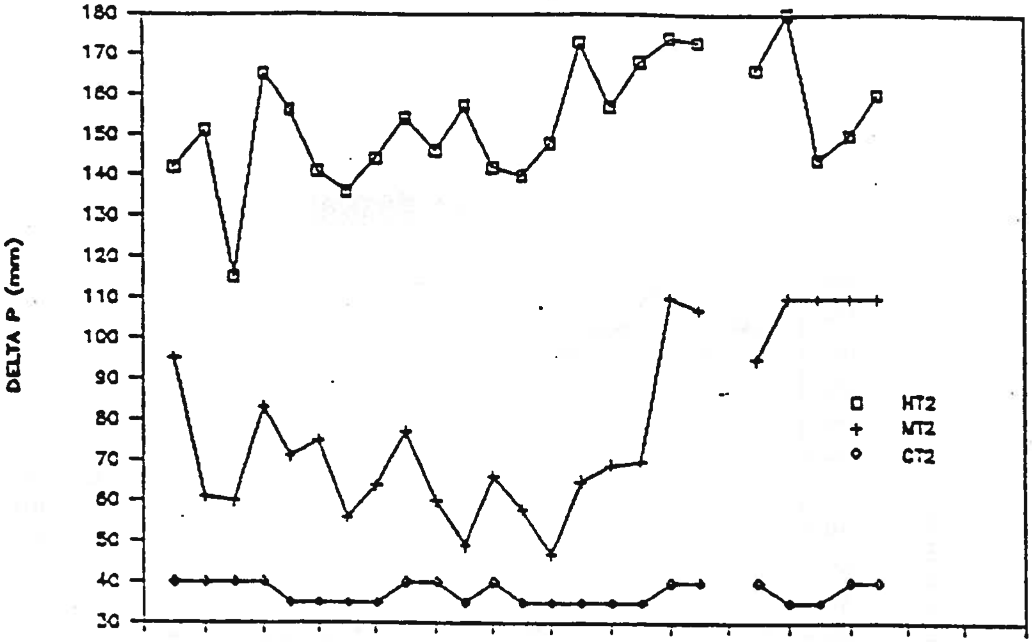

Figure 19. Delta P of water for the high (HT2; $\bar{X}$ ΔP = 153.4 mm and $\bar{X}$ % Total Saturation = 124.0), medium (MT2; $\bar{X}$ ΔP = 78.3 mm and $\bar{X}$ % Total Saturation = 112.3), and control treatments (CT2; ΔP = 37.5 mm and $\bar{X}$ % Total Saturation = 105.9) test 2 May 18 - June 11, 1986.

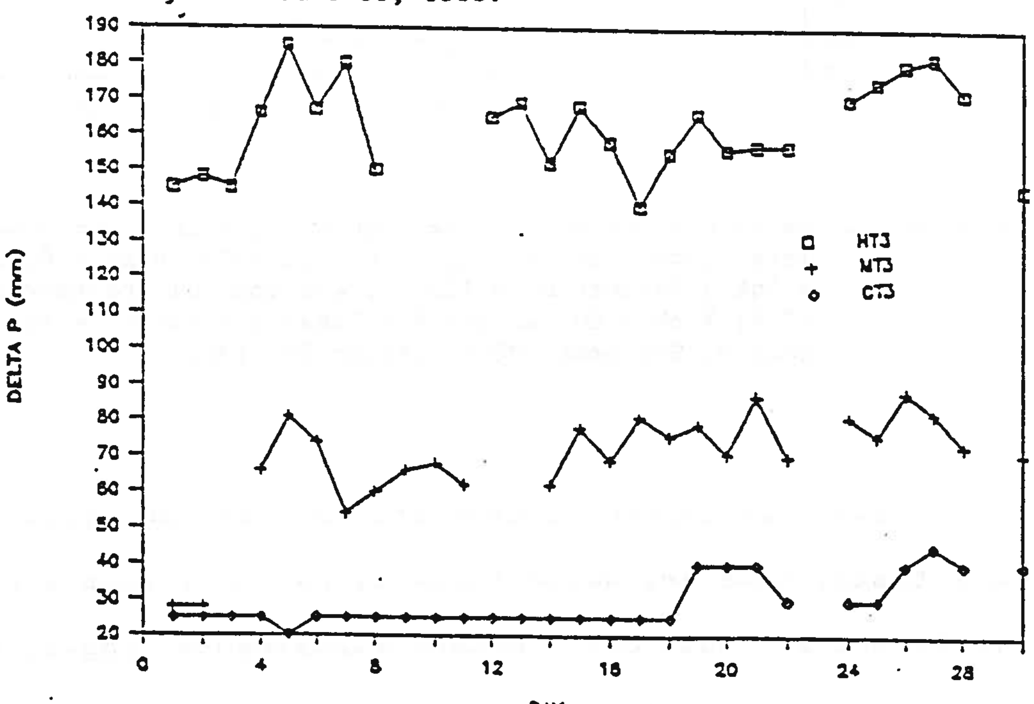

Figure 20. Delta P of water for the high (HT3; $\bar{X}$ ΔP = 162.1 mm and $\bar{X}$ % Total Saturation = 125.4 medium (MT3; X ΔP = 69.2 mm and $\bar{X}$ % Total Saturation = 110.8), and control treatments (CT3; $\bar{X}$ ΔP = 29.3 mm and $\bar{X}$ % Total Saturation = 104.5), test 3, July 17 - August 17, 1986.

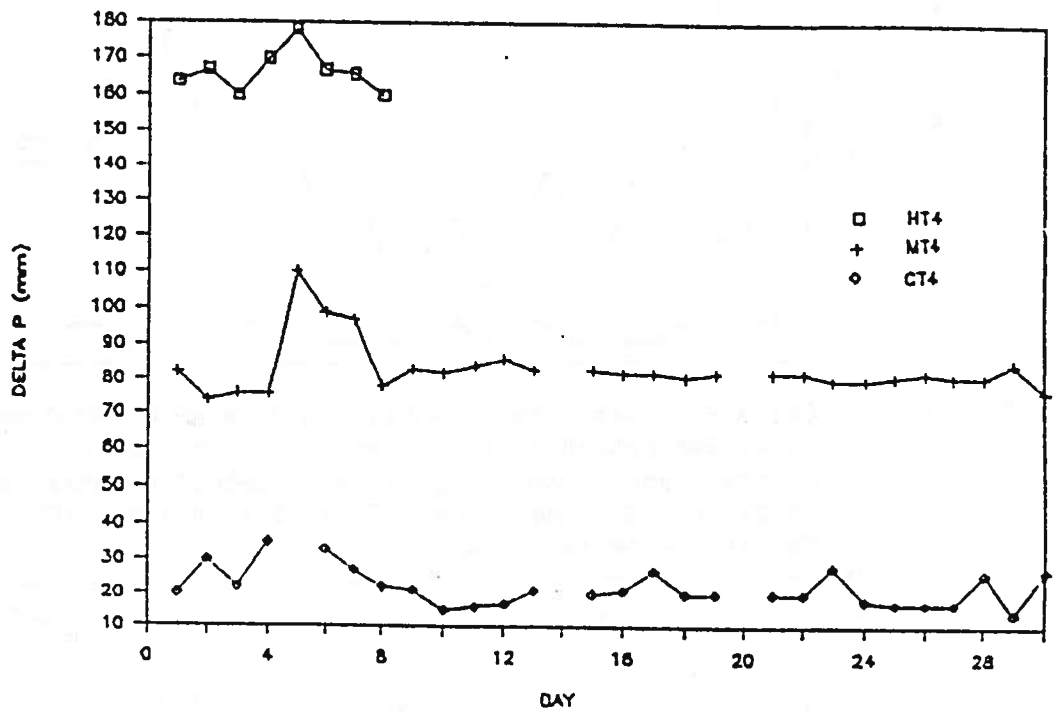

Figure 21. Delta P of water for the high (HT4; $\bar{X}$ ΔP = 166.5 mm and $\bar{X}$ % Total Saturation = 126.1), medium (MT4; $\bar{X}$ ΔP = 83.3 mm and $\bar{X}$ % Total Saturation = 113.0), and control treatments (CT4; $\bar{X}$ ΔP = 20. mm and $\bar{X}$ % Total Saturation = 103.3), test 4, September 28 - October 29, 1986.

Dissolved oxygen concentrations remained stable throughout all tests; however, water temperatures were stable only for tests 1, 2, and 3 (Table 28). Recent assessments suggest temperature has no influence ancillary to gas supersaturation (Jenkins et al. 1986).

Table 28. Mean dissolved oxygen concentrations and water temperature during tests 1-4.

Test	Treatment	Dissolved Oxygen (mg/l)	Temperature (C)
1	High	10.5	10.2
2	High	11.4	9.7
3	High	11.3	9.7
4	High	11.6	8.6
1	Medium	9.6	10.2
2	Medium	10.6	9.8
3	Medium	9.9	10.0
4	Medium	10.4	8.1
1	Control	8.9	10.1
2	Control	9.6	10.1
3	Control	9.2	10.0
4	Control	9.4	7.9

Evidence of Gas Bubble Trauma

Photographs were taken to document visible characteristics of GBT in juvenile brown and rainbow trout. Areas of occurrence included regions of the head, opercles, buccal cavity, gastrointestinal tract, gills, heart, liver, kidney, swim bladder, lateral line, occular orbits, eyeballs, and all fins. Exopthalmia became more common as fish size increased. Epidermal emphysema was often the first symptom to appear. Similar results were noted in previous studies (Wietkamp and Katz 1980).

Fish Growth

Three rearing problems were encountered to date. First, Bighorn River brown trout were hesitant to feed on pellets or to feed at a predictable rate. This was overcome by reducing the ration until brown trout consumed all the food they were offered. Secondly, the stress experienced by wild trout kept in a hatchery situatuon increased their susceptitibility to bacterial infection. A gram-negative flagelate identified in September, 1986 caused fin rot and mortality among smaller fish. Periodic treatments with 11.0 ml/hr of hyamine helped control the infection and adding Terramycin to the diet eliminated the problem. Thirdly, the domestic rainbow trout grew faster than the brown trout. The disparity was somewhat reduced by lowering the water temperature of rainbow trout, but there was still a growth difference present. Rainbow trout were heavier and longer than brown trout at the beginning of tests 2, 3, and 4 and at the end of all tests (Tables 29 and 30). We therefore made interspecific comparisons between tests (i.e. test 3 and 4) to determine the effect of size on susceptibility to GBT.

Table 29. Pre- and post-exposure mean lengths of juvenile rainbow and brown trout computed using an aggregate of fish from high, medium, and control gas treatments.

Trout species	Exposure test	Number of fish measured	Day 1 length mm/fish	Number of fish measured	Day 30 length mm/fish
Brown	1	25	35.2	32	41.5
Brown	2	25	53.1	73	55.5
Brown	3	102	62.2	62	62.8
Brown	4	73	90.7	49	93.4
Rainbow	1	25	34.0	51	45.7
Rainbow	2	25	70.5	63	76.5
Rainbow	3	74	89.5	50	97.6
Rainbow	4	39	130.5	38	140.7

Table 30. Percent increase in mean weight of juvenile rainbow and brown trout exposed to high, medium, and control levels of gas supersaturation during tests 1, 2, 3, and 4.

Species	Exposure Test	Gas Treatment	Beginning number	Beginning g/fish	End number	End g/fish	% Increase
Brown	1	High	150	0.3	117	0.4	58.3
Brown	1	Medium	150	0.3	140	0.6	85.3
Brown	1	Control	150	0.3	142	0.6	118.1
Rainbow	1	High	150	0.4	145	0.9	136.6
Rainbow	1	Medium	150	0.4	145	1.0	176.4
Rainbow	1	Control	150	0.4	145	1.1	196.4
Brown	2	High	75	1.3	5	1.4	8.4
Brown	2	Medium	75	1.2	54	1.4	11.6
Brown	2	Control	75	1.2	72	1.5	22.9
Rainbow	2	High	75	3.4	9	3.3	-9.1
Rainbow	2	Medium	75	3.6	57	4.2	16.4
Rainbow	2	Control	75	3.6	75	4.3	19.5
Brown	3	Medium	75	2.9	67	3.0	3.7
Brown	3	Control	75	2.9	73	3.0	5.3
Rainbow	3	Medium	75	8.4	71	8.6	1.7
Rainbow	3	Control	75	8.2	71	8.2	-0.2
Brown	4	Medium	75	8.3	52	8.5	3.6
Brown	4	Control	75	7.9	70	8.6	8.0
Rainbow	4	Medium	45	26.7	38	30.0	13.0
Rainbow	4	Control	45	26.1	45	29.5	13.1

Growth rate of rainbow and brown trout survivors during tests 1 and 2 varied between treatments (Table 30). In all cases control fish gained more weight than medium or high treatment fish; high treatment survivors grew slowest. This trend was not evident for either species during tests 3 or 4. Growth rate data were not calculated for tests 3 and 4 due to the rapid mortality shown by both species.

Fulton-type condition factors were calculated to compare the well-being of high, medium, and control fish (Table 31). In all cases, fish exposed to gas supersaturated water had lower condition factors, indicating poor feeding efficiency, lack of appetite, or another metabolic condition.

Table 31. Fulton-type condition factors for rainbow (RBT) and brown trout (BT) exposed during tests 1 and 2 to high, medium, and control gas treatments.

Species	Treatment	Condition Factor
BT_1	High	0.6
BT_1	Medium	0.8
BT_1	Control	0.9
RBT_1	High	0.9
RBT_1	Medium	1.0
RBT_1	Control	1.1
BT_2	High	0.8
BT_2	Medium	0.8
BT_2	Control	0.9
RBT_2	High	0.7
RBT_2	Medium	0.9
RBT_2	Control	1.0

Length and Weight Influence

Length and weight of juvenile brown and rainbow trout had a significant influence on susceptibility to supersaturated water. In the high gas treatment during test 1, when fish were smaller (Tables 29 and 30), intraspecific percent cummulative mortality (Figures 22 and 23) for both species was significantly less (Table 32) than during test 2 when fish were larger. This pattern continued in tests 3 and 4.

Medium treatment intraspecific mortality trends were not as clearly defined as high treatment trends. There was no difference between the observed mortality of control and medium treatment fish during test 1 (Table 33). In test 2, both rainbow and brown trout experienced significantly higher mortality rates than control fish (Table 32). Approximately 30% of this mortality occurred during a 10-day period when ΔP values were higher than planned (Figure 24). Chi-square tests showed that differences in mortality rates between treatments, even prior to this 10-day period, were statistically significant. This was not the case during test 4 where 100% of the mortality incurred by both species coincided with a 3 day period of uncontrolled high ΔP values (Figure 25). Mortality during test 3 was significant for brown trout only (Table 33), but only 2.9% of the mortality occurred during testing. It appears that the finding of statistical significance may be in error due to small mortality sample size.

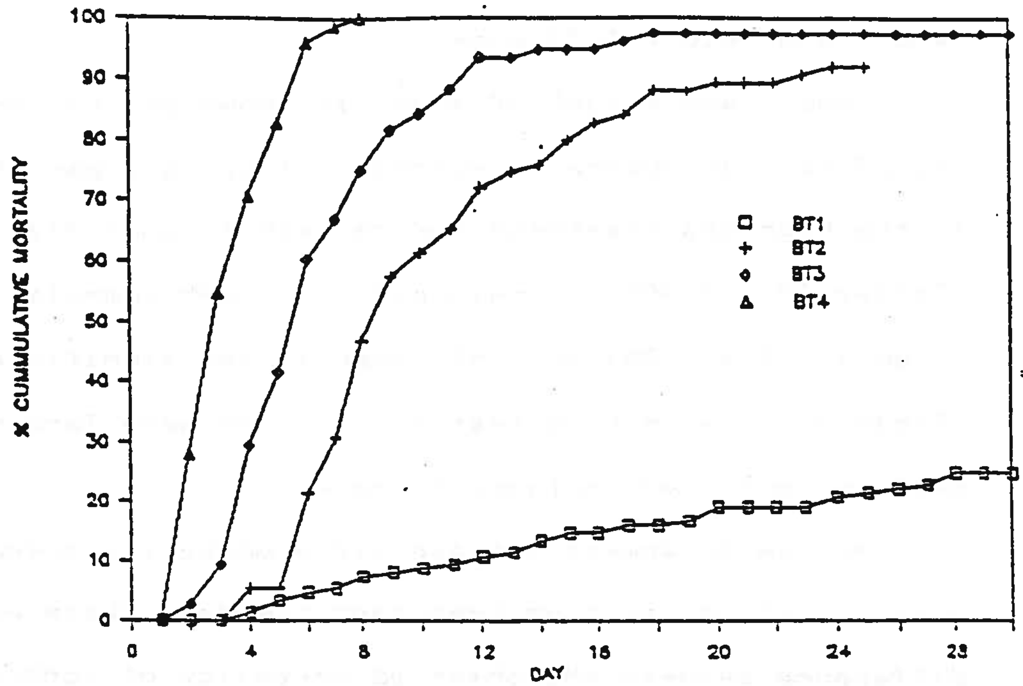

Figure 22. High treatment juvenile brown trout (BT1 = 35.2 mm, BT2 = 53.1 mm, BT3 = 62.2 mm, BT4 = 90.7 mm) percent cummulative mortality by day, during tests 1,2,3, and 4.

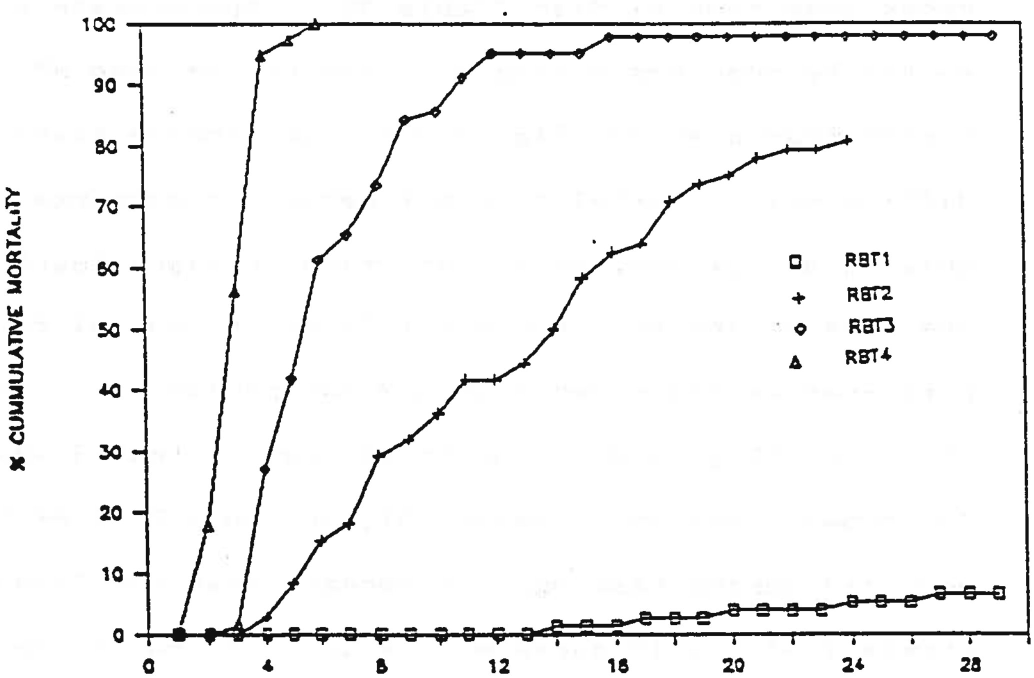

Figure 23. High treatment juvenile rainbow trout (RBT1 = 34.0 mm, RBT2 = 70.5 mm, RBT3 = 89.5 mm, RBT4 =130.5 mm) percent cummulative mortality by day, during tests 1,2,3, and 4.

Table 32. Chi-square high treatment mortality comparisons for juvenile brown and rainbow trout during tests 1,2 3, and 4.

Mortality Comparison	Conclusion (α= 0.05)
BT_1 vs BT_2	Significant Difference
BT_2 vs BT_3	Significant Difference
BT_3 vs BT_4	Significant Difference
RBT1 vs RBT2	Significant Difference
RBT_2 vs RBT_3	Significant Difference
RBT_3 vs RBT_4	Significant Difference
BT_1 vs RBT_1	Significant Difference
BT_3 vs RBT_2	Significant Difference
BT_4 vs RBT_3	Significant Difference

Table 33. Chi-square medium treatment comparisons for juvenile brown and rainbow trout (CT = control trout and MT = medium treatment trout) during tests 1,2,3, and 4.

Mortality Comparison			
Control		Medium treatment	Conclusion (α = 0.05)
CBT_1	vs	MBT_1	No Significant Difference
CBT_2	vs	MBT_2	Significant Difference
CBT_3	vs	MBT_3	Significant Difference
CBT_4	vs	MBT_4	Significant Difference*
$CRBT_1$	vs	$MRBT_1$	No Significant Difference
$CRBT_2$	vs	$MRBT_2$	Significant Difference
$CRBT_3$	vs	$MRBT_3$	No Significant Difference
$CRBT_4$	vs	$MRBT_4$	Significant Difference*

* - Eliminating the mortality incurred during a 3 day period of high ΔP values would lower the test value and there would not be a significant difference between the control and the medium gas treatment mortality.

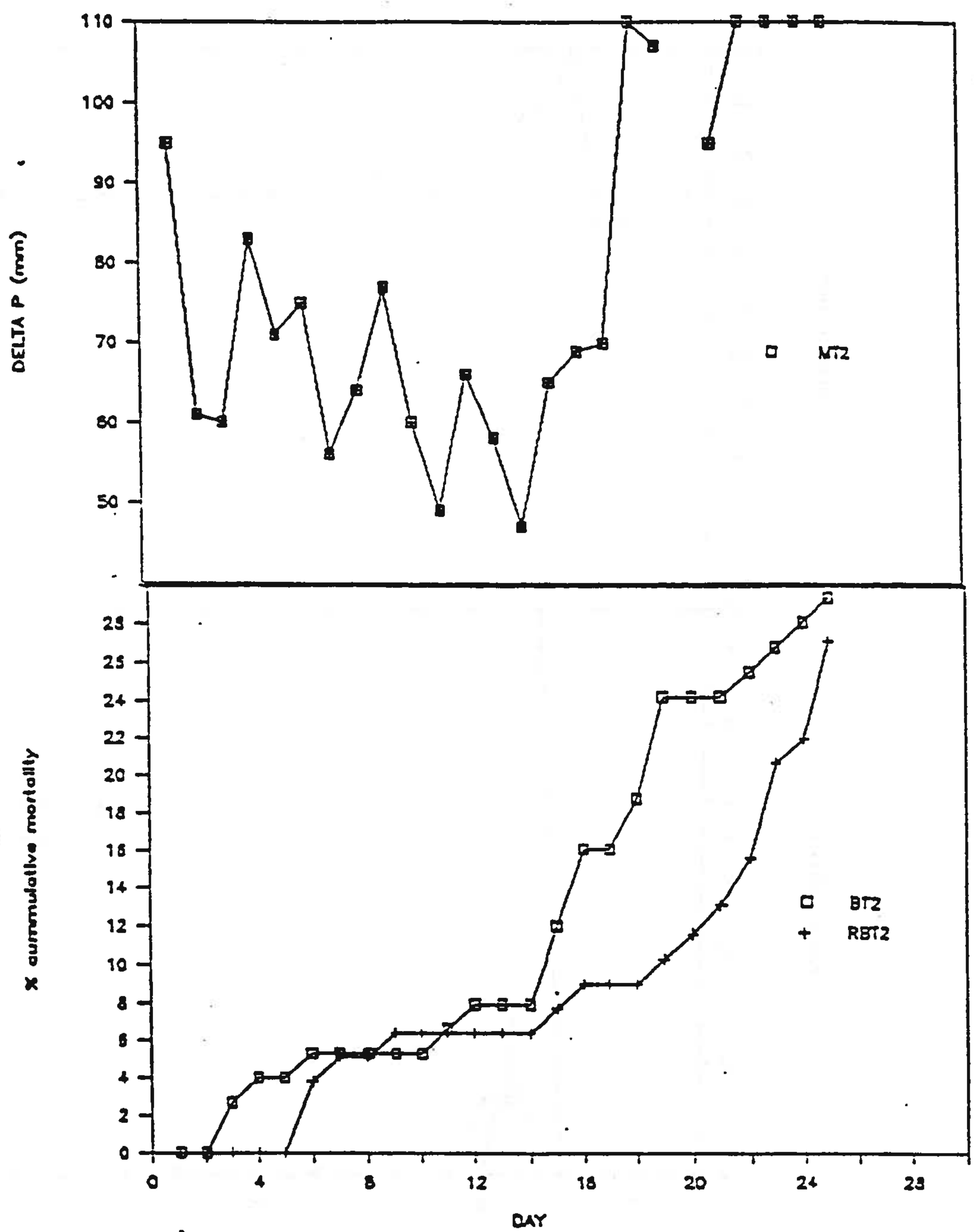

Figure 24. Comparison of medium treatment juvenile brown and rainbow trout percent cummulative mortality and associated ΔP values by day during test 2, May 18 - June 11, 1986.

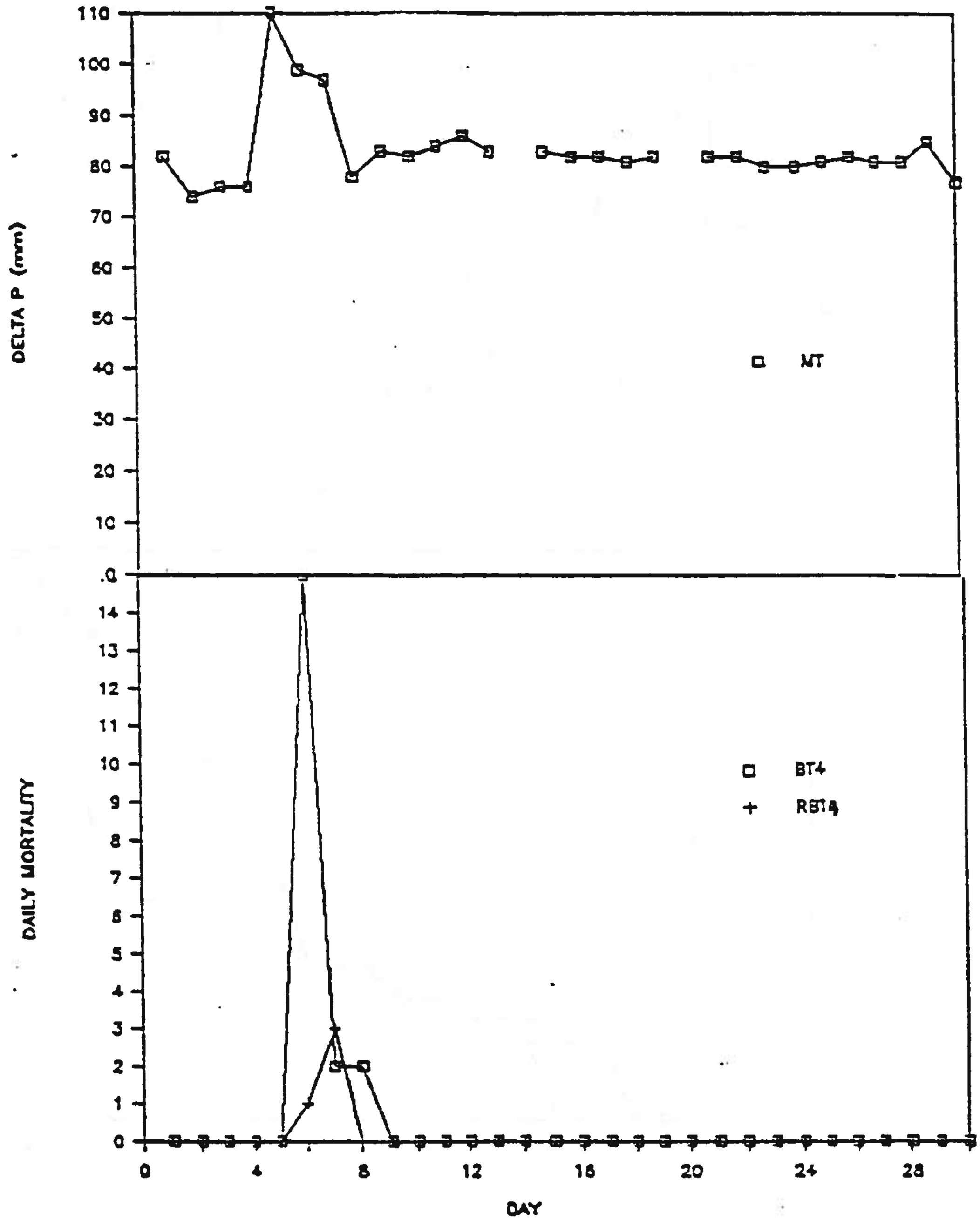

Figure 25. Comparison of medium treatment juvenile brown and rainbow trout percent cummulative mortality and associated ΔP values by day during test 4, September 28 - October 29, 1986.

Daily mortality of brown trout exposed to high gas levels (124%) was significantly greater than for rainbow trout during all comparable tests (Table 32). However, rainbow trout also experienced high mortality (Figures 26, 27, and 28). The difference in susceptibility was most evident during the earliest life stage tested (Figure 26). Differences in percent cummulative mortality between brown and rainbow trout were less in subsequent tests when fish were larger (Figures 27 and 28). This suggests that rainbow trout are less sensitive to gas supersaturated water than brown trout, but that the difference diminshes as as fish size increases.

Medium treatment interspecific mortality data were not analyzed for this report due to the complexity of the results. It appears that the threshold for inducing chronic or acute mortality is approximately 114% (ΔP = 90 mm, BP = 640.0 mm), (Figures 24 and 25).

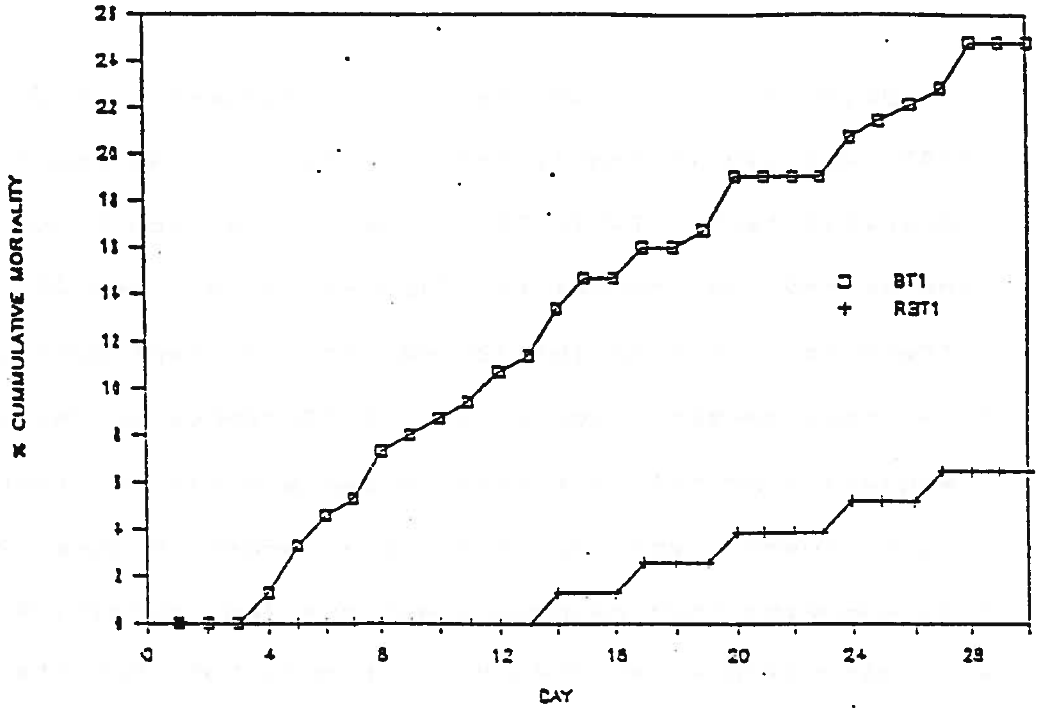

Figure 26. High treatment juvenile brown ($\bar{X}$ beginning length = 35.2 mm) and rainbow trout ($\bar{X}$ beginning length = 34.0 mm) percent cummulative mortality by day during test 1, March 15 - April 15, 1986.

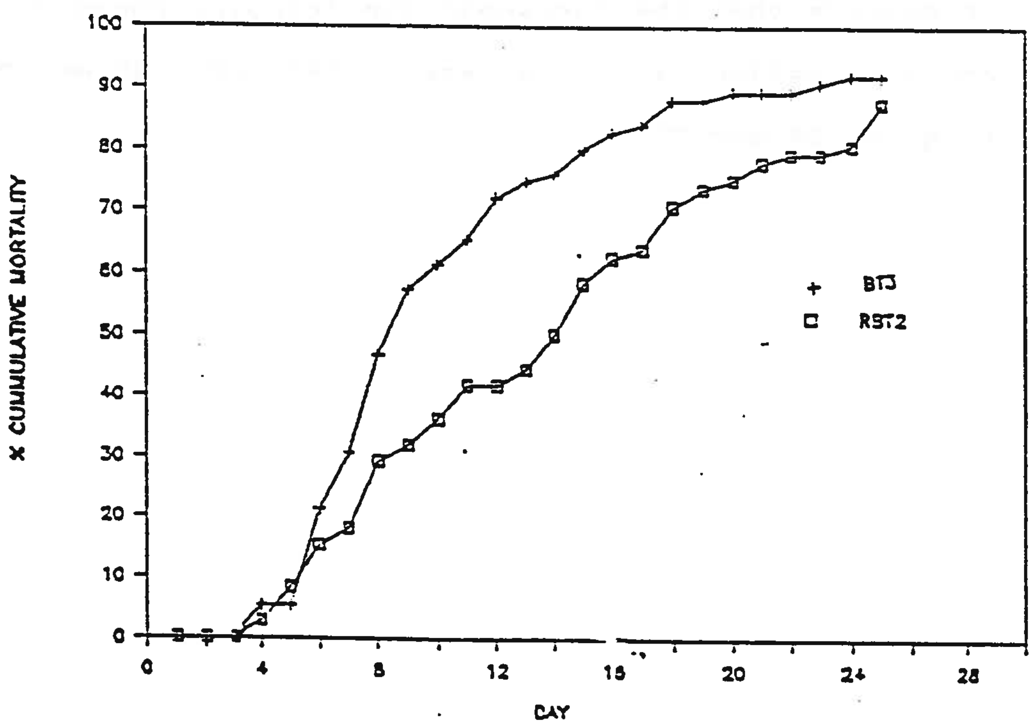

Figure 27. High treatment juvenile brown ($\bar{X}$ beginning length = 62.2 mm) and rainbow trout ($\bar{X}$ beginning length = 70.5 mm) percent cummulative mortality by day during tests 2 and 3.

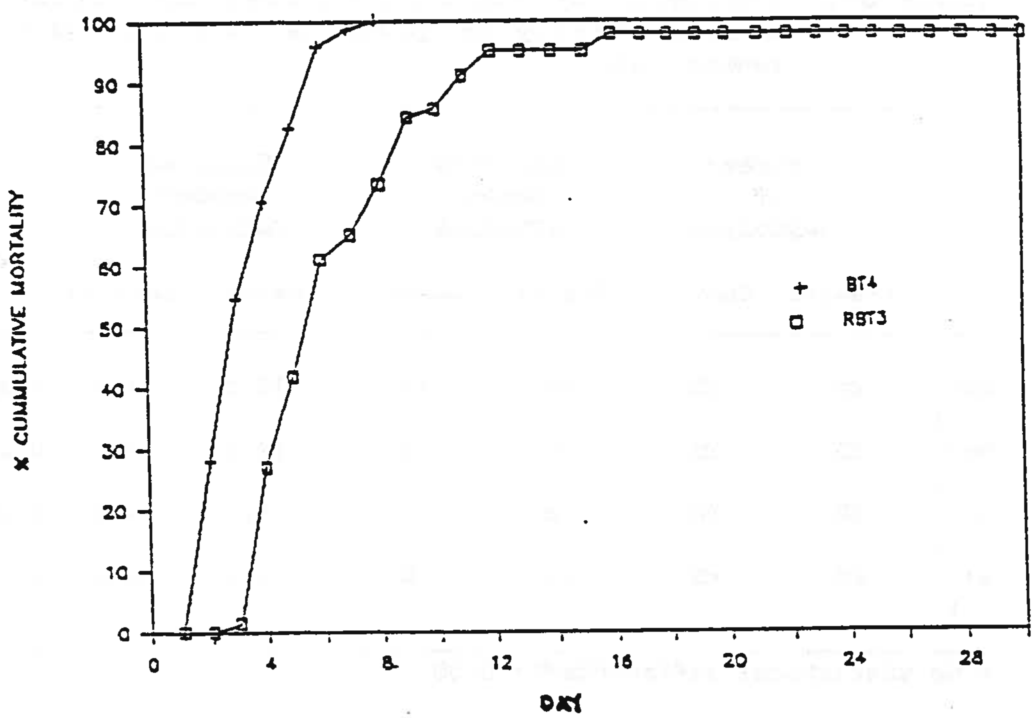

Figure 28. High treatment juvenile brown ($\bar{X}$ beginning length = 90.7 mm) and rainbow trout ($\bar{X}$ beginning length = 89.5 mm) percent cummulative mortality by day during tests 3 and 4.

Sublethal Effects on Predator Avoidance

Under laboratory conditions we found no statistical difference in the susceptibility of exposed and non-exposed fry of brown or rainbow trout to predation (Tables 34, 35, and 36). Problems with experimental technique during the first test (Table 34) resulted in reduced sample size of brown trout prey. Also the large size of the rainbow trout prey in test 2 made it difficult for predators to consume a suitable number of prey before possible recovery from the effects of GBT. Densities of predators were increased during subsequent tests to avoid this problem (Tables 35 and 36).

Table 34. Influence of exposure to 124% saturation on susceptibility of juvenile rainbow and brown trout to predation.

Test	Number of juveniles Treated	Number of juveniles Control	Observed number surviving Treated	Observed number surviving Control	Expected number surviving Treated	Expected number surviving Control	Test Value x^2	Test Value $\frac{(O-E)^2}{E}$
RBT 1	25	25	10	17	13.5	13.5	0.98	
RBT 2	25	25	16	13	14.5	14.5	0.31	4.17*
RBT 3	25	25	12	5	8.5	8.5	2.88	
BT 1	25	25	14	18	16.0	16.0	0.47	3.84*

* No statistical difference $\alpha = 0.05$

Table 35. Influence of exposure to 112% saturation on susceptibility of juvenile rainbow brown trout to predation.

Test	Number of juveniles Treated	Number of juveniles Control	Observed number surviving Treated	Observed number surviving Control	Expected number surviving Treated	Expected number surviving Control	Test Value x^2	Test Value $\frac{(O-E)^2}{E}$
RBT1	25	25	21	14	17.5	17.5	1.4	1.4*
RBT 2	25	25	14	14	14.0	14.0	0.0	
BT1	25	25	14	15	14.5	14.5	0.0	.183*
BT 2	25	25	10	12	11.0	11.0	0.18	

* No statistical difference at $\alpha = 0.05$

Table 36. Influence of exposure 130% supersaturation for 13 hours on susceptibility of juvenile rainbow and brown trout to predation.

Test	Number of juveniles: Treated	Number of juveniles: Control	Observed number surviving: Treated	Observed number surviving: Control	Expected number surviving: Treated	Expected number surviving: Control	Test Value: x^2	Test Value: $\frac{(O-E)^2}{E}$
1	25	25	12	11	11.5	11.5	.04	
2	25	25	14	8	11.0	11.0	1.60	3.74*
3	25	25	13	15	14.0	14.0	.14	
4	25	25	9	16	12.5	12.5	1.96	

* No statistical difference at $\alpha = 0.05$

Disease Resistance

The bacterial culture sent to the Fish Disease Control Center tested out to be *Aeromonas hydrophila*. Antiserum production is well underway and approximately 80 cc of antiserum has been sampled and preserved. Agglutination reactions tested positve with a titer of approximately 1/175. A titer of this strength is more than adequate for our purposes (Dr. James Cutler, pers. comm.). The antiserum gave a negative reaction when tested against *Aeromonas salmonicida* and *Renibacterium sp.* which indicates that the antiserum is probably specific for *A. hydrophila*.

Recovery Test

The GBT classification system is intended to provide a means by which chances for recovery from laboratory exposure to gas supersaturated water can be predicted. The original concept of a rating system was devised to evaluate the progression or remission of GBT symptoms of floy-marked fish in the Bighorn River (White et al. 1986) The field rating system was designed to allow rapid categorization of fish under the inherent time constraints of field work. However, under laboratory conditions, the need to expedite fish examination is unnecessary. Fish can also be viewed during exposure to gas supersaturated water until and during death. These factors make it feasible to describe fish condition in more detail.

The GBT classification system described in Table 37 is being used to quantify recovery data. A hypothetical example of how the rating system is used follows: after 10 days of exposure to 115% supersaturated water a brown trout is removed from the tank. The fish has emphysema on its pelvic fins, anal fin, caudal fin, exopthalmia of one eye, but there are no signs of occular hemoraging or cloudy coloration on the surface of the eye and no swimming impairment is observed. Fish with the symptoms described above are given a rating of 3. The rating is recorded on a data form and the fish is placed in a recovery tank containing unsupersaturated water. On consecutive days the fish will be re-examined and rescored. This process will be followed until a large sample size of fish is acquired. The data will then be expressed graphically to evaluate recovery.

Table 37. Gas Bubble Trauma description and status system for quantification of laboratory recovery data.

Description and Status	Rating
- No visible external symptoms. Fish which have developed no external symptoms of GBT during exposure to gas supersaturated water or which have lost all previously acquired symptoms during recovery	0
- Emphysema present in 1 - 4 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, dorsal fin, left pelvic fin, right pelvic fin, anal fin, adipose fin, caudal fin, and trunk. Fish may be experiencing minor stress	1
- Emphysema present on more than 4 of the above regions. Fish may be experiencing stress capable of reducing feeding activity	2
- Severe emphysema, exopthalmia in one or both eyes, but no evidence of impaired swimming. Impaired vision combined with a scant appetite may eliminate feeding activity and lead to poor nutritional status	3
- Severe emphysema, exopthalmia in one or both eyes, eyes maybe cloudy or hemoraging, may lack response to visual stimuli, noticeable swimming impairment. Poor nutritional status and energy inefficient swimming may combine to seriously threaten fish survival. Blinded fish physiologically deteriorate (embedded scales and lowered condition factors) and starve	4
- Obvious loss of equilibrium accompanied by convulsions and uncontrolled bursts of speed, emphysema and/or exopthalmia may be present depending on duration of exposure period. Fish in this stage most likely will not survive. In many cases, complete loss of equilibrium and convulsions are followed by death	5

The most important aspect of the GBT classification system is the status rating (Table 37). Laboratory and field findings suggest that fish with ratings 0-3 will survive exposure to gas supersaturated water if removed to unsupersaturated conditions, but fish which are given a ratings of 4 or 5, have a poor long-term chance of survival. Work being done in the laboratory will test these preliminary hypotheses. Ancillary factors such as total gas pressure, water depth, temperature, and fish size may affect mortality and recovery (Jensen et al. 1986). If feasible, these factors will be incorporated into the GBT classification system to increase its predictive ability.

IMPACT OF GAS SUPERSATURATION ON INVERTEBRATE COMMUNITY STRUCTURE

Gas Bubble Trauma has been well documented in invertebrates. Marsh and Gorham (1905) observed its occurrence in American lobsters (*Homarus americanus*), horseshoe crabs (*Limulus* sp.) and bivalve mollusks. Evans and Walder (1969) induced GBT in shrimp (*Crangon crangon*) in the laboratory and Malouff et al. (1972) reported symptoms in oysters (*Crassostrea gigas* and *C. virginiana*), and clams (*Mercenaria mercenaria*) exposed to supersaturated water created by warming.

Information concerning aquatic insects, however, is quite limited. Nebeker (1976) noted body distension and gas bubbles throughout the body fluids of stoneflies at 125% saturation but no mortality occurred. Fickeisen and Montgomery (1975) conducted bioassays on effects of gas supersaturated water on six families (representing four orders) of aquatic insects and reported that *Pteronarcys californica* acquired GBT when exposed to 140% and 132% supersaturation for 10 days.

Although aquatic insects have been shown to be susceptable to GBT, zoobenthic impacts from gas supersaturation in rivers has not been documented. For this reason invertebrate community structure is being examined at two sites on the Bighorn River in an effort to determine gas supersaturation influences on the zoobenthos. The sites were selected to provide a comparison of benthic communities at two levels of gas saturation and represent areas where high and low levels of GBT are occurring in fish.

Methods

Site Selection

To examine the effect of gas supersaturation on invertebrate communities in the Bighorn River, we sampled two physically similar riffles, one near Afterbay Dam (Rkm 2.4; high saturation) and one 14.5 Rkm downstream (lower saturation). Sampling areas were selected after comparing physical characteristics of two upstream and two downstream riffles. These comparisons were made by establishing three transects, perpendicular to the current, at each potential site. Transect locations were chosen so that the upper, mid, and lower portions of each riffle were sampled. Water velocity (0.6 depth) and depth were measured at 1.5 m intervals along each transect using a Marsh-McBurney current meter and a top-setting rod. Substrate analysis was made by placing a modified Hess sampler over substrate at 2 m intervals; the longest axis of each rock, > 6.0 cm , in the upper 10 -15 cm of the stream bed was measured. Subtrate was grouped by size: large gravel, 6.0 - 10.0 cm; small cobble, 10.1 - 14.0 cm; medium cobble, 14.1 - 18.0 cm; and large cobble, 18.1 cm and larger. Percent composition for each substrate group along with mean water velocity and depth were calculated for individual transects, and for the three transects representing a riffle. Sample site selection was based on similarity of physical characteristics.

Sampling

Twenty-five invertebrate sampling sites in each riffle were randomly selected by dividing the riffles into 1 m^2 grids, numbering each grid, and using a computer-generated list of random numbers to choose sites. Benthos were sampled with a modified Hess sampler, 0.085 m^2 in area, which was embedded into the substrate to a depth of 5 - 10 cm. All rocks with periphytic algae were brushed with a soft bristled brush, examined to ensure that all invertebrates were absent, and removed from the sampler. A metal rod was then used to agitate the remaining substrate to a depth of 7 - 10 cm. Samples were preserved in Kahle's solution containing rose bengal dye (to enhance sorting efficiency) and labeled (date, collection site, and sample site) for sorting and identification in the lab. Before each sample was taken, site specific water velocities were taken. Thirteen invertebrate samples were collected from each site on August 21, and the remaining 12 were collected the following day.

Results and Discussion

To date, 25 samples have been sorted but not quantitatively analyzed. Taxonomic diversity at each site appears low. Low diversity is characteristic below deep release impoundments due to increased environmental constancy and lowered temperature regimes (Ward 1976).

Mayflies (Ephemeroptera) are represented almost exclusively by three genera: *Ephemerella*, *Tricorythodes*, and *Baetis*. These collector-gatherers, are apparently favored by the high productivity of the Bighorn River below Afterbay Dam. Most midges (Chironomidae) appear to be in the subfamily Orthocladinae, and the simulids (Simulidae) are represented by the single genus *Simulium*. Caddis fly larvae (Trichoptera) were rare but caddis cases were abundant at both sites, suggesting that these insects had emerged prior to sampling. A single stone fly (Plecoptera) was found in a preliminary sample collected in June 1986, however none have been found in the August collection. Spence and Hynes (1971) reported a general loss of invertebrate predators below dams due to thermal regimes, and that plecopterans are often the taxa most widely affected. Amphipods (primarily *Gammarus*), turbellarians, oligochaetes, and gastropods are relatively abundant in the Bighorn.

Preliminary evaluation indicates a difference in community structure between sites. *Baetis* is the most abundant mayfly at the upper site, although *Ephemerella* and *Tricorythodes* are common. *Ephemerella* and *Tricorythodes* are dominant at the lower site, with *Baetis* being common. Simulids appear more numerous downstream than upstream. Ward (1975) suggested that a hypolimnial release may not provide a sufficiently reliable food source to support large populations of invertebrates dependent exclusively on suspended matter, and reductions in filter-feeders may occur. Williams and Winget (1979), however, found increased abundance of Simulids and filter-feeding Trichoptera below Soldier Creek Dam in the Strawberry River. They attributed the increase to added drift from enriched epilithic algae from the river. Given the extreme productivity of the Bighorn River between the upper and lower sites, the increase in downstream simulids may be a response to a more consistent food source. Another possible explanation may be a response to decreasing gas saturation levels.

Chironomidae and *Gammarus* decreased in abundance downstream. This may be attributable to habitat changes. Variations in the numbers of oligochaetes, turbellarians, and gastropods, if present, are not large enough to be noticeable without quantification and analysis of the data.

PHYSIOLOGY VERSUS HABITAT SELECTION

Brown trout in the Bighorn River have a higher incidence of external symptoms of GBT than observed in rainbow trout. We hypothesized that this could be due to physiological differences between the species or, alternatively, to differnces in habitat preferences.

Physiology studies are being conducted at the University of British Columbia to determine thresholds of total gas pressure (TGP) required to 1) initiate bubble growth in the vascular system, 2) produce over-inflation of the swim bladder, and 3) develop bubbles in the buccal cavity of small fish.

Physiological Studies

In a detailed study of the transport of supersaturated gases from the environmental water to the vascular system of fish and the subsequent development of bubbles in that system, Fidler (1985) developed equations that relate the threshold for bubble growth to a number of physical and physiological parameters. These paramaters include the environmental water total gas pressure, depth, oxygen partial pressure ratio, temperature, barometric pressure, and the physiological parameters of nucleation site size, the surface tension of fish blood, oxygen and nitrogen transport parameters in the blood, vascular system pressure, and gill oxygen uptake ratio. The development of bubbles in the vascular system of fish exposed to supersaturated water has been identified as the principal cause of death at TGP levels above 110% (Weitkamp and Katz 1980). The cause of death is considered to be the stoppage of blood flow caused by bubble blockage (hemostatis), with ensuing hypoxia. The condition is considered to be acute with mortality occurring quickly (Weitkamp and Katz 1980). Using the same analysis technique, a simpler equation was developed for predicting threshold total gas pressures for over-inflation of the swimbladder. The relationship developed relates environmental water oxygen partial pressure ratio, temperature, depth, barometric pressure, O_2 and N_2 blood transport parameters and oxygen uptake ratio across the gill membrane to threshold TGP. The consequence of an over-inflated swimbladder is mainly one of excess buoyancy which must be compensated for by actively swimming in a head down position. The swimming energy required will be related directly to the degree of over-inflation. An important aspect of this work included a study of the pressure in the swimbladder required to force gas out the pneumatic duct. The expulsion of gas through the pneumatic duct would reduce the extent of swimbladder over-inflation and excess buoyancy. The consequences of swimbladder over-inflation in terms of fish survival will be described in more detail below.

Finally, the theoretical analysis was applied to the environmental water itself as a means of examining the growth of bubbles in the buccal cavity of small fish. Jensen (1980) and Shirahata (1966) have reported this type of bubble formation being responsible for mortality in newly emerged salmonid fry. The general observation has been that bubbles form in the mouth and completely fill the buccal cavity stopping respiratory water flow. It is observed only in very small fish and, as suggested by Fidler (1985) is probably related to high surface tension forces on small bubbles which prevent the fish from deforming the bubbles, a condition which is necessary for dislodging them from the mouth.

The results of these initial theoretical studies suggested that fish exposed to supersaturated water would experience very different problems depending on the level of supersaturation and the size of the fish. The analyses indicate that, in general, the TGP threshold required to over-inflate the swimbladder was low (TGP $\geq$ 103%) compared to the TGP threshold required to produce bubbles in the vascular system (TGP = $\geq$ 112%). This implies that swimbladder over-inflation and the associated excess buoyancy would be a larger problem for juvenile fish than for adult fish. As mentioned above, over buoyancy must be corrected for by continuous swimming in a head down position. Since juvenile fish are growing and their swimming performance is less than that of adult fish, their basal energy demands will exceed those of adult fish. Add to this handicap the possibility that feeding and the ability to escape predation may be compromised by excess buoyancy, the chances of survival are reduced even further.

The study of pneumatic duct release pressure suggested further threats to the survival of juvenile fish. Because of their small size and the correspondingly small dimensions of the pneumatic duct, surface tension forces should result in a higher release pressure than for a large fish (Fidler 1985). This suggests that small fish could experience a greater buoyant force per unit body mass than large fish. The effects of surface tension could explain the observation in several hatcheries of ruptured swimbladders in small fish (Jensen 1984). The same observation has been made in experimental studies at the University of British Columbia and at the Fish Technology Center in Bozeman. Except for cases involving swimbladder rupture, the problem of swim-bladder over-inflation is interpreted to be one of chronic rather than acute proportions. Mortalities resulting from this condition could be the result of many indirect factors involving combinations of general swimming stress, disease, predation, inability to feed and other unknown stresses.

The analysis of bubble growth in the buccal cavity of small fish indicates that the TGP threshold for this condition was also low, compared to that for vascular system bubble growth. Although the size of critical nucleation sites in the mouth of small fish was unknown, a size corresponding to that of cellular dimensions yielded thresholds similar to those for swimbladder over-inflation, (TGP $\geq$ 103%).

As described earlier, bubble growth in the vascular system involves a physiological insult more traumatic than swimbladder over-inflation and is interpreted as an acute effect of supersaturation. Although the thresholds required to produce vascular system bubbles are considered to be higher, the ensuing mortality occurs quickly. That being the case, small fish may have an advantage over large fish in that the dimensions of their vascular systems do not allow large nucleation sites on which bubbles can grow (Fidler 1985).

The analysis of bubble growth in the vascular system involves physiological parameters that are difficult to measure directly and the form of the resulting equations is necessarily more complex than that for swimbladder over-inflation or buccal cavity bubble formation. However, an indirect experimental means of evaluating these parameters was developed and is currently being pursued. Once the parameters have been evaluated, the equations can be applied to any aquatic environment to predict thresholds required to initiate vascular system bubble growth.

In the following discussion, the predictive equations applicable to swimbladder over-inflation, vascular system bubble formation and buccal cavity bubble formation will be described along with the experimental work being performed at UBC. Also described will be the manner in which the results can be applied to the Bighorn River.

Results and Discussion

<u>Swimbladder</u> <u>Over-Inflation</u>: Equation 1 describes the mathematical relationship between the major parameters that cause swimbladder over-inflation (see Fidler 1985).

$$TGP_{sb} = [F*X'O(1 - K) + K]*P_E / [X'_o(F - K) + K] \quad \text{Eq. 1}$$

where: TGP_{sb} = total gas pressure at which swimbladder over-inflation begins.
F = oxygen uptake ratio across gill membrane.
X'O = oxygen partial pressure ratio in environmental water, PO_2/TGP.
K = transport ratios = $H_N D_N / H_O D_O$,

H_N = Henrys constant for nitrogen in fish blood.
H_O = Henrys constant for oxygen in fish blood.
D_N = diffusivity of nitrogen in fish blood.
D_O = diffusivity of oxygen in fish blood.
$P_E = P_A + P_H$
P_A = atmospheric pressure.
P_H = hydrostatic pressure.

From Fidler (1985), the value of K is approximately 0.4 over a range of water temperatures from 0 to 12 C. Based on work currently being conducted at UBC, the oxygen uptake ratio across the gill membrane for resting Rainbow trout is approximately 0.67. Using these values, Equation 1 can be written as:

$$TGP_{sa} = (P_E - .675*P\text{-}O_2)/2$$

$$+ [SQRT((.675*P\text{-}O_2 - {}_{P}E)2 + 4.02*P\text{-}O_2*P_E)]/2 \quad \text{Eq. 2}$$

Using Equation 2 with an atmospheric pressure of 685 mm Hg (typical or the altitude of the Bighorn River), Figure 29 shows the threshold TGP for swimbladder over-inflation for a range of water depths and water dissolved oxygen partial pressure.

In terms of the Bighorn River, Equation 2 can be applied directly since TGP, $P\text{-}O_2$ and barometric pressure are known from satellite data. For example, using data for Rkm 4.8 from 17 September 1986 (Figures 30 through 32), where the barometric pressure is 685 mm Hg, TGP is 805 mm Hg and $P\text{-}O_2$ is 210 mm Hg; all fish below a depth of 0.95 m will not experience swimbladder over-inflation while all those above that depth will experience some form of excess buoyancy (see Figure 29). In other words, fish in locations at Rkm 4.8, where the depth is less than 0.95 m will be exposed to a condition of swimbladder over-inflation.

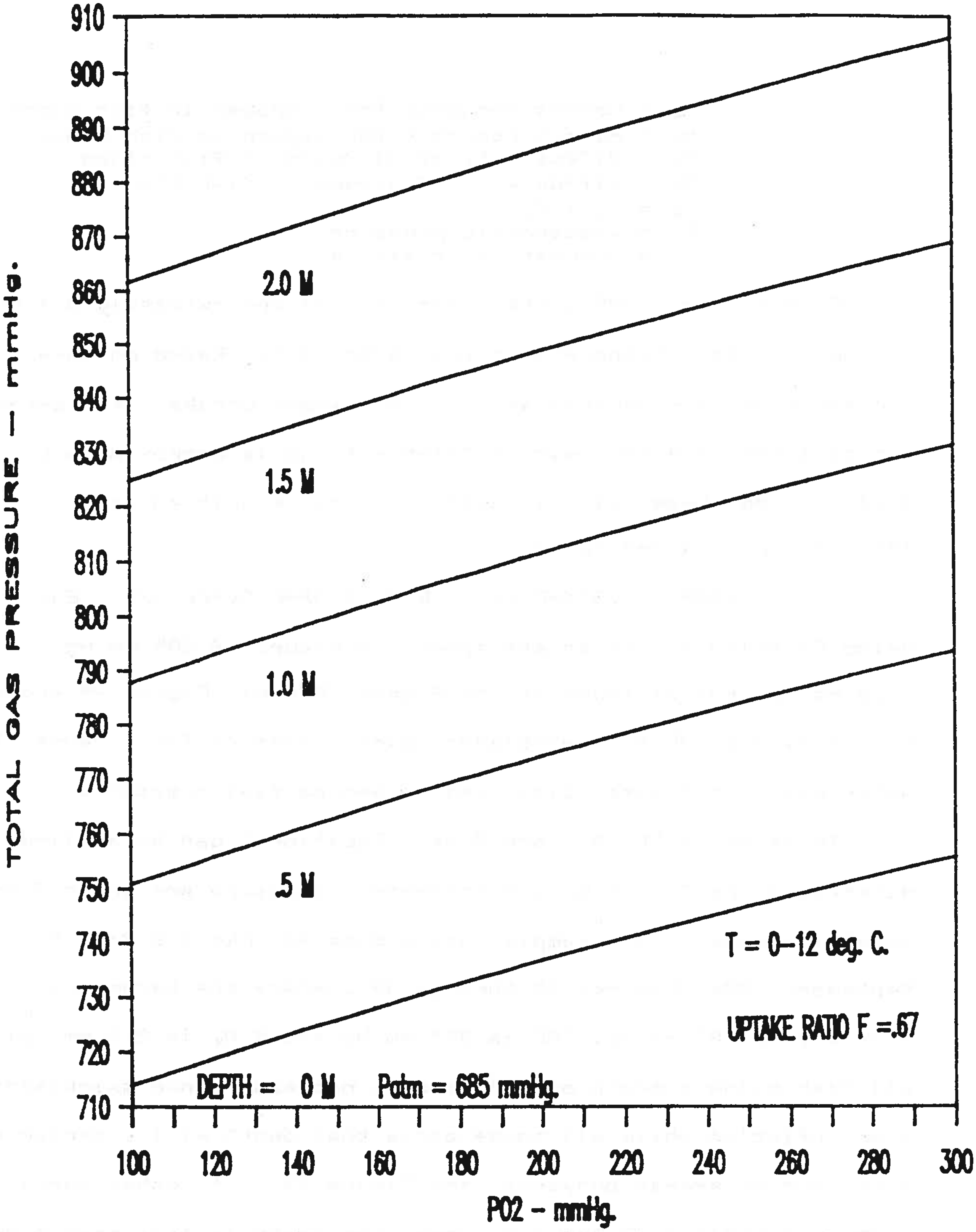

Figure 29. Predicted total gas pressure thresholds for swimbladder over-inflation as a function of water depth and dissolved oxygen partial pressure.

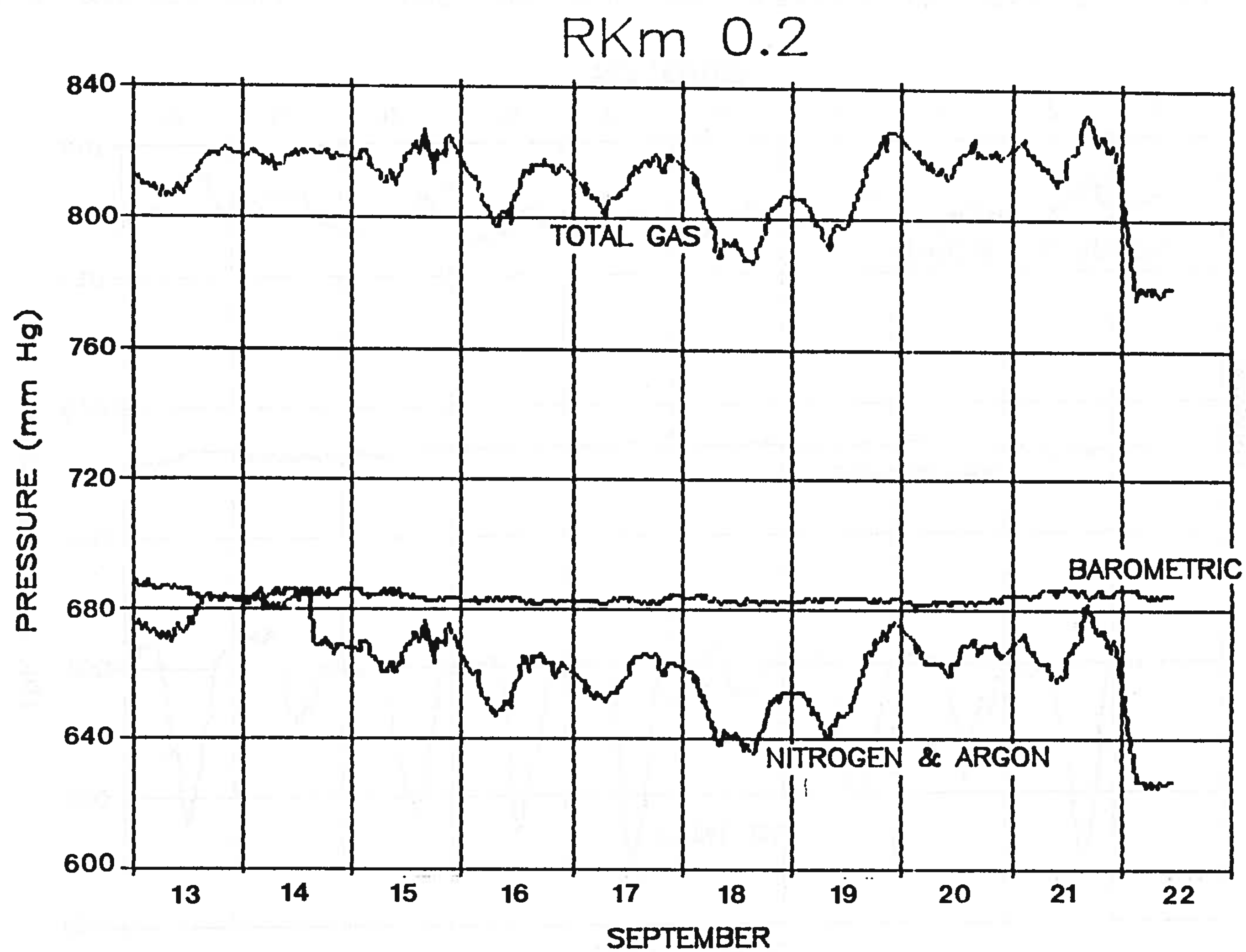

Figure 30. Total gas pressure, nitrogen and argon gas pressure and barometric pressure at Rkm 0.2, 13-22 September 1986.

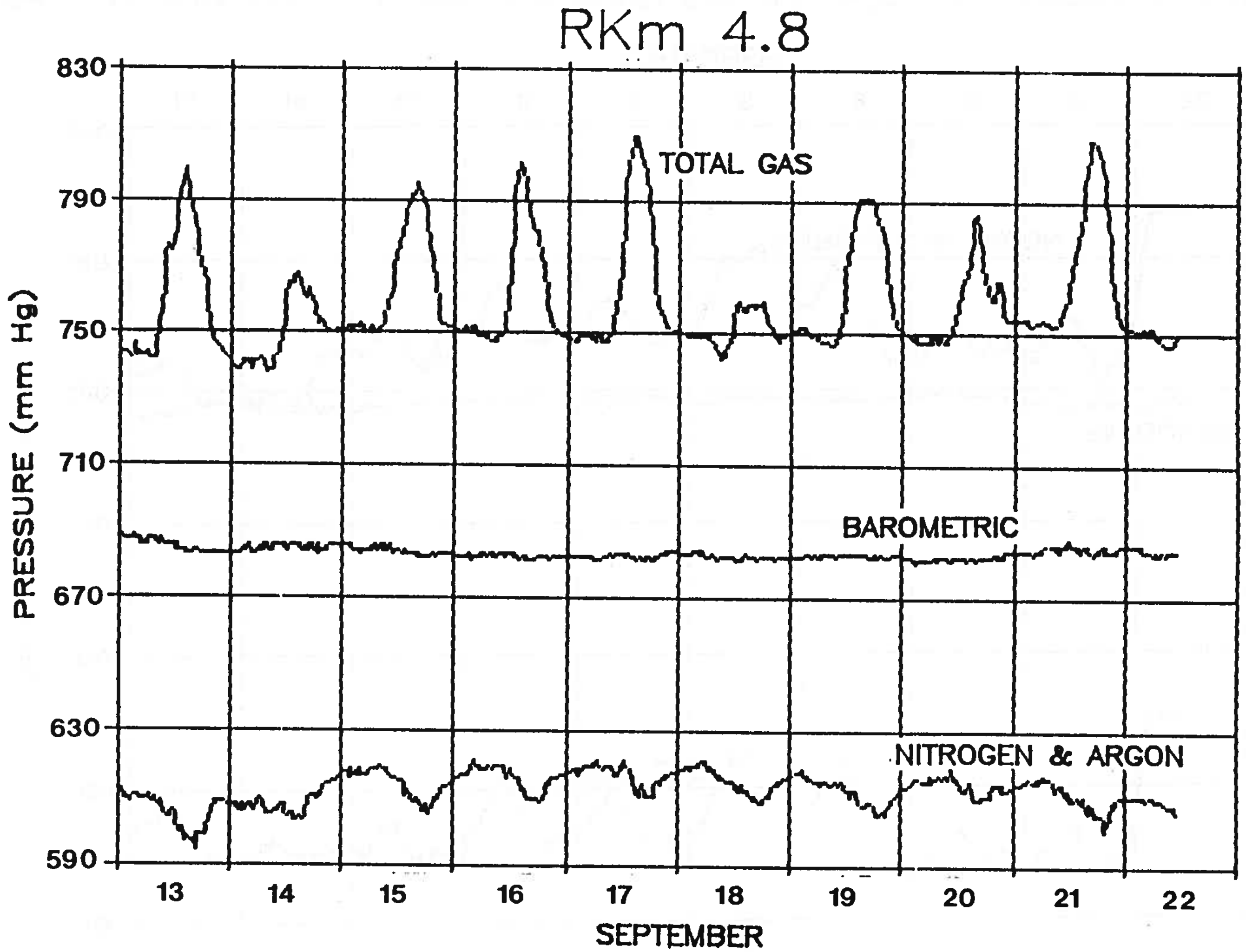

Figure 31. Total gas pressure, nitrogen and argon gas pressure and barometric pressure at Rkm 4.8, 13-22 September 1986.

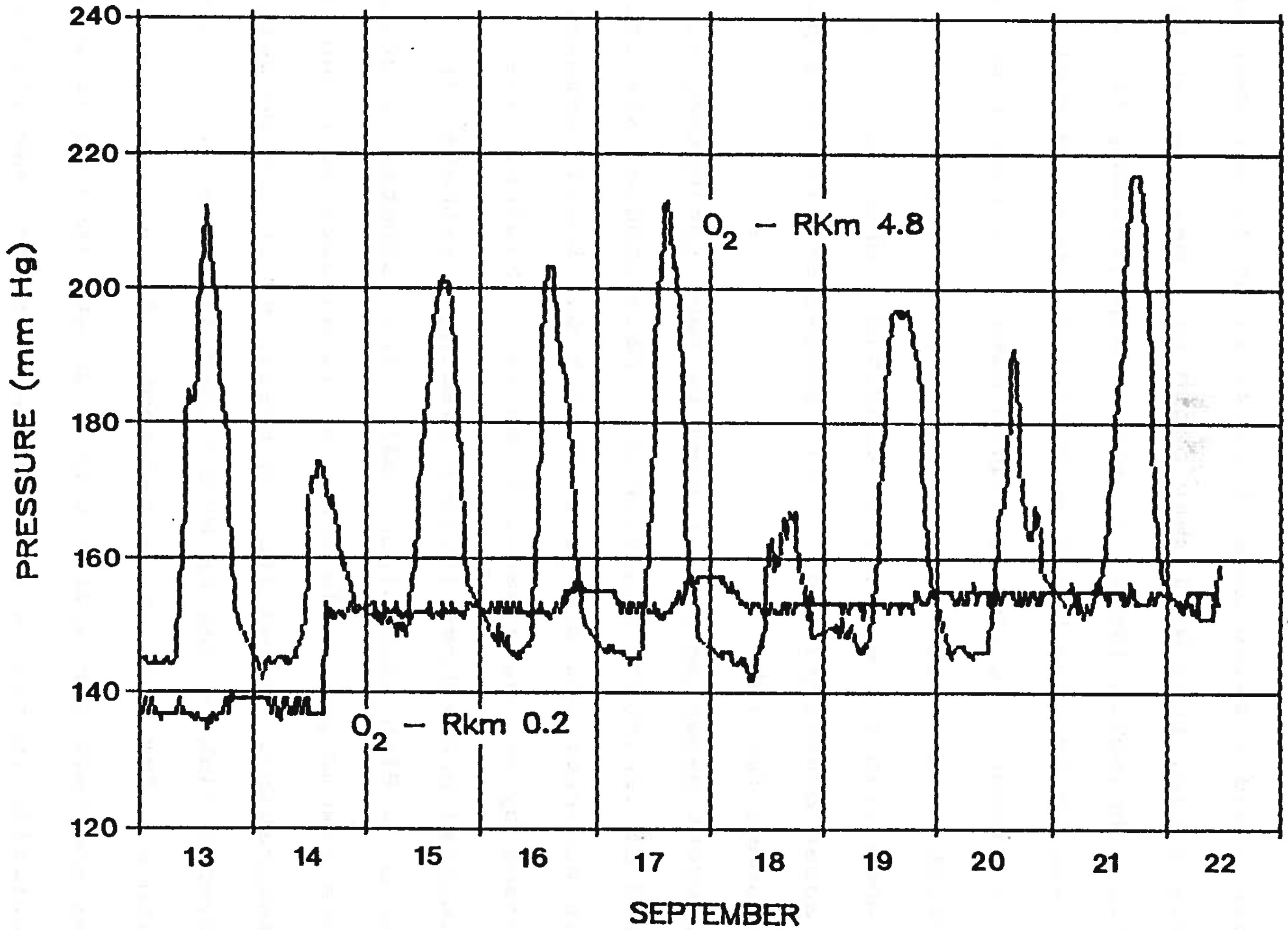

Figure 32. Comparison of dissolved oxygen pressure at Rkm 0.2 and Rkm 4.8, 13-22 September 1986.

More importantly, if at least 0.95 m of water depth is present, fish can avoid a swimbladder problem since it has been shown that when available, they will seek depth as a means of avoiding over buoyancy (Shrimpton 1984). In actual application, it is more convenient to program Equation 2 into a Lotus 123 worksheet and apply it directly to the satellite data on a continuous basis. With depth information on the river, predictions can then be made as to when fish are exposed to conditions which will lead to swimbladder over-inflation. This procedure will be a part of the work planned for 1987.

Current experimental studies by Mark Shrimpton, at UBC, are directed at verifying Equation 2. These studies are independent of this contract and are part of an M.Sc. thesis program being supervised by Dr. Dave Randall and Larry Fidler. The experimental procedures involve placing a catheter in the swim bladder of a fish (see Figure 33). The catheter is attached to a pressure transducer while the fish is exposed to known levels of supersaturation. By monitoring pressure, it can be determined when over-inflation begins which then allows a direct check of Equation 2. The complete experimental system used for these studies and vascular system bubble growth studies is shown schematically in Figure 34. The swimbladder experimental studies are expected to be completed in 1987.

In other experimental work, Shrimpton is examining the pressure at which gas in the swimbladder vents through the pneumatic duct. As already discussed, Fidler (1985) suggested that this pressure would increase as the size of the fish

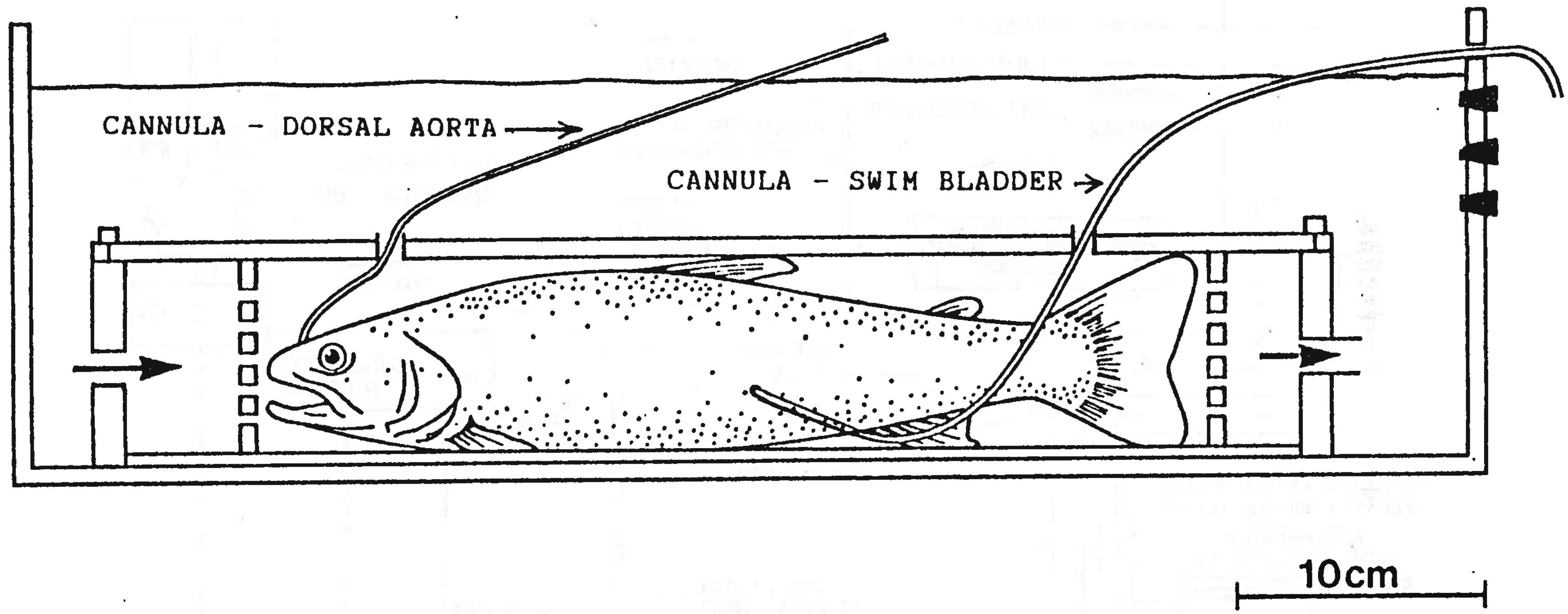

Figure 33. Schematic of experimental container and placement of swimbladder and dorsal aorta cannula.

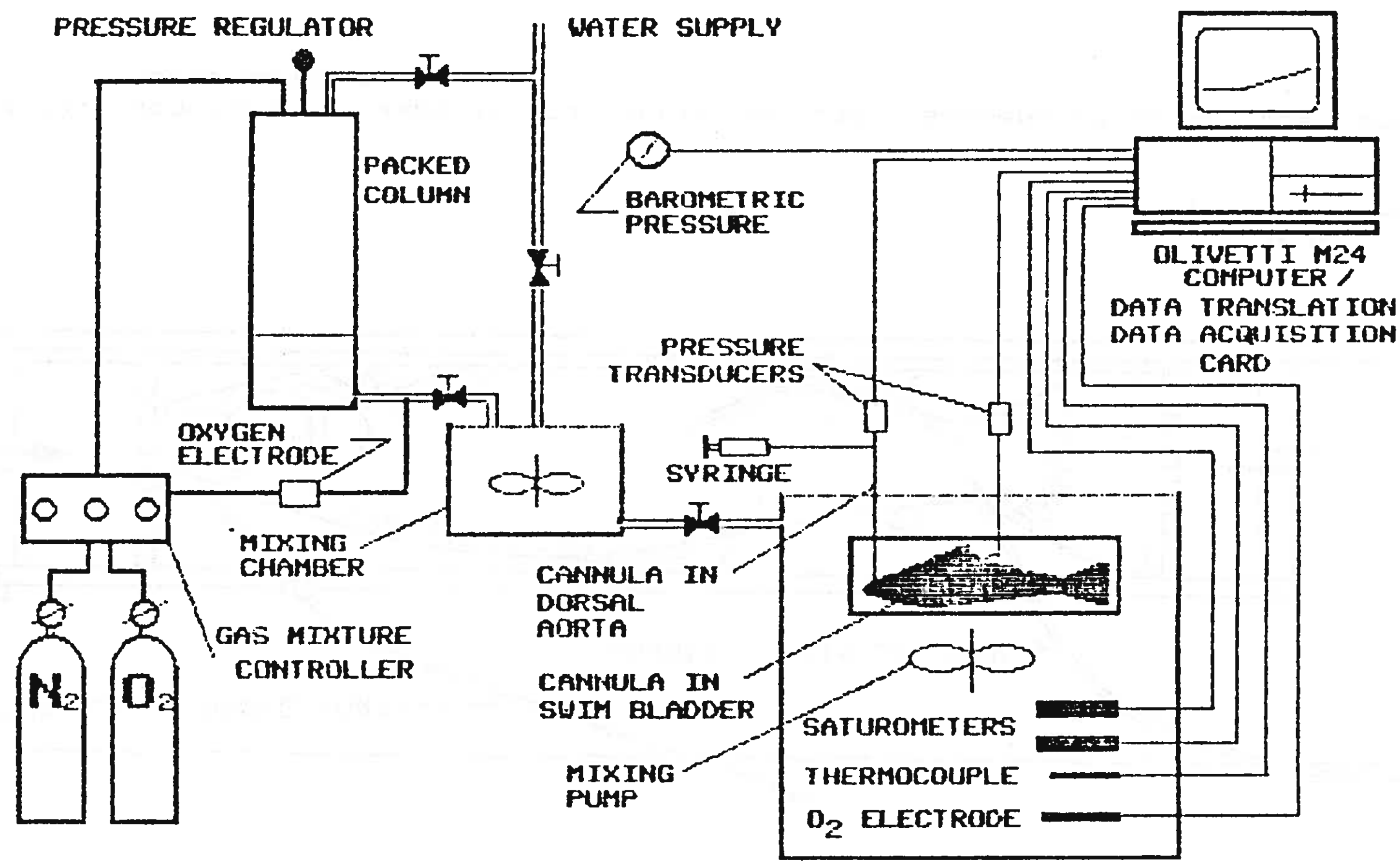

Figure 34. Schematic of experimental system for swimbladder inflation and vascular system bubble growth experiments, University of British Columbia.

decreased due to surface tension forces at the gas/liquid interface in the pneumatic duct. For large fish the experimental procedure again involves a catheter in the swimbladder which is pressurized until gas is vented through the pneumatic duct (Method 1). The release pressure is recorded as a function of the size of the fish. For small fish that cannot be cannulated, a hyperbaric chamber is used to establish release pressure (Method 2). The results of these experiments are shown in Figure 35. Here release pressure is plotted as a function of fish size. For comparison, the Laplace relationship for surface tension is also shown. The scaling factor between Laplace's equation and fish weight is as indicated in the figure. It would appear that the surface tension hypothesis is confirmed. In terms of the effects of supersaturation on fish this means that small fish will experience a greater buoyant force, per unit body weight, than large fish under the same conditions of supersaturation, providing delta P is above the release pressure for the large fish. For example, in a condition where the Delta P (TGP - $P_{t.}$ is 30 mm Hg, a fish of 10 g will experience a swimbladder overpressure of 30 mm Hg while a fish of 200 g will experience a swimbladder overpressure of only 10 mm Hg.

At this time the smallest fish studied in this series of experiments is about 5 g. Many times during these experiments it has been found that the swimbladder of the small fish (5 g) rupture before the pneumatic duct releases the gas pressure. This result suggests that mortalities in small fish can occur at very low Delta P levels (i.e. TGP of approximately 105%). It

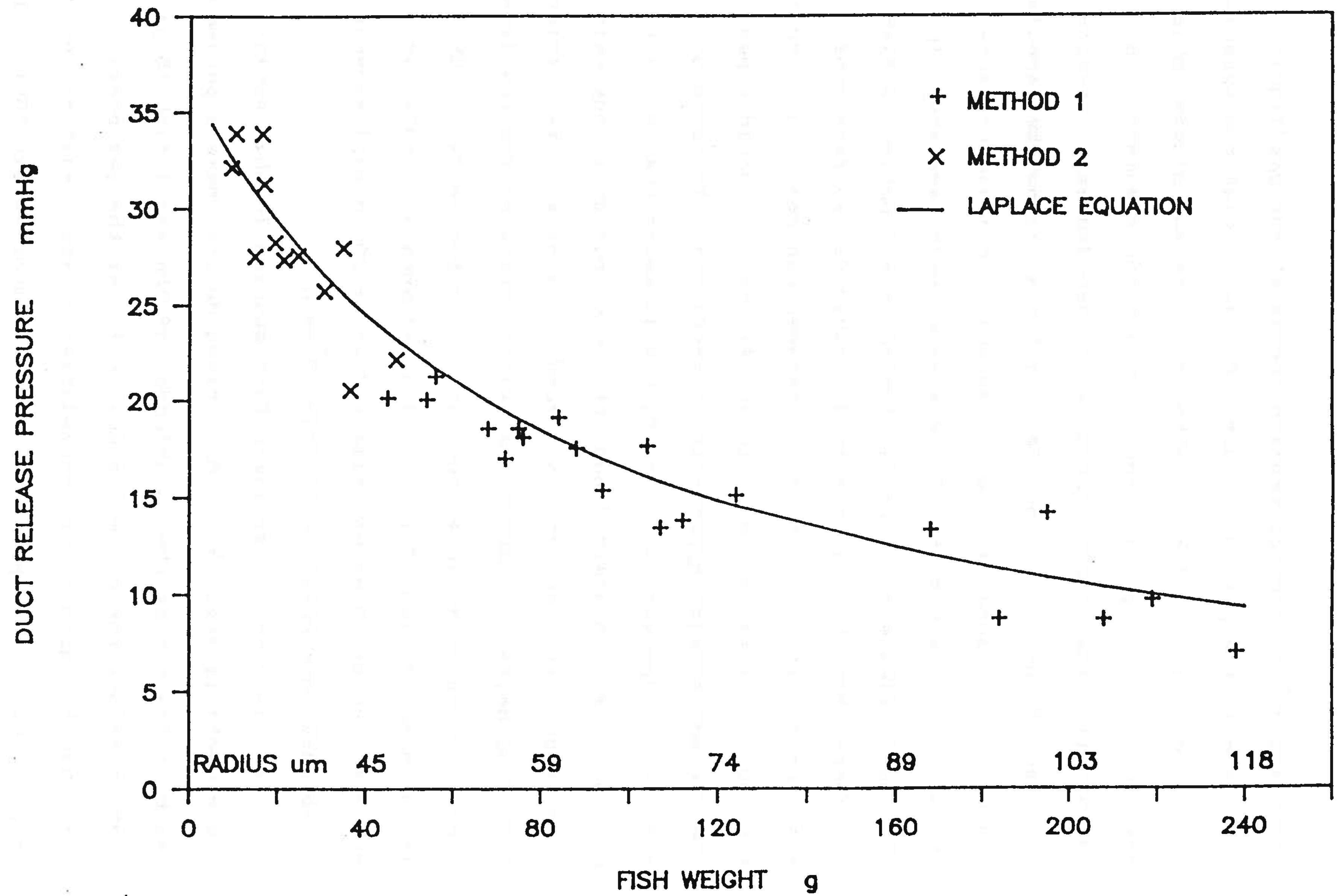

Figure 35. Average pneumatic duct release pressure as a function of rainbow trout weight and probable radius of pneumatic duct.

will be important to apply these observations and the predictive equations to the Bighorn River during 1987. The UBC studies will continue during 1987 and include fish as small as 1.0 g.

<u>Buccal Cavity Bubble Growth</u>: The equation which describes the threshold total gas pressure for bubble growth in the buccal cavity (see Fidler 1985) is given as:

$$TGP_W = P_E + (2\sigma/r) \qquad \text{Eq. 3}$$

where: TGP_W = threshold TGP for bubble growth in water
$P_E = P_A + P_H$
σ = the surface tension of water
r = the size of a critical nucleation site

It will be noted that this equation shows the threshold TGP is independent of water oxygen partial pressure, transport parameters or respiratory parameters. In the equation P_A and P_H are usually known and σ can be obtained from standard physical properties data, (Welty et al. 1976). The only unknown is the size of critical nucleation sites in the buccal cavity. Using a radius on the order of the dimensions of epithelial tissue cells, (approximately 20 um), Equation 3 can be plotted as shown in Figure 36. It is clear that buccal cavity bubble growth thresholds will be a function primarily of atmospheric pressure and water depth. Or, Delta P (TGP - P_{atm}) is constant at any given depth. There is a slight variation with temperature due to its effect on surface tension; however, it is small in relation to the other parameters. At this time no experimental work is being conducted to evaluate this relationship.

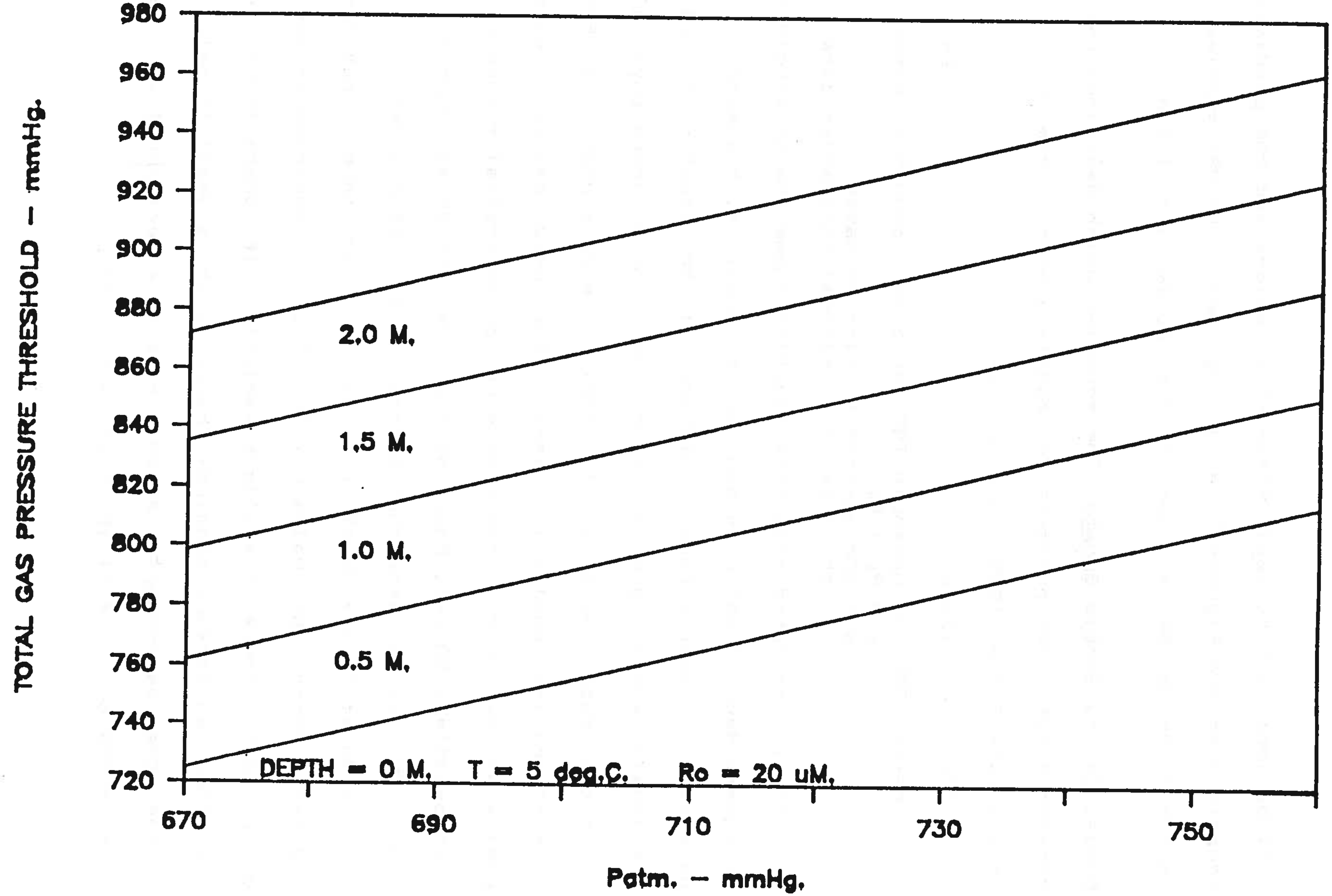

Figure 36. Predicted buccal cavity bubble growth threshold as a function of atmospheric pressure and water depth.

Vascular System Bubble Growth: For bubble growth thresholds in the vascular system, Equation 4 describes the mathematical relationship between the important physical and physiological parameters (see Fidler 1985).

$$TGP_A = [F*X'O(1 - K) + K]*[P_E + (2o/r)]/[X'_o(F - K) + K] \quad \text{Eq. 4}$$

where: TGP_A = total gas pressure at which a nucleation site grows into a bubble.
o = surface tension of fish blood.
r = radius of nucleation site from which bubble grows.
$P_E = P_A + P_H + P_B$
P_A = atmospheric pressure.
P_H = hydrostatic pressure.
P_B = pressure in vascular system where bubble growth begins.

In applying this equation to the vascular systems of fish, Fidler (1985) showed that because arterial blood had significantly higher levels of dissolved oxygen than venous blood (100 mm Hg compared to 15 mm Hg), bubble growth would begin on the arterial side of the vascular system at much lower TGP levels than required for bubble growth on the venous side. In general, the physical parameters for the environmental water, TGP, X'_o, P_H, and P_A, can be measured while the physical parameters of H_N, H_o, D_N, D_o for fish blood are known from standard physical data tables, (see Altman and Dittmer 1961, 1971). The principal unknowns in the equation are the size of critical nucleation sites, r; the pressure in the vascular system where bubble growth begins, P_B; the gill oxygen uptake ratio, F; and the surface tension of fish blood, o. Of these unknowns, the surface tension of fish blood was recently determined (Fidler 1985) and the gill oxygen uptake ratio, described below, can be determined experimentally. The remaining unknowns, r and P_a, are nearly impossible to determine by direct measurement. Although blood pressure, P_a, can be measured, the difficulty lies in knowing exactly where the nucleation site, that will grow into a bubble, is located in the arterial system. Although this appears to be a major problem, it will be noted in Equation 4 that the term P_a and the 2σ/r terms can be added together to yield a pseudo parameter which will be called $2σ/R_0$. This term is an effective critical radius which combines the effects of blood pressure and actual nucleation site radius. It is interesting to note that if the value of F is taken as 0.67, a condition observed in experiments at UBC, and the critical TGP is taken as between 110 and 112% at sea level conditions, the value of R_0, back calculated from Equation 4, is 20 umM. The range of 110 to 112% is often stated in the literature as a critical value for mortalities in adult fish (see Weitkamp and Katz 1980, for example). This effective radius, interestingly enough is on the order of the size of fish erythrocytes, (Mott 1957 and Heming 1984). Fidler (1985) argued that if nucleation sites were free in the blood, then their maximum size must be no greater than the size of erythrocytes; otherwise, the nucleation sites would block capillary beds even under conditions of dissolved gas equilibrium. This argument does not necessarily confirm the maximum size of nucleation sites, for it is possible that the sites are attached to the walls of the vascular system which would allow them to be larger. As will be described in a subsequent section, Larry Fidler is engaged in experimental studies at UBC which will establish the size of these effective nucleation sites. First, however, the 20 um value for R_0 will be used to demonstrate how Equation 4 can be applied to the Bighorn River to establish thresholds for vascular system bubble growth.

As described above, most of the physical parameters in Equation 4 are available from satellite data. The transport parameters contained in the K term are known from standard physical properties tables, the surface tension of fish blood is known from Fidler (1985), and the oxygen uptake ratio F is known from current experimental studies at UBC. Using this information, Equation 4 can be written as:

$$TGP_A = (P_E - .675*P\text{-}O_2)/2$$
$$+ [SQRT((.675*P\text{-}O_2 - P_E)2 + 4.02*P\text{-}O_2*P_E)]/2 \quad \text{Eq. 5}$$
$$\text{where: } P_E = P_A + P_H + 2\sigma/R_0$$

Taking R_0 as 20 um and a barometric pressure of 685 mm Hg, Equation 5 can be plotted as shown in Figure 37 for various water depths and water oxygen partial pressures. More specifically, taking data from September 17, 1986, at Rkm 4.8 (see Figures 30 through 32), where $P\text{-}O_2$ is 210 mm Hg and TGP is 805 mm Hg, a river depth of 0.1 m is enough to prevent bubble growth in the vascular system. Comparing this to the earlier example for swim-bladder over-inflation, a fish at a depth of 0.1 m will be protected from vascular system bubbles but may have a swimbladder over-inflated by as much as 75 mm Hg. In the case of a small fish this would surely lead to swimbladder rupture. As mentioned earlier, a

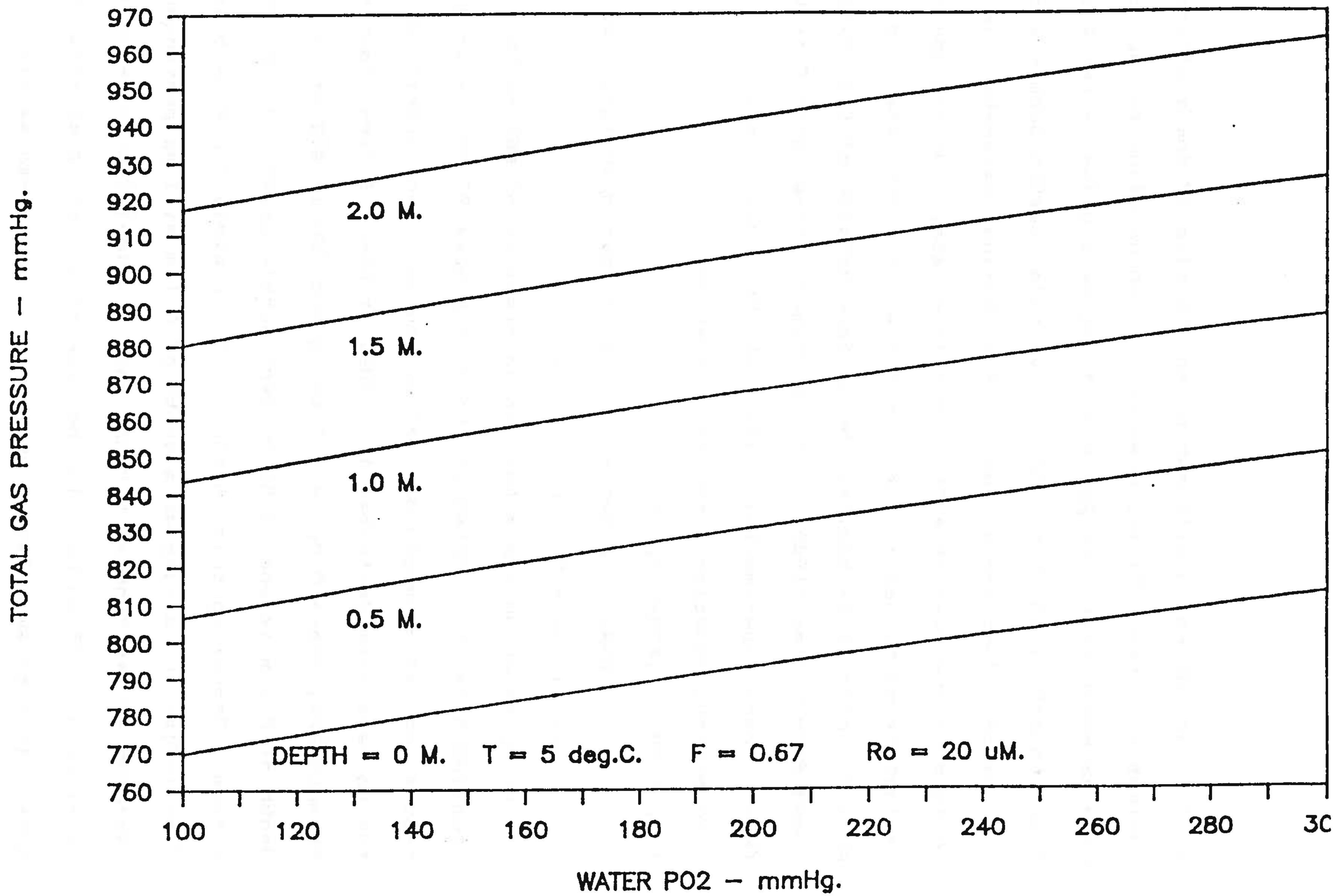

Figure 37. Predictions of total gas pressure thresholds resulting in arterial bubble formation as a function of water PO_2 and depth.

200 g fish would have a swimbladder over-inflated by no more than 10 mm Hg; however, it would experience vascular system bubbles if it spent much time above the 0.1 m depth. Again, as in the case of the swimbladder equation, the application of the vascular system threshold equation would best be accomplished by programming it into a Lotus 123 worksheet and applying it directly to the satellite data on a continuous basis.

To establish the value of the R_0 term in Equation 5, Larry Fidler is conducting a series of experiments at UBC wherein fish are subjected to known levels of supersaturation. Blood pressure is monitored during exposure, along with arterial $P\text{-}O_2$. Figure 34 shows a schematic drawing of the experimental setup, while Figure 33 shows how the fish is held captive with a dorsal aorta cannula. The object of the experiment is to establish the threshold conditions for bubble growth. The TGP at which bubbles appear will be observed as a change in the response of blood pressure. For example, Figures 38 through 41 show the response of blood pressure in a fish exposed to a TGP of 940 mm Hg and a water $P\text{-}O_2$ of 225 mm Hg. It will be observed that for several hours (10:23 AM to 1:24 PM), no major change in blood pressure occurred. Eventually, at 1:24 there was a rise in blood pressure from 25 to 50 mm Hg and at 1:33 PM there was a dramatic increase in blood pressure which was is associated with the sudden appearance of large quantities of gas in the vascular system. At the time of this change, the fish went to its side and attempted violent swimming motions. The fish did not recover after the level of dissolved gas tension was reduced back to equilibrium conditions

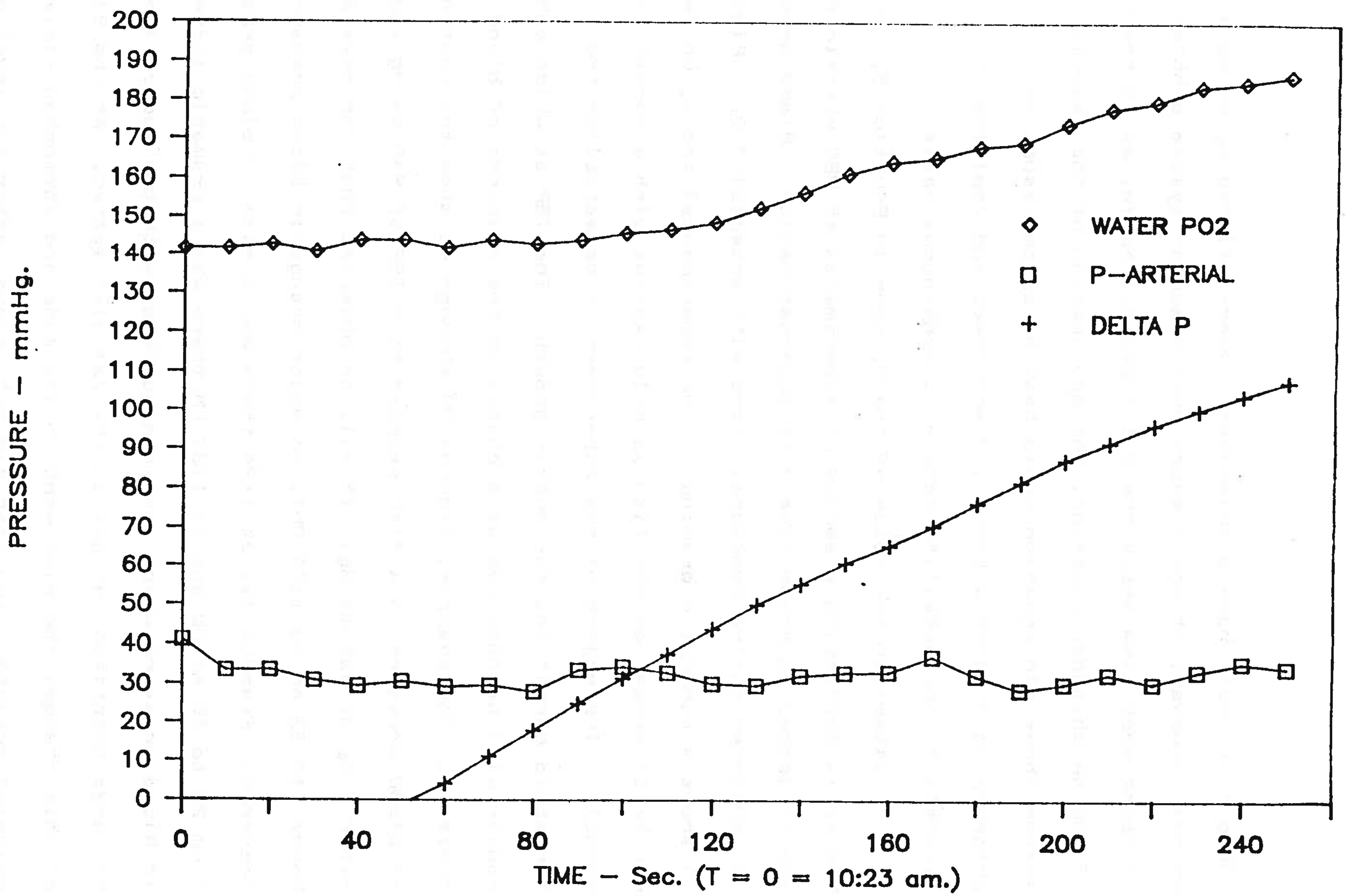

Figure 38. Response of arterial pressure of a rainbow trout exposed to 940 mm Hg total gas pressure and water $P\text{-}O_2$ of 225 mm Hg, 10:23-10:27 am, 28 June 1986.

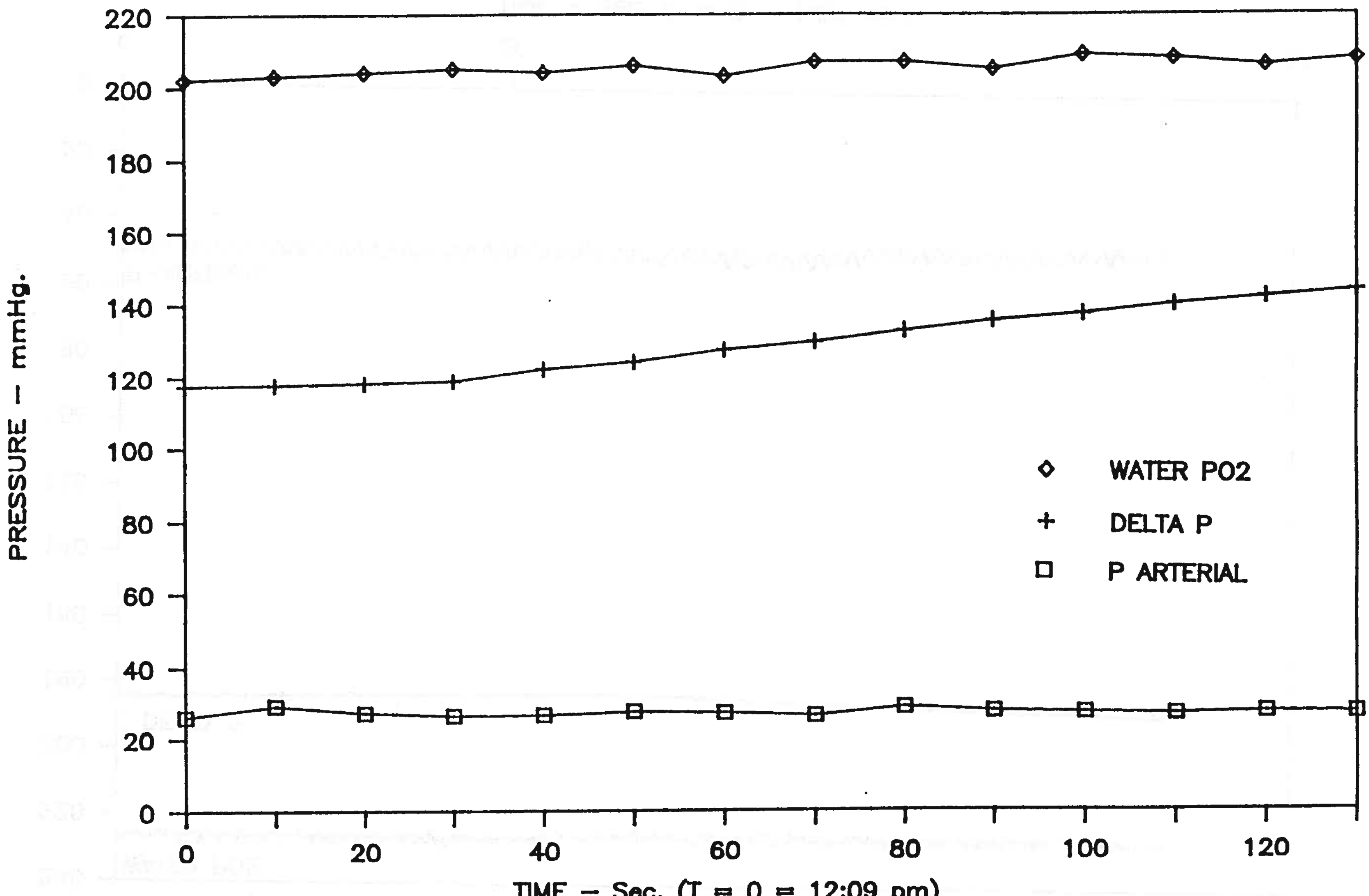

Figure 39. Response of arterial pressure of a rainbow trout exposed to 940 mm Hg total gas pressure and water P_{O_2} of 225 mm Hg, 12:09-12:11 pm, 28 June 1986.

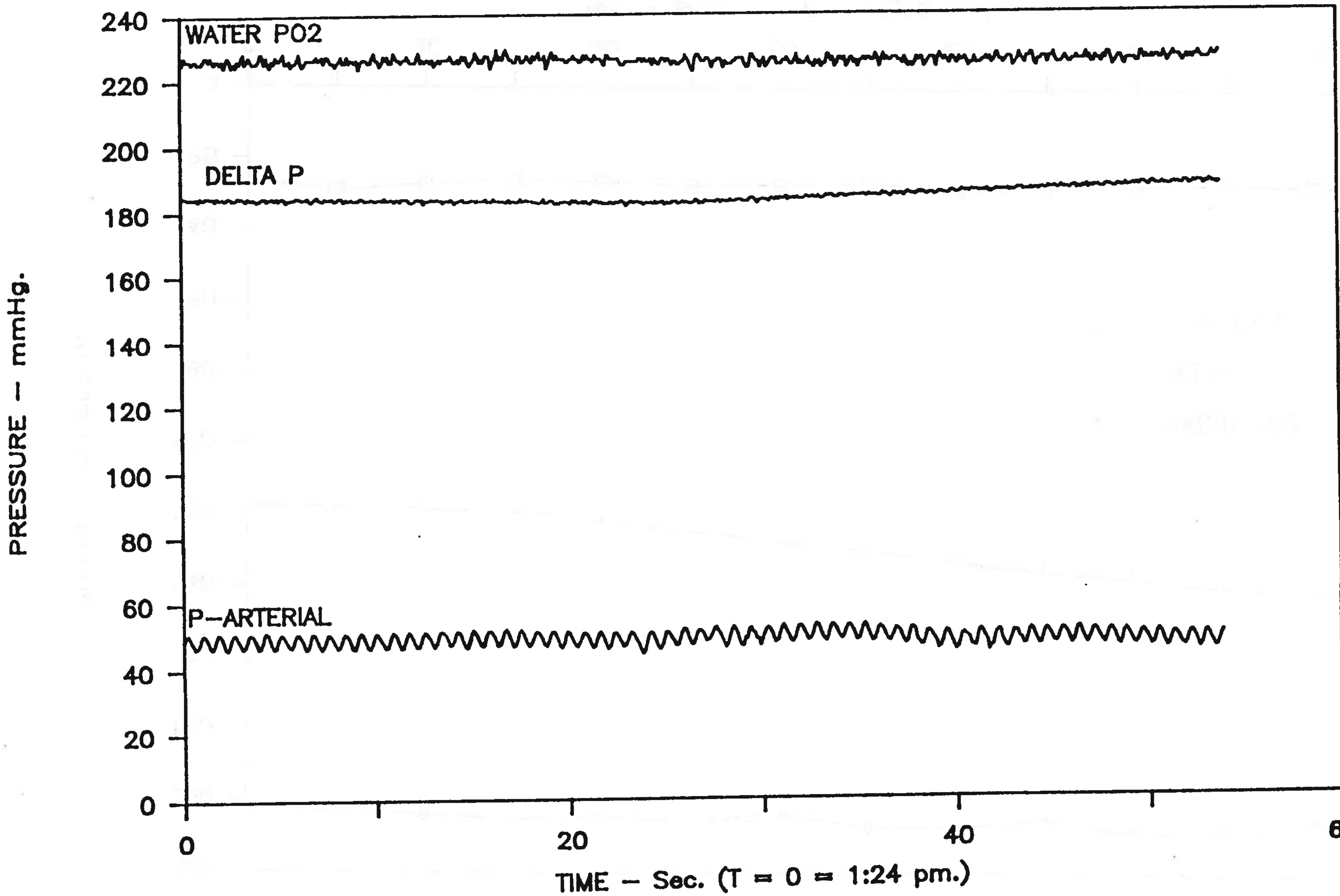

Figure 40. Response of arterial pressure of a rainbow trout exposed to 940 mm Hg total gas pressure and water $P\text{-}O_2$ of 225 mm Hg, 1:24-1:25 pm, 28 June 1986.

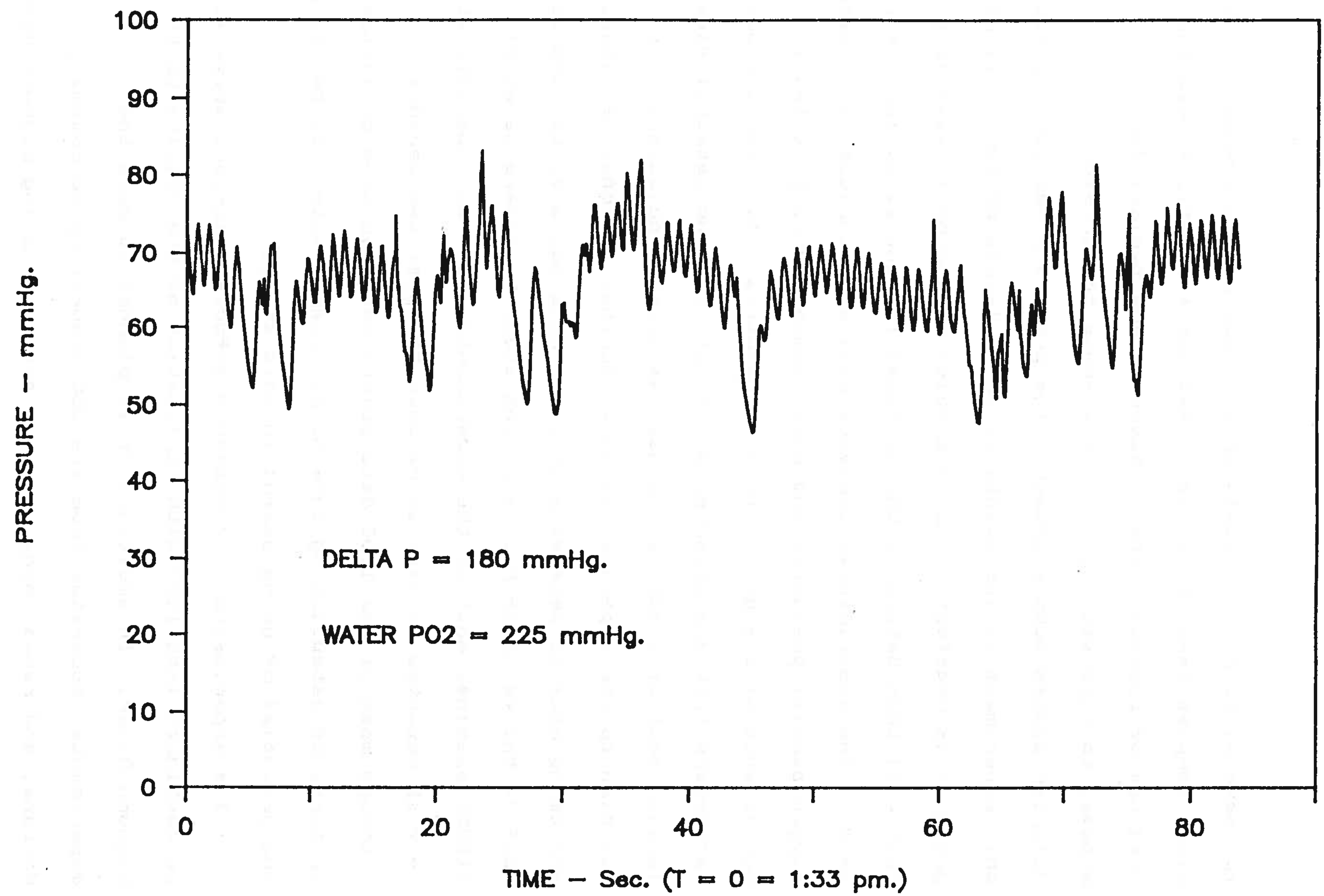

Figure 41. Response of arterial pressure of a rainbow trout exposed to 940 mm Hg total gas pressure and water P-O2 of 225 mm Hg, 28 June 1986; total exposure time, approximately 191 minutes.

for several hours. In addition to blood pressure measurements, blood samples taken from the dorsal aorta clearly showed the presence of bubbles. These responses are typical for fish exposed to supersaturation levels above the threshold for vascular system bubble growth. The procedure has been to repeat this experiment at incrementally lower levels of TGP until blood pressure is unaffected and no bubbles are found in sampled blood. This will then determine the critical TGP and establish the value of R_0. The experimental sequence will also include other water oxygen partial pressures and water temperatures to establish the consequence of changes in these parameters. Additional blood parameters that are measured are hematocrit and catacholamine levels, both of which can be used as stress indicators. In addition to the experimental work, further searches of literature are being made to determine if other data may also be used to confirm the value of R_0. A recent study by Alderdice et al. (1985) examines most of the experimental data on supersaturation in fish reported in literature over the past two decades. Although most of the 1200 data points examined were of little use in terms of establishing thresholds, approximately 90 points have the potential of being useful in this study.

The experimental and research efforts described above will be continued into 1987 with application of the results to the Bighorn River. In addition, it is planned to move the experimental apparatus from the UBC laboratory to Bozeman, Montana, and repeat many of the experiments using Bighorn River fish. It is anticipated that some of the Bighorn species have, and one perpendicular transect could be examined on a single dive. Approximately 55 hours of snorkeling was done to observe general fish distribution.

Direct observation methods tested were found to be ineffective in the Bighorn River. When transects were dove, few fish were located. Fausch and White (1981) state that stream salmonids maintain relatively fixed positions with respect to the stream bed and make short forays from them to feed. This type of holding behavior has been described in other streams (Bachman 1984; Jenkins 1969). Brown and rainbow trout in the Bighorn River did not show this type of behavior. With the 2-3 minute criterion, only eight trout remained in position long enough for data to be collected. The lack of stationary positions has also been observed in other large rivers (Griffith 1987, pers. comm.).

Habitat use data were collected for seven brown trout and one rainbow trout (Table 38). These data and general snorkel observations indicate that brown trout selected areas of moderate depth and velocity during midday. Very few brown trout were seen in depths less than 1.0 m.

All observations were made during midday hours when light conditions were optimum. Diurnal changes in habitat use were not examined. It has been suggested that brown trout may move into shallow water during the night to feed (Griffith 1987, pers. comm.). The depth of the fish below the water surface is critical in supersaturated water. Each meter of increased depth provides a 10% reduction in saturation due to increasing hydrostatic pressure (Haynes 1978). Salmonids exposed to gas

Table 38. Fish habitat use observations measured along transects in the Bighorn River, July - September 1986.

Species	Fish length (cm)	Water depth (m)	Fish depth (m)[a]	Focal point velocity (cm/s)	Velocity within 0.3 m or 0.6 m (cm/s)[b]	GBT signs	Relation to cover	Activity	Substrate	Time of day
brown	15.0	2.10	1.90	21.51	37.53	no	substrate	feeding	cobble	11:30
brown	30.0	2.30	2.20	31.83	57.77	no	substrate	feeding	cobble	11:30
brown	20.0	2.00	1.95	21.51	21.85	no	substrate	feeding	cobble	11:00
brown	27.0	2.15	2.08	23.23	70.56	yes[c]	substrate	feeding	gravel	11:30
brown	18.0	0.45	0.40	22.89	23.23	no	substrate	resting	cobble	13:30
brown	11.0	1.40	1.20	21.85	29.77	no	vegetation	feeding	cobble	12:00
rainbow[d]	12.0	1.00	0.75	28.05	58.25	no	substrate	feeding	cobble	16:30

a - Fish depth is the depth the fish is below the water surface.
b - Maximum velocity within 0.3 m (age 0), or 0.6 m (>age 0).
c - Pop eye left, fish tilted with right eye tilted towards on coming drift items.
d - Hatchery fish, pelvic clip.

supersaturation within cages and tanks usually survived when allowed to sound to depth (Chamberlain et al. 1980). Perhaps a shift in habitat use by brown trout at night in the Bighorn River would expose them to high gas levels and may accont for the higher incidence of GBT seen in brown trout compared to rainbow trout. This hypthesis will be investigated during the upcoming field season with night-time snorkeling in shallow areas and radio telemetry studies.

SUMMARY

1. Trends of gas tensions in the Bighorn River during 1986 were similar to those of 1985. Peak gas levels were observed in late June - early July. Gas dissipation, as shown by mean delta P of nitrogen and argon, occurs at a high rate from Afterbay Dam down to Rkm 4.8, decreases between Rkm 4.8 and 8.0, and increases again between Rkm 8.0 and 14.5. Gas levels were least variable immediately below Afterbay Dam. The effects of gas entrainment at Afterbay Dam were observed throughout the entire length of the study area.

2. Oxygen becomes an increasingly larger component of TGP in the Bighorn River with distance downstream. This is beneficial to larger fish in the lower reaches since the threshold for bubble growth in the vascular system rises along with the proportion of oxygen to TGP.

3. Internal pressures in salmonid eggs and their incubation in the stream bottom affords them more protection from GBT than other life stages. Development of bubbles in the buccal cavity may be the most serious threat to swim-up fry. Growth of buccal cavity bubbles is dependent on TGP and hydrostatic pressure and does not vary with oxygen pressure.

4. Permanently installed Common Sensing tensionometers appear to be a feasible method for remote, long term monitoring of gas levels provided instruments are calibrated and Silastic tubing is changed on a regular basis.

5. Construction of a powerhouse at Afterbay Dam could result in substantial reduction in dissolved oxygen in the upper portion of the study area during some periods of the year.

6. Cross-river transect data indicate that when the sluiceway is in operation, fish have the opportunity to minimize exposure to high gas levels by making short, lateral movements and by occupying deeper habitat.

7. In Section 1, incidence of GBT was higher on the right bank than on the left bank during spring and fall sampling. GBT incidence in Section 2 showed similar trends but was lower. In Section 3, incidence remained consistently low.

8. In the upper portion of the river, peak GBT incidence in adult rainbow trout coincided with spawning activity. Incidence for rainbow trout in Section 1 was about half that observed in brown trout. The peak incidence of GBT

among rainbow in Section 2 was the highest for either species in any section.

9. Mountain whitefish are more sensitive to supersaturated conditions than trout. The mountain whitefish population below Afterbay Dam may increase after a permanent solution to the air entrainment problem is achieved.

10. GBT incidence increased most rapidly among large fish. This may be related to larger nucleation site radii resulting from repeated exposure to supersaturated conditions.

11. Seasonal changes in percentage of total discharge passed through the sluice gates would help reduce TGP. These changes may be feasible while minimizing wear on the sluice gates and preventing them from binding. However, changes may decrease the stability of river discharge.

13. Juvenile brown and rainbow trout became more sensitive to gas supersaturated water as they increased in length and weight. More fish died in a shorter period of time as size increased. External symptoms developed more quickly and were more severe in larger fish.

14. Juvenile brown trout were more sensitve to gas supersaturated water than juvenile rainbow trout of similar size. Brown trout mortality occurred faster, but the total mortality after 30 days was similar.

15. Juvenile brown and rainbow trout can recover from GBT. Further work is needed to determine exposure period thresholds and sublethal effects. Fish which have been exposed to gas supersaturated water, but did not develop GBT are not more susceptible to predation under laboratory conditions.

16. Trout exposed to supersaturated water experience different physiological problems depending on saturation level and fish size. The TGP threshold required to over-inflate the swimbladder and for bubble growth in the buccal cavity is low (103%) compared to the TGP threshold required to produce bubbles in the vascular system ($\geq$ 112%).

17. Swimbladder over-inflation, and buccal cavity bubble growth and associated excess buoyancy is a larger problem for juvenile trout than for adult trout. Swimbladder over-inflation may affect juvenile fish feeding and ability to escape predation; in addition, buccal cavity bubble growth in fry may result in hypoxia.

18. **Rainbow and brown trout in the Bighorn River do not maintain fixed positions typical of salmonid behavior in smaller streams. Brown trout selected areas of moderate depth during midday.**

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APPENDIX A

Gas Monitoring Data

Table 5. Means and range of water temperature and dissolved gas levels at five sites on the Bighorn River below Afterbay Dam, 16 July - 15 October, 1986. (Does not include data from 4 - 20 August.)

River km	Sample size	Temp. (C)	D.O. (mg/l)	% saturation Oxygen	N_2+Ar	Total
0.6	27	17.3 (14.4-18.9)	8.6 (7.7-9.7)	99.8 (89.3-110.1)	120.2 (113.4-126.9)	115.6 (108.9-122.3)
2.4	26	17.6 (15.0-18.9)	9.1 (8.2-10.2)	106.0 (94.5-115.8)	116.5 (111.3-120.1)	113.9 (118.8-107.6)
4.8	27	17.8 (14.4-20.6)	10.4 (8.5-12.5)	122.2 (98.3-145.2)	109.5 (103.8-112.4)	111.8 (107.0-118.0)
8.0	26	18.0 (14.7-20.6)	10.2 (8.5-11.5)	120.0 (96.8-138.7)	109.6 (106.4-112.4)	111.5 (106.3-114.8)
14.5	26	18.3 (14.4-21.1)	10.8 (8.5-12.4)	128.7 (99.5-148.8)	105.9 (101.7-110.1)	110.4 (104.2-114.7)

Table 6. Means and ranges of barometric pressure and delta P's (mm Hg) at five sites on the Bighorn River below Afterbay Dam, 16 July - 15 October, 1986. (Does not include data from 4 - 20 August.)

River km	B.P. (mm Hg)	ΔP (mm Hg)	ΔP-O_2 (mm Hg)	ΔP-N_2+Ar (mm Hg)
0.6	681 (664-687)	106 (61-152)	-0.3 (-15-14)	106 (71-142)
2.4	681 (664-688)	95 (52-128)	8 (-8-22)	87 (60-106)
4.8	682 (664-690)	81 (48-123)	31 (-2-63)	50 (20-66)
8.0	682 (664-689)	78 (43-100)	28 (-4-54)	50 (33-66)
14.5	682 (663-690)	71 (29-100)	40 (-1-68)	31 (9-53)

Table 7. Means and ranges of water temperature and dissolved gas levels at five sites on the Bighorn River below Afterbay Dam, 16 October - 31 December, 1986.

River km	Sample size	Temp. (C)	D.O. (mg/l)	% saturation Oxygen	N2+Ar	Total
0.6	22	10.1 (6.1-13.9)	10.9 (9.7-12.3)	107.8 (98.7-111.2)	120.1 (111.4-124.0)	117.2 (109.2-120.0)
2.4	22	10.2 (6.1-14.2)	11.1 (9.8-12.4)	109.4 (101.5-114.7)	117.0 (110.4-120.4)	114.7 (105.0-118.0)
4.8	19	10.2 (6.1-14.4)	12.3 (11.2-13.4)	121.7 (106.4-140.6)	111.5 (107.4-114.8)	113.4 (107.8-118.5)
8.0	22	10.3 (6.4-14.4)	12.1 (10.7-13.5)	119.7 (98.7-133.2)	110.7 (107.7-113.8)	112.4 (107.6-115.8)
14.5	22	10.3 (6.4-15.0)	13.2 (11.4-14.9)	131.4 (109.3-157.4)	106.4 (103.2-109.4)	111.4 (105.0-116.9)

Table 8. Means and ranges of barometric pressure and delta P's (mm Hg) at five sites on the Bighorn River below Afterbay Dam, 16 October - 31 December, 1986.

River km	B.P. (mm Hg)	ΔP (mm Hg)	ΔP-O2 (mm Hg)	ΔP-N2+Ar (mm Hg)
0.6	682 (674-697)	118 (63-135)	11 (-2-16)	107 (60-127)
2.4	683 (674-697)	103 (60-123)	13 (2-20)	90 (55-108)
4.8	682 (674-689)	91 (53-127)	31 (9-57)	61 (39-78)
8.0	683 (674-697)	85 (52-108)	28 (-2-47)	57 (41-73)
14.5	684 (675-697)	78 (34-116)	44 (13-81)	34 (17-49)

Table 3. Chi-square distribution (α=0.05) for high gas treatment (ΔP3=162.1mm and ΔP4=166.5mm) brown trout tests 3 and 4 (BT3=62.1mm and BT4=90.7mm).

Test Day	Observed % Mortality BT 3	Observed % Mortality BT 4	Expected % Mortality BT 3	Expected % Mortality BT 4	X2	Test Value $\frac{(O-E)^2}{E}$	X^2 Distribution	Conclusion
1	0.0	0.0	0.0	0.0	0.0			
2	2.7	28.0	15.35	15.35	20.9			
3	9.4	54.7	32.1	32.1	31.8			
4	29.4	70.7	50.1	50.1	16.9	101.2	14.1	Significant Difference
5	41.4	82.7	62.1	62.1	13.7			
6	60.1	96.0	78.1	78.1	8.2			
7	66.8	98.7	82.8	82.8	6.1			
8	74.8	100.0	87.4	87.4	3.6			

Table 4. Chi-square distribution (α=0.05) for high gas treatment (ΔP=155.0mm) brown and rainbow trout from test 1 (BT_1=35.2mm and RBT_1=34.0mm).

Test Day	Observed % Mortality BT_1	Observed % Mortality RBT_1	Expected % Mortality BT_1	Expected % Mortality RBT_1	X2	Test Value $\frac{(O-E)^2}{E}$	X^2 Distribution	Conclusion
5	3.3	0.0	1.7	1.7	3.3			
10	8.7	0.0	4.4	4.4	8.7			
15	14.7	1.3	8.0	8.0	11.2	42.9	9.5	Significant Difference
20	19.0	3.9	11.5	11.5	9.8			
25	21.4	5.2	13.3	13.3	9.9			

Table 5. Chi-square distribution (α=0.05) for high gas treatment ($\Delta P2$=153.4mm and $\Delta P3$=162.1mm) brown and rainbow trout from tests 2 and 3 (BT_3=62.2mm & RBT_2=70.5mm).

Test Day	Observed % Mortality BT_3	Observed % Mortality RBT_2	Expected % Mortality BT_3	Expected % Mortality RBT_2	X2	Test Value $\frac{(O-E)^2}{E}$	X^2 Distribution	Conclusion
5	41.4	8.3	24.9	24.9	21.9			
10	84.2	36.1	60.2	60.2	19.1			
15	94.8	58.3	76.6	76.6	8.7	53.1	9.5	Significant Difference
20	97.4	75.0	86.2	86.2	2.9			
25	97.4	87.5	92.5	92.5	0.5			

* Note similarity in final mortality value.

Table 6. Chi-square distribution (α=0.05) for high gas treatment (ΔP3=162.1mm and ΔP4=167.0) brown and rainbow trout from tests 3 and 4 (BT4=90.7mm and RBT3=89.5mm).

Test Day	Observed % Mortality BT 4	Observed % Mortality RBT 3	Expected % Mortality BT 4	Expected % Mortality RBT 3	X2	Test Value $\frac{(O-E)^2}{E}$	X^2 Distribution	Conclusion
1	0.0	0.0	0.0	0.0	0.0			
2	28.0	0.0	14.0	14.0	28.0			
3	54.7	1.4	28.1	28.1	50.4			
4	70.7	27.0	48.9	48.9	19.4	129.5	14.1	Significant Difference
5	82.7	41.9	62.3	62.3	13.4			
6	96.0	61.3	78.7	78.7	7.6			
7	98.7	65.4	82.1	82.1	6.7			
8	100.0	73.5	86.8	86.8	4.0			

Table 7. Chi-square distribution (α= 0.05) for high gas treatment (ΔP1=1554.0mm and ΔP2=153.4mm) rainbow trout tests 1 and 2 (RBT1=34.0mm and RBT2=70.5mm).

Test Day	Observed % Mortality RBT 1	Observed % Mortality RBT 2	Expected % Mortality RBT 1	Expected % Mortality RBT 2	X2	Test Value $\frac{(O-E)^2}{E}$	X^2 Distribution	Conclusion
5	0.0	8.3	4.2	4.2	8.0			
10	0.0	36.1	18.1	18.1	35.8			
15	1.3	58.3	29.8	29.8	54.51	235.5	19.5	Significant Difference
20	3.9	75.0	39.4	39.4	64.3			
25	5.2	87.5	46.4	46.4	72.8			

Table 8. Chi-square distribution (α=0.05) for high gas treatment (ΔP2=153.4mm and ΔP3=162.1mm) rainbow trout tests 2 and 3 (RBT2=70.5mm and RBT3=89.5mm).

Test Day	Observed % Mortality RBT2	Observed % Mortality RBT3	Expected % Mortality RBT2	Expected % Mortality RBT3	X2	Test Value $\frac{(O-E)^2}{E}$	X^2 Distribution	Conclusion
5	8.3	41.9	25.1	25.1	22.5			
10	36.1	85.7	60.9	60.9	20.2			
15	58.3	95.1	76.7	76.7	8.3	54.6	19.5	Significant Difference
20	75.0	97.8	86.4	86.4	3.1			
25	87.5	97.8	92.65	92.65	0.6			

Table 9. Chi-square distribution (α=0.05) for high gas treatment (ΔP3=162.1mm and ΔP4=166.5mm) rainbow trout tests 3 and 4 (RBT3=89.5mm and RBT4=126.0mm).

Test Day	Observed % Mortality RBT 3	Observed % Mortality RBT 4	Expected % Mortality RBT 3	Expected % Mortality RBT 4	X2	Test Value $(O-E)^2$	X^2 Distribution	Conclusion
1	0.0	0.0	0.0	0.0	0.0			
2	0.0	17.9	8.9	8.9	17.9			
3	1.4	56.4	28.9	28.9	52.3			
4	27.0	94.9	60.9	60.9	39.3	140.9	11.1	Significant Difference
5	41.9	97.4	69.6	69.6	22.1			
6	61.3	100.0	80.65	80.65	9.3			

Table 10. Chi-square distribution (α=0.05) for medium gas treatment (ΔPmedium=81.6mm and ΔPcontrol=15.6mm) brown trout test 1 (BT1=35.2mm).

Test Day	Observed % Mortality control	Observed % Mortality Treatment	Expected % Mortality control	Expected % Mortality treatment	X2	Test Value $\frac{(O-E)^2}{E}$	X^2 Distrib.	Conclusion
10	.68	1.3	1.0	1.0	.2			
20	2.72	5.3	4.0	4.0	.9	2.1	6.0	No Significant Difference
30	4.09	6.7	5.4	5.4	.6			

Table 11. Chi-square distribution (α=0.05) for medium gas treatment (ΔPmedium=78.3mm and ΔPcontrol=37.5mm) brown trout test 2 (BT2=53.1mm).

Test Day	Observed % Mortality control	Observed % Mortality Treatment	Expected % Mortality control	Expected % Mortality treatment	X2	TestValue $\frac{(O-E)^2}{E}$	X^2 Distrib.	Conclusion
5	0.0	4.0	2.0	2.0	4.0			
10	0.0	5.3	2.7	2.7	5.3			
15	0.0	12.0	6.0	6.0	12.0	71.2	19.5	Significant Difference
20	0.0	24.2	12.1	12.1	24.4			
25	1.4	29.4	15.4	15.4	25.5			

* In this test eliminating the mortality incurred during the period of high ΔP days does not influence the conclusion.

Table 12. Chi-square distribution (α=0.05) for medium gas treatment (ΔPmedium=69.2mm and ΔPcontrol=29.3mm) brown trout test 3 (BT3=62.2mm).

Test Day	Observed % Mortality control	Observed % Mortality Treatment	Expected % Mortality control	Expected % Mortality treatment	X2	TestValue $\frac{(O-E)^2}{E}$	X^2 Distrib.	Conclusion
10	0.0	2.9	1.5	1.5	2.9			
20	0.0	2.9	1.5	1.5	2.9	8.7	6.0	Significant Difference
30	0.0	2.9	1.5	1.5	2.9			

Table 13. Chi-square distribution (α=0.05) for medium gas treatment (ΔPmedium=83.3mm and ΔPcontrol=20.9mm) brown trout test 4 (BT4=90.7mm).

Test Day	Observed % Mortality control	Observed % Mortality Treatment	Expected % Mortality control	Expected % Mortality treatment	X2	Test Value $\frac{(O-E)^2}{E}$	X^2 Distrib.	Conclusion
10	0.0	25.9	13.0	13.0	25.9			
20	0.0	25.9	13.0	13.0	25.9	77.7	6.0	Significant Difference
30	0.0	25.9	13.0	13.0	25.90			

* Eliminating the mortality from days 6,7, and 8 would lower the test value and their would be no significant difference between the treatment and control mortality values.

Table 14. Chi-square distribution (α=0.05) for medium gas treatment (ΔPmedium=81.62m and ΔPcontrol=15.6mm) rainbow trout test 1 (RBT1=34.0mm).

Test Day	Observed % Mortality control	Observed % Mortality Treatment	Expected % Mortality control	Expected % Mortality treatment	X2	Test Value (O-E)2 / E	X^2 Distrib.	Conclusion
10	1.3	0.0	.7	.7	1.3			
20	2.0	1.3	1.7	1.7	.1	1.9	6.0	No Significant Difference
30	2.7	1.3	2.0	2.0	.5			

Table 15. Chi-square distribution (α=0.05) for medium gas treatment (ΔPmedium=78.3mm and ΔPcontrol=37.5mm) rainbow trout test 2 (RBT2=70.5mm).

Test Day	Observed % Mortality control	Observed % Mortality Treatment	Expected % Mortality control	Expected % Mortality treatment	X2	Test Value (O-E)2 / E	X^2 Distrib.	Conclusion
5	0.0	0.0	0.0	0.0	0.0			
10	0.0	6.4	3.2	3.2	6.4			
15	0.0	7.7	3.9	3.9	7.7	52.8	19.5	Significant Difference
20	0.0	11.6	5.8	5.8	11.6			
25	0.0	27.1	13.6	13.6	27.1			

* In this case elimination of high ΔP days does not influence the statistical significance of the Chi-square test.

Table 16. Chi-square distribution (α=0.05) for medium gas treatment (ΔP_{medium}=69.2mm and $\Delta P_{control}$=29.3mm) rainbow trout test 3 (RBT3=89.5mm).

Test Day	Observed % Mortality control	Observed % Mortality Treatment	Expected % Mortality control	Expected % Mortality treatment	X2	Test Value $\frac{(O-E)^2}{E}$	X^2 Distrib.	Conclusion
10	0.0	0.0	0.0	0.0	0.0			
20	0.0	0.0	0.0	0.0	0.0	1.4	6.0	No Significant Difference
30	0.0	1.4	0.7	0.7	1.4			

Table 17. Chi-square distribution (α=0.05) for medium gas treatment (ΔP_{medium}=83.3mm and $\Delta P_{control}$=20.9mm) rainbow trout test 4 (RBT4=126.0mm).

Test Day	Observed % Mortality control	Observed % Mortality Treatment	Expected % Mortality control	Expected % Mortality treatment	X2	Test Value $\frac{(O-E)^2}{E}$	X^2 Distrib.	Conclusion
10	0.0	9.4	4.7	4.7	9.4			
20	0.0	9.4	4.7	4.7	9.4	28.2	6.0	No Significant Difference
30	0.0	9.4	4.7	4.7	9.4			

* Eliminating the mortality attributed to days 6 and 7 would reduce the significance of the test value making the difference in mortality insignificant.