Effects of Supersaturated Water on Reproductive Success of Adult Salmonids

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William L Gale*, Alec G. Maule#, Ann Postera, Mary Peter-Swihart[1]

U.S. Geological Survey, Western Fisheries Research Center
Columbia River Research Laboratory
5501A Cook-Underwood Rd, Cook, WA, 98605

[1] U.S. Fish and Wildlife Service, Lower Columbia Fish Health Laboratory
61552 SR 14, Underwood, WA, 98651

*Current address: U.S. Fish and Wildlife Service, Spring Creek National Fish Hatchery
61552 SR 14, Underwood, WA 98651

#Corresponding author: alec_maule@usgs.gov

Executive Summary

In 1995 the voluntary spill program was initiated in order to provide a spill-bay route of passage for juvenile salmonids emigrating past hydropower dams on the Columbia and Snake rivers. There were concerns that increased total dissolved gas supersaturation (TDGS) caused by spilling water would have a net negative effect on juvenile salmonid survival. As a result, from 1995 through 1999, considerable research was conducted to determine the effects of gas-supersaturated water on survival of juvenile salmonids. The possible effects of gas supersaturated water on adult salmonids, however, were not examined. The success of management actions to restore salmonid populations depends on the reproductive success of adults returning from the ocean to spawn in their natal streams. Reproductive success of salmonid populations is determined by the number of adults that spawn and the number of their surviving progeny.

The goal of this project was to determine if acute exposure to TDGS affects the reproductive performance of female spring chinook salmon. To accomplish this goal we had several objectives: (1) determine the effects of acute exposure to various TDGS levels on pre-spawning mortality and fecundity of spring chinook salmon, (2) determine the impact of maternal exposure to TDGS on the egg quality, fertilization rate and short-term survival of progeny of spring chinook salmon and (3) determine the effects of TDGS on disease resistance in maturing female spring chinook salmon. All of these objectives were addressed using fish from the Little White Salmon National Fish Hatchery; experimental fish received short-term (< 72 h), sub-lethal exposures to gas supersaturated water in shallow tanks.

- During this study, adult female spring chinook salmon were exposed to mean TDGS levels of 114.1% to 125.5%.

- We ended exposure as soon as the first fish in the tank died. Time to first mortality in six trials was from 10 to 68 h and was inversely related to TDGS.

- There was no effect of TDGS on pre-spawning mortality (after exposure) or fecundity when comparing treatment fish to experimental controls or the general hatchery population.

- Egg quality, based on egg weight and egg diameter, did not differ between treatment and control fish.

- Fertilization rate and survival to eyed-stage was high (> 94%) for all groups.

- With the exception of *Renibacterium salmoninarum* (the causative agent of bacterial kidney disease; BKD), no viral or bacterial fish pathogens were isolated from experimental fish. The prevalence (about 45%) and severity of *R. salmoninarum* did not differ among the groups or the general hatchery population.

- We conclude that these acute exposures to moderate levels of gas-supersaturated water—perhaps similar to that experienced by immigrating adult salmon as they approach and pass a hydropower dam on the Columbia River—did not affect reproductive success of female spring chinook salmon. To determine the full impact of TDGS on reproductive success of immigrating chinook salmon, it would be necessary to duplicate inriver exposure scenarios obtained from archival depth tags.

Introduction

Total dissolved gas (TDG) concentrations in the Columbia and Snake rivers have recently been elevated due to involuntary spill from high spring runoff and voluntary spill used as a method to pass juvenile salmonids over dams. The voluntary spill program is based on data indicating that passage survival is greater in juvenile salmonids passing via spill than via turbine or bypass routes (Muir et al. 1998). However, as water spills over the dam (or a waterfall), it becomes mixed with atmospheric air that can be forced into solution as the water hits the plunge-pool. If this hydrostatic pressure is great enough, the water can become supersaturated with atmospheric air—a condition that can injure or kill fish. The effects on fish of exposure to gas supersaturated water can vary from relatively benign to acutely fatal depending on several factors, including water temperature, duration of exposure, and species-specific sensitivity. The goal of the voluntary spill program is to reap the benefits of increased fish passage survival without losing those benefits via mortality due to exposure to TDG supersaturation (TDGS).

Many studies have been conducted to assess the direct and sub-lethal effects of exposure of fish to TDGS. Significant acute effects of gas bubble trauma (GBT) in fish have been reported, but little is known about the sub-lethal effects in adult salmonids. Studies describing signs and direct mortality of adult salmonids exposed to TDGS are common in the literature (Gorham 1901, Beiningen and Ebel 1970, Ebel 1971, Nebeker et al. 1976). Westgard (1964) found an 88% pre-spawning mortality in adult chinook salmon (*Oncorhynchus tshawytscha*) blinded by exposure to supersaturated water in a spawning channel near McNary Dam. He also reported other sub-lethal effects in blinded fish,

including females that did not dig redds and males that were unable to successfully cover eggs with milt.

There is additional evidence that sub-lethal exposure to TDGS could affect the reproductive performance of adult salmonids. Montgomery and Becker (1980) reported intra-vascular bubbles in ovarian arteries of adult smallmouth bass (*Micropterus dolomieui*) exposed to TDGS in the Columbia River; these bubbles could reduce or eliminate the blood supply to developing eggs and affect reproductive success. It is also possible that TDGS could indirectly affect reproductive success via a stress response. It has been shown that changes in water quality, such as temperature, water hardness, and dissolved oxygen, can cause a stress response in fish (see review: Wendelaar-Bonga 1997), so it is reasonable to assume that exposure to some level of TDGS will result in stress responses in adult salmon. Studies have also demonstrated that stress can alter levels of reproductive hormones in fish (Pickering et al. 1987; Carragher et al. 1989; Carragher and Pankhurst 1991; Melotti et al. 1992; Pankhurst and Dedual 1994). Cortisol, the corticosteroid hormone secreted by fish in response to a variety of environmental stressors, has been shown to inhibit production of estradiol and testosterone by ovarian follicles of rainbow trout (*O. mykiss*; Carragher and Sumpter 1990). Campbell et al. (1992, 1994) reported that exposure of female hatchery rainbow trout and brown trout (*Salmo trutta*) to acute and chronic stressors resulted in smaller egg size, delayed ovulation, and lower survival of larvae compared to unstressed fish.

The goal of this project was to determine if acute exposure to TDGS affects the reproductive performance of female spring chinook salmon. To accomplish this goal we had several objectives: (1) determine the effects of acute exposure to various TDGS levels on pre-spawning mortality and fecundity of spring chinook salmon, (2) determine the impact of maternal exposure to TDGS on egg quality, fertilization rate and short-term survival of progeny of spring chinook salmon and (3) determine the effects of TDGS on disease resistance in maturing female chinook salmon. All of these objectives were addressed using fish from Little White Salmon National Fish Hatchery that received short-term (< 72 h), sub-lethal exposures to gas supersaturated water in shallow tanks.

Methods

On June 30, 2000, adult female spring chinook salmon were selected from the 2000 brood-year at the U.S. Fish and Wildlife Service, Little White Salmon National Fish Hatchery (Cook, WA). To distinguish males from females, sex of the fish was determined on by visualizing the gonads using a portable, Aloka ultrasound machine (Evans and Beaty 2000). Before and after the gas exposure trials, fish were maintained in the hatchery adult holding facility, but were separated from the general hatchery broodstock by the use of mechanical crowders. The fish received care similar to the general hatchery broodstock. At the end of the spring adult migration season (1 June), all brood fish received an injection of erythromycin (10 mg / kg) and all fish were treated with formalin at 167 ppm for one hour three times weekly until spawning began.

Dissolved Gas Exposure System

Gas supersaturated water was generated by injecting air into pressurized ambient river water. See Appendix 1 for a detailed drawing of the dissolved gas system (DGS). Water was pumped (¾-HP, self-priming pump) out of the adult return ladder, and into a sump tank (1600 l), which fed the gas injection system and the control tank. From the sump tank, water flowed into a centrifugal pump (¾ HP). Atmospheric air, provided by a 2-HP compressor (output at 70 psi) and regulated by a flowmeter, was injected directly into the water line on the intake side of the pump. From the pump, water (ca. 38 psi) flowed through 15.3 m of garden hose, which increased backpressure and retention time for the air and water mixture. To allow excess gas bubbles to escape and provide a reservoir of treated water, water was pumped into a 169-l tower constructed of 25-cm (10-inch) diameter PVC (pump head ca. 3.7 m). Water exited the bottom of the tower and flowed to the treatment tanks. The level of dissolved gas in the water exiting the tower was monitored continually with a Hydrolab. Water flow into all of the treatment tanks was regulated to 15.1 l (4 gallons) per minute, and entered the tanks at the bottom to prevent degassing. The treatment and control tanks were 2.4-m diameter, and water depth was only 0.5 m to minimize available hydrostatic pressure and the possibility that fish could depth-compensate for the effects of increased dissolved gas. Total water volume in each tank was 2,100 l. This system was set up to run trials with TDGS levels at 115, 120, 125 and 130%.

Dissolved Gas Exposure Trials

We initially stocked 12 fish into each of two treatment tanks in which the TDGS was 128%. However, within 2 h the TDGS dropped to ≤115% and we were unable to increase it—perhaps because the activity of the fish or their respiration reduced TDGS. As a result, all subsequent trials were conducted with six fish per tank (two treatment and one control tank). For each trial, 18 fish were removed from the adult holding facility and rapidly anaesthetized using tricaine methanesulfonate (MS-222; 86 ppm). Individual fish were tagged with color-coded, external anchor tags in order to visually identify treatment groups. Each fish also received a passive integrated transponder (PIT) tag that was injected into the dorsal sinus. The fish were then randomly assigned to one of the treatment tanks. Fish were allowed to acclimate in the tanks with untreated (i.e., TDG ≤ 100%) water for 24 hr prior to beginning the dissolved gas exposure. During the acclimation period, aeration was provided to all tanks by an air pump and air stones. After 24 hr, the air stones were removed and airflow into the DGS was started to increase the TDGS to two tanks, while the third tank continued to receive untreated water as a control. Dissolved gas levels in all tanks were recorded at regular intervals (every 30 min to 2 hr) using a Weiss saturometer. Tank water temperatures and oxygen levels were also monitored, and were stable throughout all of the trials (9 ± 1 ºC; $[O_2] > 7.0$ ppm). The dissolved gas exposure continued until signs of near mortality (due to TDGS exposure) began to occur in at least one fish. These signs were typified by rapid and erratic swimming and jumping, followed by a complete loss of equilibrium. Once these signs were present in one fish, all of the fish in that tank were rapidly netted, anaesthetized, and transported to the adult holding facility. After the signs of near mortality had occurred in

both of the treatment tanks, the control fish were transported to the adult holding facility as well.

Spawning

After the final dissolved gas exposure trial, male fish were stocked with the experimental fish at a ratio one male to one female. Fish were checked weekly for readiness to spawn beginning on August 8, 2000. Spawning was done using standard hatchery techniques, in which fish were crowded, rapidly anaesthetized (86 ppm MS-222) and checked for spawning readiness by palpating the fish's abdomen. Unripe fish were immediately returned to the adult holding facility. Ripe females were killed by a blow to the head, and bled by severing the caudal peduncle; males were killed with a blow to the head but were not bled. Prior to fertilization, a sample of eggs in ovarian fluid was removed and held on ice for measurement of egg diameter and weight. Eggs were fertilized with a 1:1 male / female ratio; fertilized eggs were transported to the incubation facility, where an equal volume of water was added to eggs and allowed to stand for one minute for fertilization and water hardening. The eggs were then washed twice with about 19 l of water, placed in Heath incubation trays and treated in an iodophor (Argentyne, Argent Chemical Co., Redmond, WA; 75 ppm) bath for 30 min. After iodophor treatment, eggs were washed with fresh water, and placed in an incubation system. For each female, mean egg diameter (n = 30) and total egg weight was determined using a dissecting microscope (10x) with an ocular micrometer and an analytical balance.

Disease in Spawners

To determine if an acute TDGS exposure altered disease resistance, study fish were tested for the presence of fish pathogens by the U.S. Fish and Wildlife Service, Lower Columbia River Fish Health Center using standard fish pathology protocols. Briefly, samples were collected aseptically from ovarian fluid (females) or kidney, spleen and gills (males) and inoculated on cell cultures to test for the presence of viruses. Approximately 0.5 g of anterior and posterior kidney were collected aseptically for bacteriological analyses. A subsample of the kidneys was inoculated onto tryptic soy agar media to test for the presence of bacteria. All of the kidney samples were processed and tested for the presence of *Renibacterium salmoninarum* (the causative agent of bacterial kidney disease; BKD) using the double-antibody, enzyme-linked immunosorbent assay (ELISA) of Pascho et al. (1991).

Egg Rearing

Between fertilization and eye-up, eggs were treated 2-3 times a week with formalin (1673 ppm) as part of standard hatchery procedures. After eye-up (about 30 days post spawning) developing embryos were shocked by pouring them into an egg basket (suspended in water) from a height of about 0.5 m. Four to six hours later dead eggs were removed, and remaining live eggs were counted using an automated counter (Jensorter Inc., Bend, OR). The total of all eggs counted—including the sub-sample at the time of spawning, dead eggs after fertilization, and those counted by the automated counter—was used to calculate absolute fecundity. Relative fecundity was determined by dividing absolute fecundity by kg body weight. Formalin treatments were halted in all

trays after eggs from the first week of spawning became eyed. Following eye-up, trays were checked weekly for dead eggs. The study was halted at the button-up stage (about 4 months post spawning).

Statistical Analysis

Differences in egg size within a single TDGS trial were assessed using ANOVA, with $p \leq 0.05$ for all tests of significance. There were no significant differences between the replicates (A and B) in any trial, so treatment group values within a trial were combined and used for subsequent analyses. Values for the control groups from all six trials were compared using ANOVA. No significant differences were found with the exception of the control group from trial 5. However, this difference was dependent on the inclusion of a single data point. We combined the data from all of the control groups, and compared the treatment values from each trial with both the combined control values as well as the control group alone from that trial using ANOVA. We used a similar analytical scheme for fecundity, mean BKD ELISA values, and the cumulative mortality (at eye-up and button-up stages) data. With the exception noted above, there were no differences between treatments and individual trial controls or combined controls.

Results

Exposure Trials

The exposure trials began on July 17, 2000 and concluded on August 3, 2000. Because we had a limited number of fish and limited time (spawning began on August 8), we were only able to conduct six separate trials. Target TDGS treatment level was chosen

randomly, without replacement, for each trial. The target levels were 115, 120, 125 and 130 %; however, we were unable to obtain the highest target TDGS levels before mortality occurred. Five of the six trials were conducted at mean TDGS levels of between 114.1 and 118.8%, and the sixth was 125.5% (Fig. 1). The time to first mortality was closely related to the level of TDGS (Fig. 1). In all but two cases (replicate B in trials 4 and 6) the first fish died before or shortly after transport back to the adult holding tank. At the highest TDGS level (≥ 125%), mortalities in the treatment groups occurred within 10 h (Fig. 1, Trials 1 and 5). Conversely, at the lowest TDGS level (≤ 115%) (Fig. 1, Trial 6) first mortalities did not occur until about 46 h after the start of the treatment.

Reproductive success

One fish that was exposed to TDGS was later determined to be a male; thus, the ultra-sound technique for selecting females was 98.8% accurate. A low level of adult mortality was observed during the period between the end of the exposure trials and spawning. Mortality (excluding mortalities immediately after or during TDGS exposure) for all of the groups was 11.0 ± 2.9 %. This level of mortality was not consistently related to TDGS exposure and included some control fish. Comparing this mortality to the general hatchery population is difficult because most of the fish that returned to the hatchery were surplus and were not held until spawning. There were, however, 830 fish spawned at the hatchery and 58 (6.5%) died in the ponds before spawning (Jim Rockowski, Little White Salmon NFH, unpublished data).

In our study, a total of 82 females were spawned on five days (8, 15, 22, 28 August and 5 September). The time of ovulation (readiness to spawn) did not vary consistently between the control and treatment groups (Fig. 2). There were no significant relationships between relative fecundity (Fig. 3A), gonadsomatic index (Fig. 3B), average egg weight (Fig. 4) or egg diameter (Fig. 4), and TDGS exposure. The absolute fecundity of all fish (treatment and controls) in the study was 3937 $\pm$ 82 eggs per female (mean $\pm$ SE; n = 82), as compared to 4,135 eggs per female spawned for hatchery production (1,732,592 eggs from 419 females; Jim Rockowski, Little White Salmon NFH, unpublished data). The percent fertilization was consistently high (>95%) and did not vary significantly between the control and treatment groups. There was no significant relationship between TDGS exposure and cumulative mortality at eyed-egg and button-up stages (Fig. 5). Survival to the eyed stage was 94.1% for eggs from treatment fish and 95.1% for eggs from controls; survival to eyed stage in the hatchery was 95.1% (Jim Rockowski, Little White Salmon NFH, unpublished data).

Disease

All spawned females were screened for the presence of *R. salmoninarum* and subsamples of spawned females were screened for the presence of viral (n = 35) and bacterial (n = 8) pathogens. No fish pathogens, with the exception of *R. salmoninarum*, were found in this subsampled group. The level of R. *salmoninarum* was determined in 78 of the 82 fish in the study and in all fish spawned for hatchery production; there was no significant relationship between prevalence or severity of *R. salmoninarum* and TDGS exposure. The overall prevalence of fish used in this study was 44.2%, while prevalence in all other

females spawned at the hatchery was 46.3%. Severity of R. *salmoninarum*, as measured by average optical density in the ELISA (Fig. 6), was 0.248 $\pm$ 0.055 (mean $\pm$ SE; n = 78) in all study fish, as compared to 0.402 $\pm$ 0.032 for hatchery fish (n = 419); both of these mean values suggest medium severity of infection. Median optical density was 0.085 for both groups, which is considered a low level of infection.

Discussion

In the present study we exposed female adult spring chinook salmon to water in which total dissolved gas was supersaturated between 114.1 and 125.5%, and found that times to first mortality were positively related to TDGS level (Fig. 1). Our results are consistent with other studies of both juvenile and adult chinook salmon, although most other studies report time to 20% or 50% mortality (LT20; LT50). Nebeker (1973, as cited by Ebel et al. 1975) reported a LT50 for adult chinook salmon held in 125% TDGS of about 17 h; Mesa et al. (2000) estimated LT20 for juvenile chinook salmon at 130% TDGS to be 3 to 6 h. We found that these acute exposures to gas supersaturated water had no effect on selected measures of reproductive success in maturing female spring chinook salmon. The variables that we examined included pre-spawning mortality, and addressed possible effects on maturational processes (i.e., fecundity, GSI and egg diameter), fertilization success, and survival of embryos to the button-up stage.

Several earlier studies examined mortality of adult salmon relative to TDGS in laboratory experiments (Nebeker 1973, as cited by Ebel et al. 1975) and in the field (Beiningen and Ebel 1970) but in very few studies was TDGS correlated to some measure of

reproductive success. Ebel et al. (1975) reported that in years in which nitrogen supersaturation was ≤ 110% the chinook salmon redd count indices (redds/fish passing Ice Harbor Dam) in the Salmon River, ID were from 10 to 19; whereas, when nitrogen supersaturation was 125–135% the indices were only 6 to 8. These results suggest that in the wild, TDGS can reduce reproductive success via pre-spawning mortality or disrupting the fish's ability to build redds. Westgard (1964) reported that TDGS blinded some adult chinook salmon in a spawning channel near McNary Dam, and the blinded fish suffered 88% pre-spawning mortality. Fish in the same environment that were not blinded had only 6% pre-spawning mortality. The surviving blinded females could not make redds, while blinded males apparently could not find the eggs to fertilize them. Westgard (1964) reported that TDGS-related blinding also lead to a proliferation of fish pathogens (bacterial, fungal, and protozoan). In the present study, however, short-term exposure to TDGS had no effect on the presence of viral or bacterial fish pathogens, or the presence and severity of *R. salmoninarum*—the causative agent of BKD.

One of our early hypotheses was that the stress of exposure to TDGS might affect reproductive success independent of the physiological effects of TDGS. In our experiments, control fish were handled in the same way as fish exposed to TDGS (i.e., anesthetized, tagged, transported etc.), which should have caused a stress response in the control fish. Because there were no differences between control fish and TDGS-exposed fish for any of the variables tested, one might conclude that the effects of handling stress overpowered the effects of TDGS. However, measurements taken from the general hatchery population (i.e., pre-spawning mortality, absolute fecundity, % fertilization,

survival to eyed-egg) did not differ from those in our study, suggesting that the combined stress of our handling and TDGS did not affect reproductive success. This is consistent with reports on the effects of stress on reproduction (see review: Schreck et al. 2001). In several studies, fish were stressed repeatedly with little, or no, effect on measures of reproductive success (Contreras-Sanchez 1995, Morgan et al. 1999). It appears that the timing relative to maturation, the severity, and duration of the stresses are important variables regulating stress effects on reproduction. Moreover, Schreck et al. (2001) recently proposed physiological mechanisms through which eggs are protected from maternal responses to stress by mitigating the amount, availability and biological activity of cortisol—the primary stress hormone.

Even though we report that acute exposure to TDGS (up to 125.5%) did not affect reproductive success of spring chinook salmon, this does not mean that TDGS does not have population-level effects on salmonids in the wild. We selected spring chinook salmon for these experiments because they typically enter the freshwater 3 to 6 months before spawning—allowing an extended time for the adverse effects of TDGS to become evident. However, chinook salmon are perhaps the salmonid most resistant to TDGS—fewer than 0.2% of 4,667 chinook salmon examined by Backman and Evans (in press) had signs of GBT, as compared to 5.6% of sockeye and 1.5% of steelhead. While the differences in prevalence reported by Backman and Evans (in press) could be the result of differing exposure histories, earlier laboratory experiments also suggested that sockeye salmon and steelhead are more susceptible to TDGS than are chinook salmon (Nebeker 1973).

Many have suggested that interpreting the significance of signs of GBT in individual fish in the wild without exposure history is impossible (Mesa et al. 2000, Ryan et al. 2000, Backman and Evans in press). The results of our study, however, may be applicable to adult chinook salmon as they approach and pass hydropower dams. This passage requires fish to enter the relatively shallow water below the dam (tailrace), find and enter an adult fish ladder, and swim to the top of the ladder. Bjornn et al. (2000) reported that in 1996 the median time taken by adult chinook salmon passing Columbia and Snake river dams (i.e., from the tailrace to the top of the fish ladder at the same dam) was between 18.0 to 38.2 h, which is very similar to the duration of exposures in our study (Fig. 1). Thus, it would appear that potential TDGS exposure while passing one dam would not adversely affect chinook salmon reproductive success. Moreover, recent studies using archival, pressure-sensitive depth tags suggest that adult chinook salmon and steelhead immigrating between dams in the Columbia River spend 60% of their time below 2 m deep (personal communication Dr. T. Bjornn, USGS-BRD, University of Idaho). Hydrostatic compensation at this depth means that fish should be able to resist the adverse effects of TDGS as high as 120%, and may also offer protection when the fish are in shallower water with high TDGS (Knittel et al. 1980). The next step in elucidating the effects of TDGS on reproductive success of adult salmonids is to duplicate, in the laboratory, migration scenarios based on archival tag records and in-river TDGS monitoring records, and monitor the reproductive success in fish exposed chronically to TDGS.

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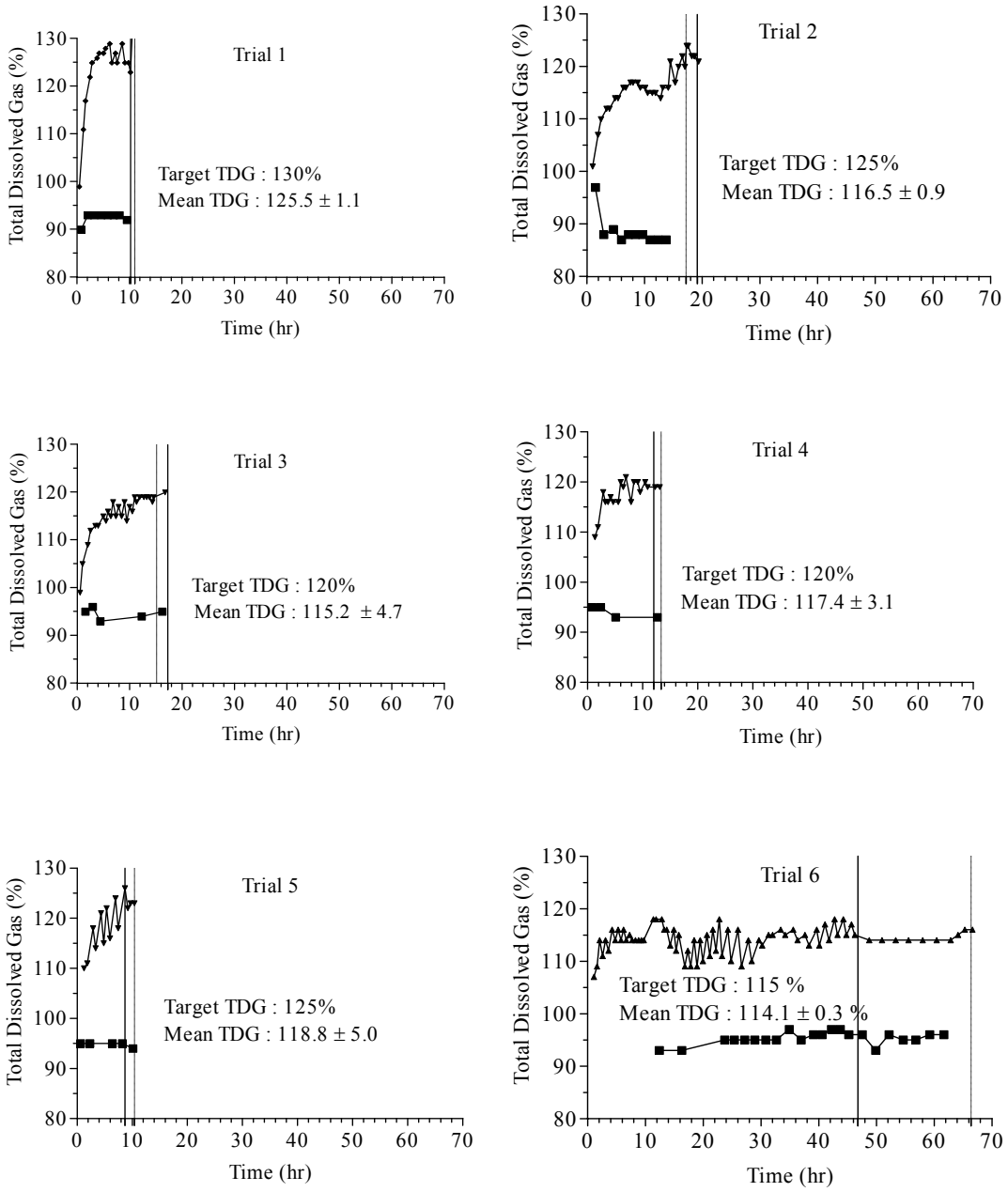

Figure 1. Exposure of adult female chinook salmon to increasing levels of total dissolved gas (% saturation; TDG). The two treatment tanks combined (▲) and a control group (■) are shown for each trial. The vertical solid line is the time to the signs of first mortality in treatment group A, and the dashed line is for the time to the signs of first mortality in treatment group B. Sample sizes were six fish in each tank. The target TDG level and the observed mean (± SE) TDG level in the treatment tanks is given for each trial.

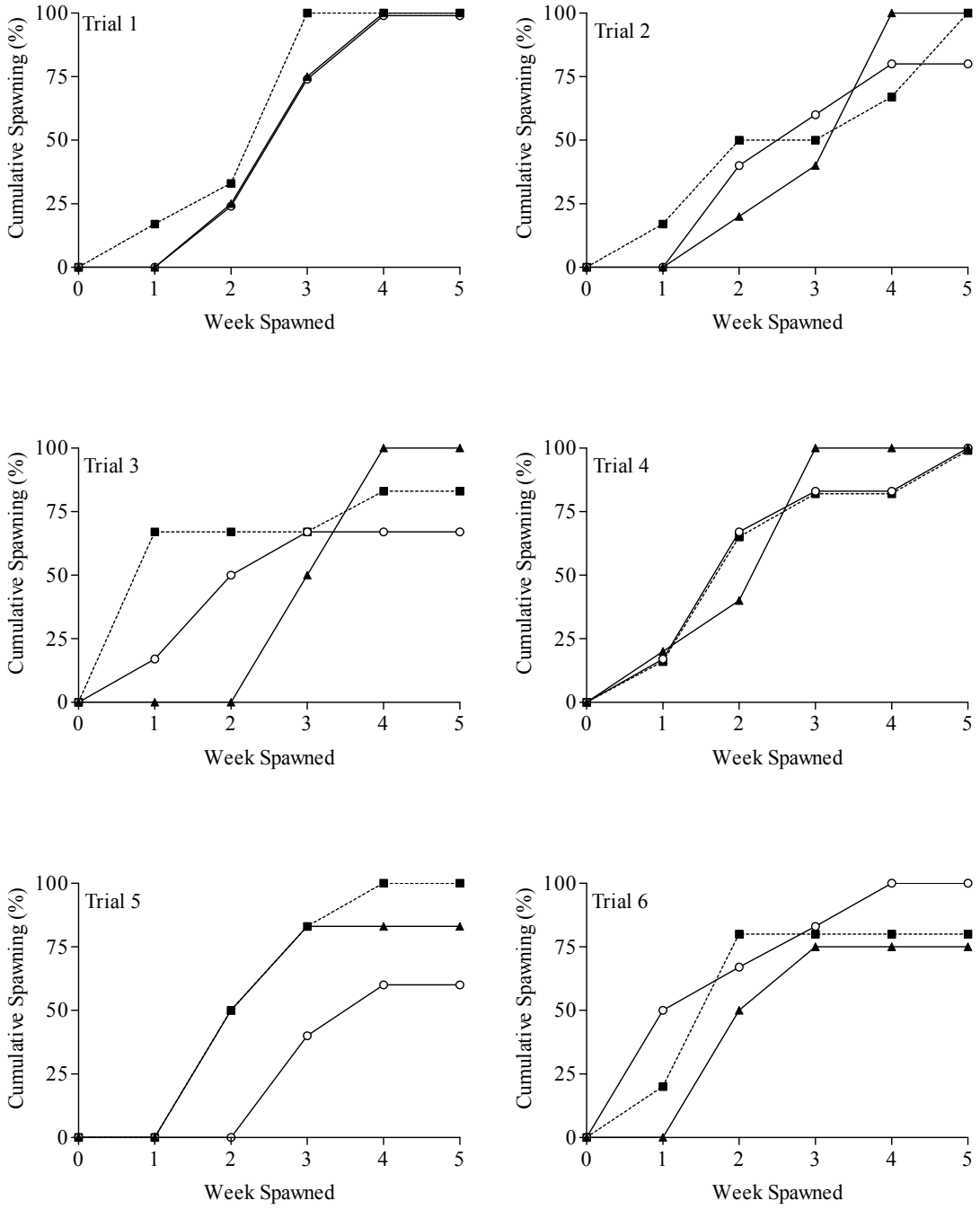

Figure 2. Time of spawning for adult female chinook salmon exposed to increasing levels of total dissolved gas (N = 6 fish/tank). Trial designations are the same as for Fig. 1. Treatment groups A (▲) and B (○) and the control group (■) are shown for each trial.

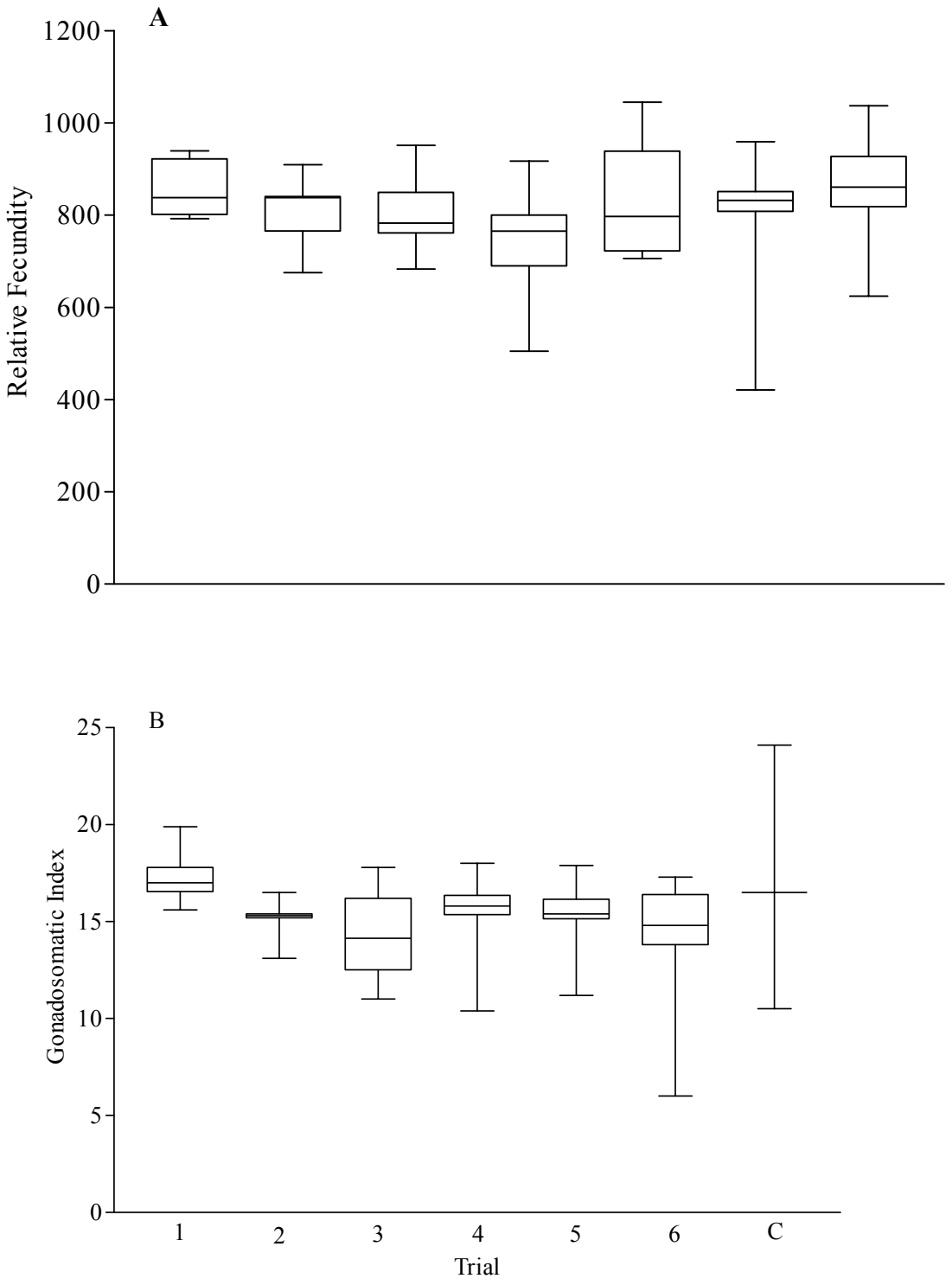

Figure 3. Relative fecundity (eggs/kg body wt; A) and gonadosomatic index (B; total egg wt / body wt x 100) of spawned female chinook salmon. The trial numbers are defined in Fig. 1. The treatment groups (A & B) were combined, and C is the control group for all trials combined. The plotted boxes contain data values within the 25th and 75th percentile, the horizontal lines are the median values, and the vertical lines represent the entire range of the data.

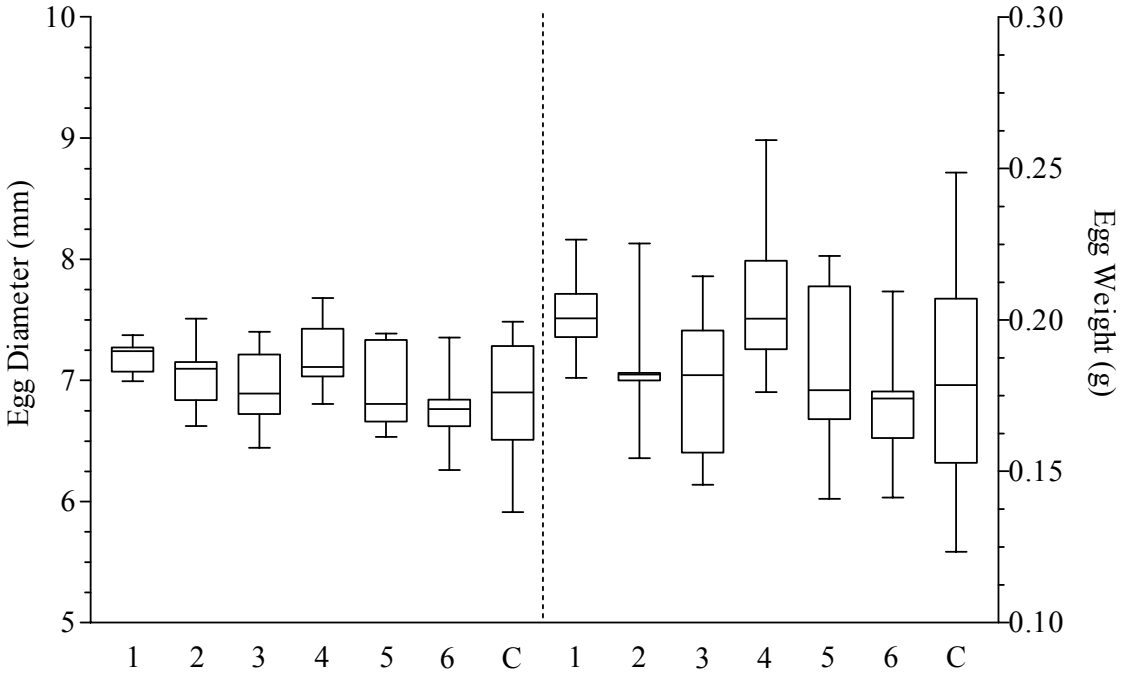

Figure 4. Mean egg diameter and mean egg weight of spawned chinook salmon. The trial number given on the X-axis is the same as in Fig. 1. The treatment groups (A & B) were combined, and C is the control value for all trials combined. The mean diameter and weight were determined on a sample of 30 eggs from each individual. The plotted boxes contain data values within the 25th and 75th percentile, the horizontal lines are the median values, and the vertical lines represent the entire range of the data.

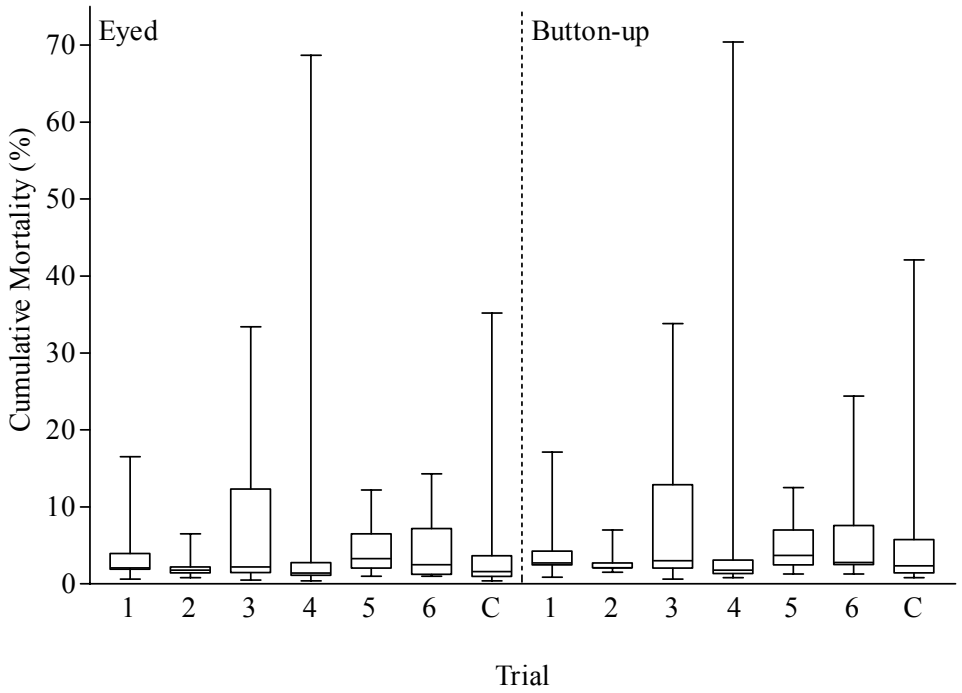

Figure 5. Cumulative mortality at the eyed and button-up stages. The number under each error bar denotes the trial number. The treatment groups (A & B) were combined, and C are the control group values for all of the trials. The plotted boxes contain data values within the 25th and 75th percentile, the horizontal lines are the median values, and the vertical lines represent the entire range of the data.

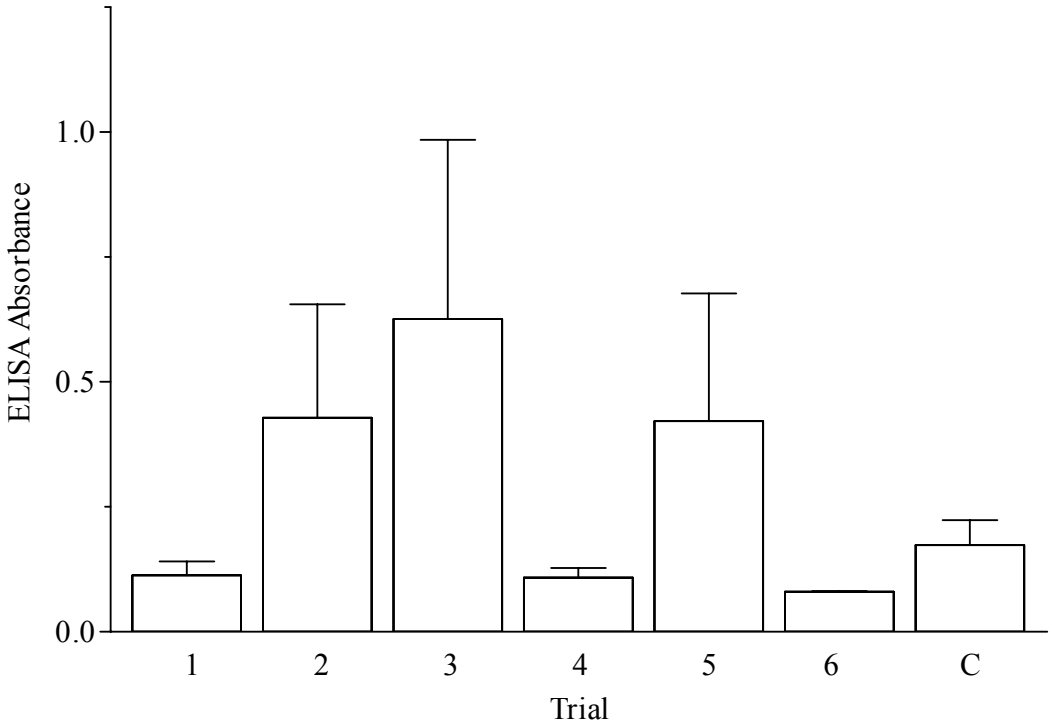

Figure 6. Mean (± SE) ELISA absorbances of spawned females. Kidney samples were processed and assayed for the presence of *Renibacterium salmoninarum*. The number under each bar denotes the trial number as given in Fig. 1. The treatment groups (A & B) were combined, and C is the control group value for all of the trials.

Appendix A.

Diagrams of the dissolved gas system and exposure tanks built at Little White Salmon National Fish Hatchery to create gas-supersaturated water. Adult spring chinook salmon were given acute exposures to gas-supersaturated water to determine the effects of exposure on reproductive success.

List of Figures

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Figure A-2. North-facing view of tower of dissolved gas system built at Little White Salmon National Fish Hatchery.

Figure A-3. East-facing view of tower of dissolved gas system built at Little White Salmon National Fish Hatchery.

Figure A-4. South-facing view of tower of dissolved gas system built at Little White Salmon National Fish Hatchery.

Figure A-5. West-facing view of tower of dissolved gas system built at Little White Salmon National Fish Hatchery.

Figure A-1. Plan view of dissolved gas system built at Little White Salmon NFH.

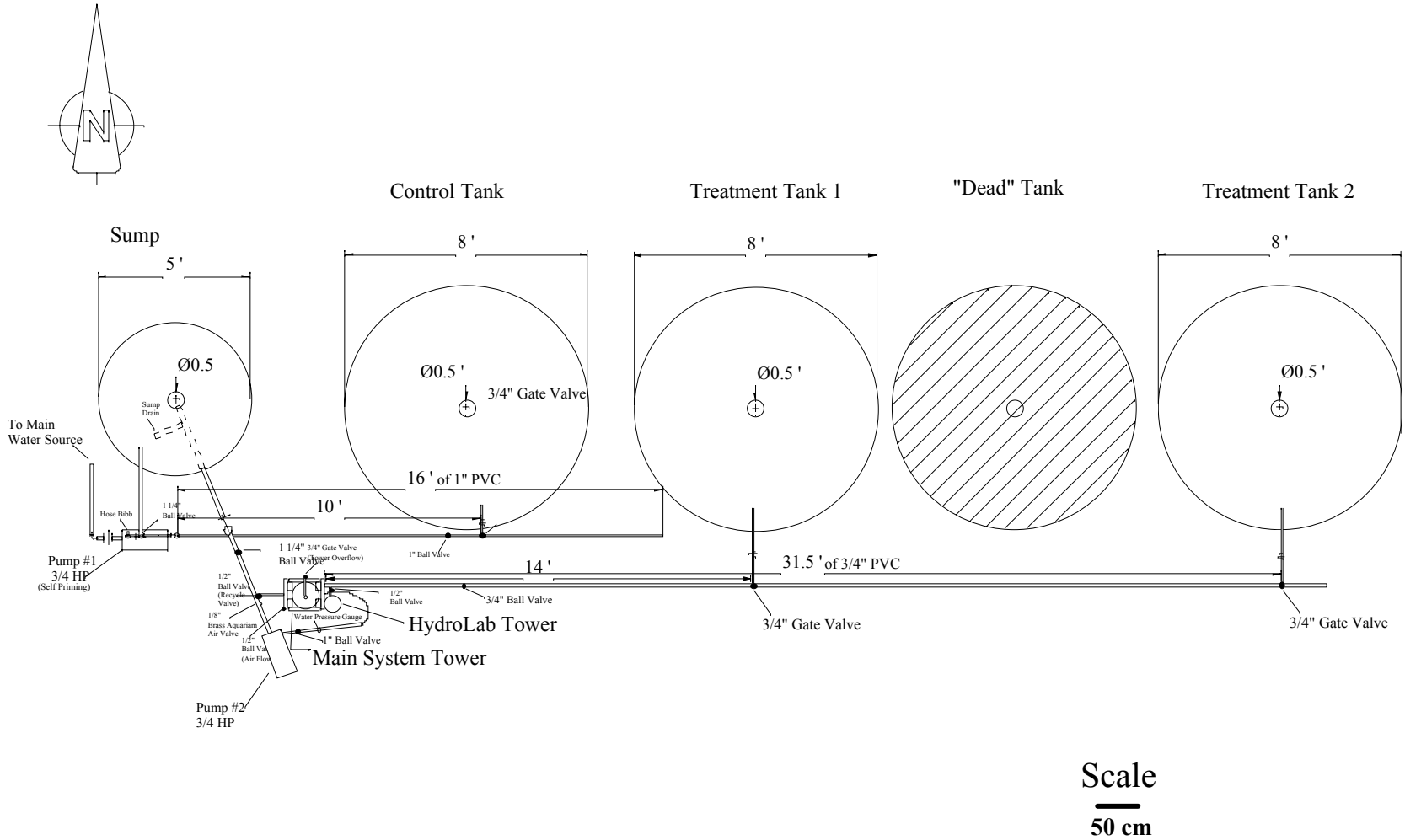

Figure A-2. North-facing view of tower of dissolved gas system built at Little White Salmon NFH.

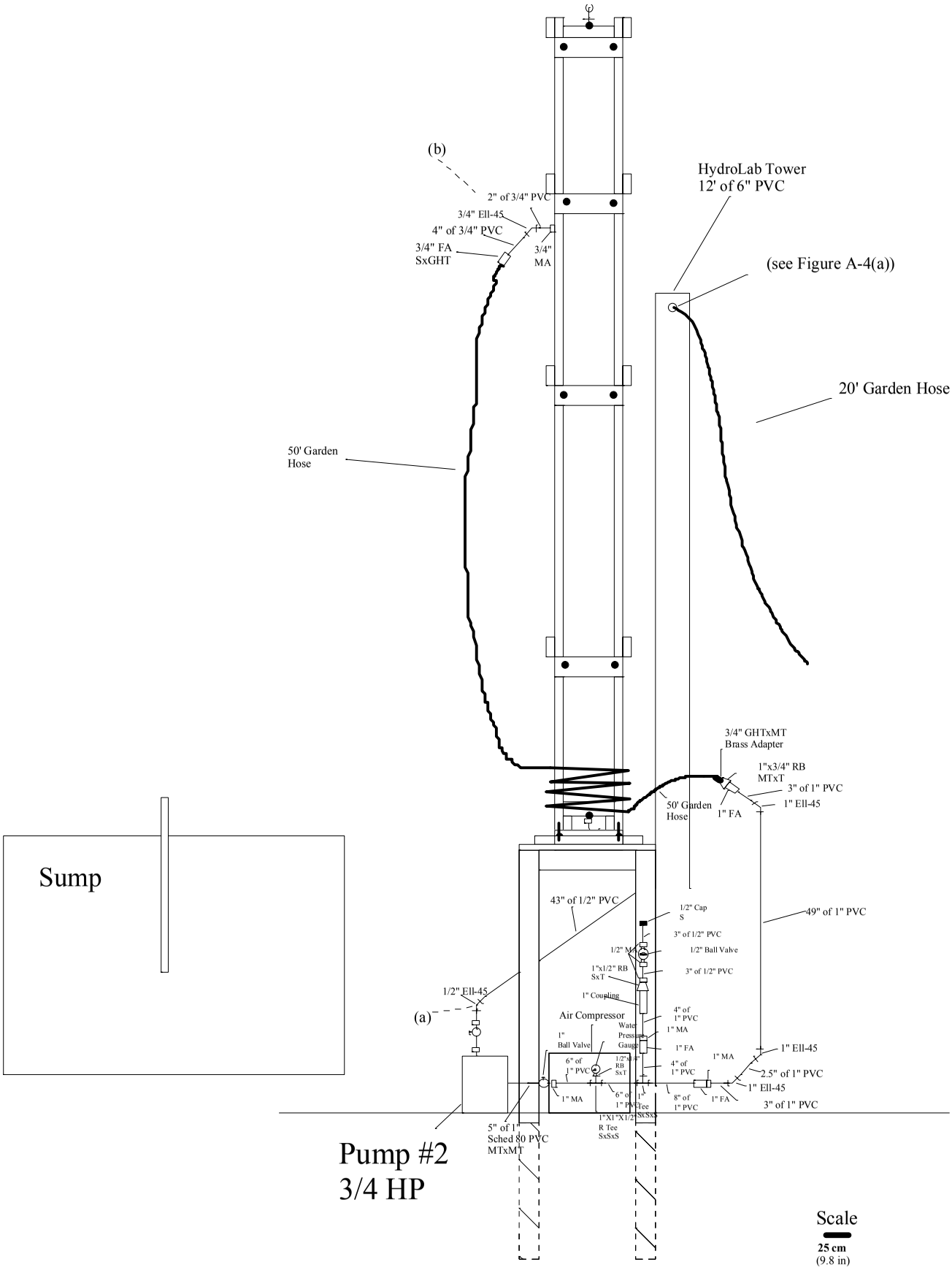

Figure A-3. East-facing view of tower of dissolved gas system built at Little White Salmon NFH.

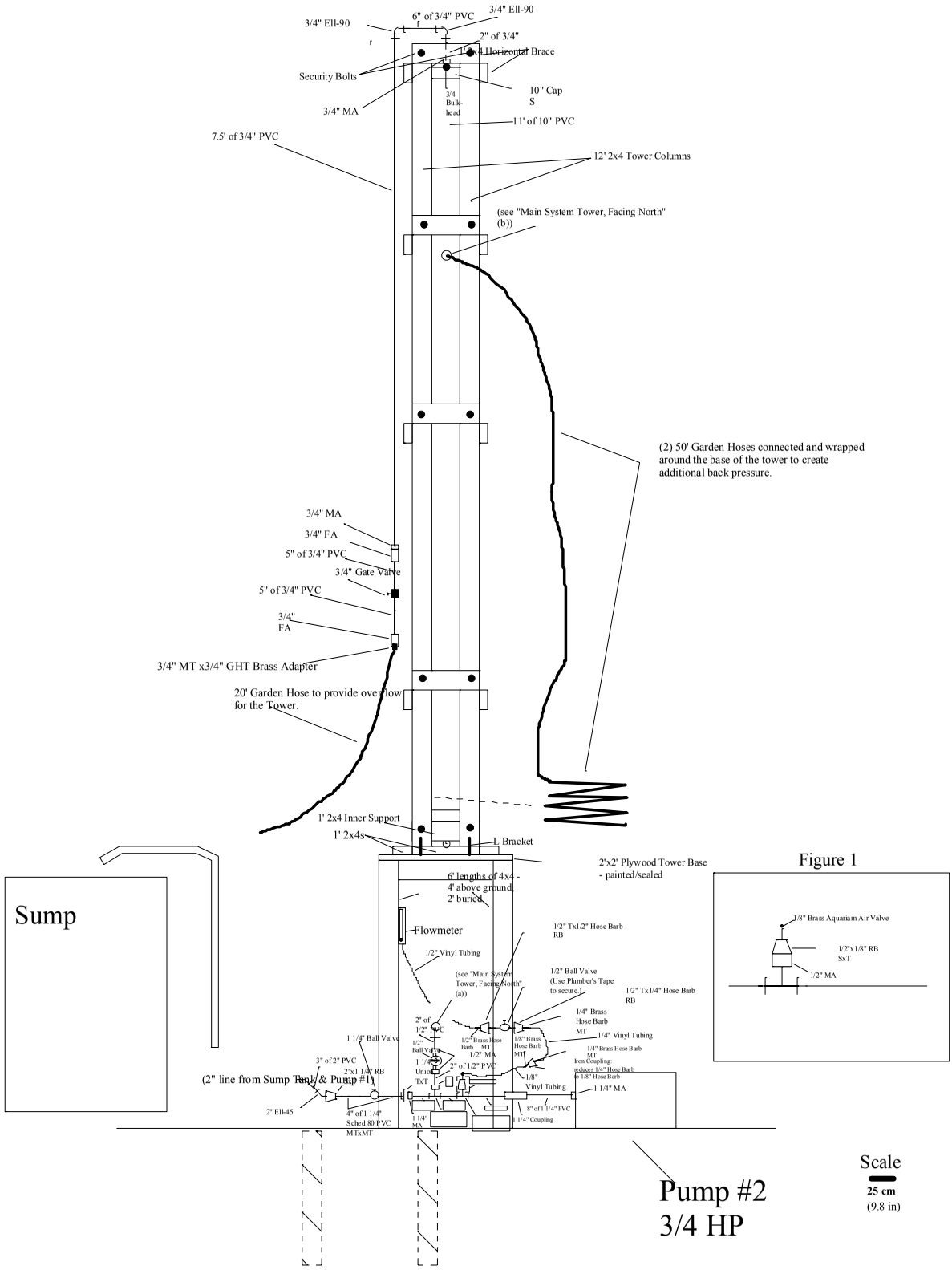

Figure A-4. South-facing view of tower of dissolved gas system built at Little White Salmon NFH.

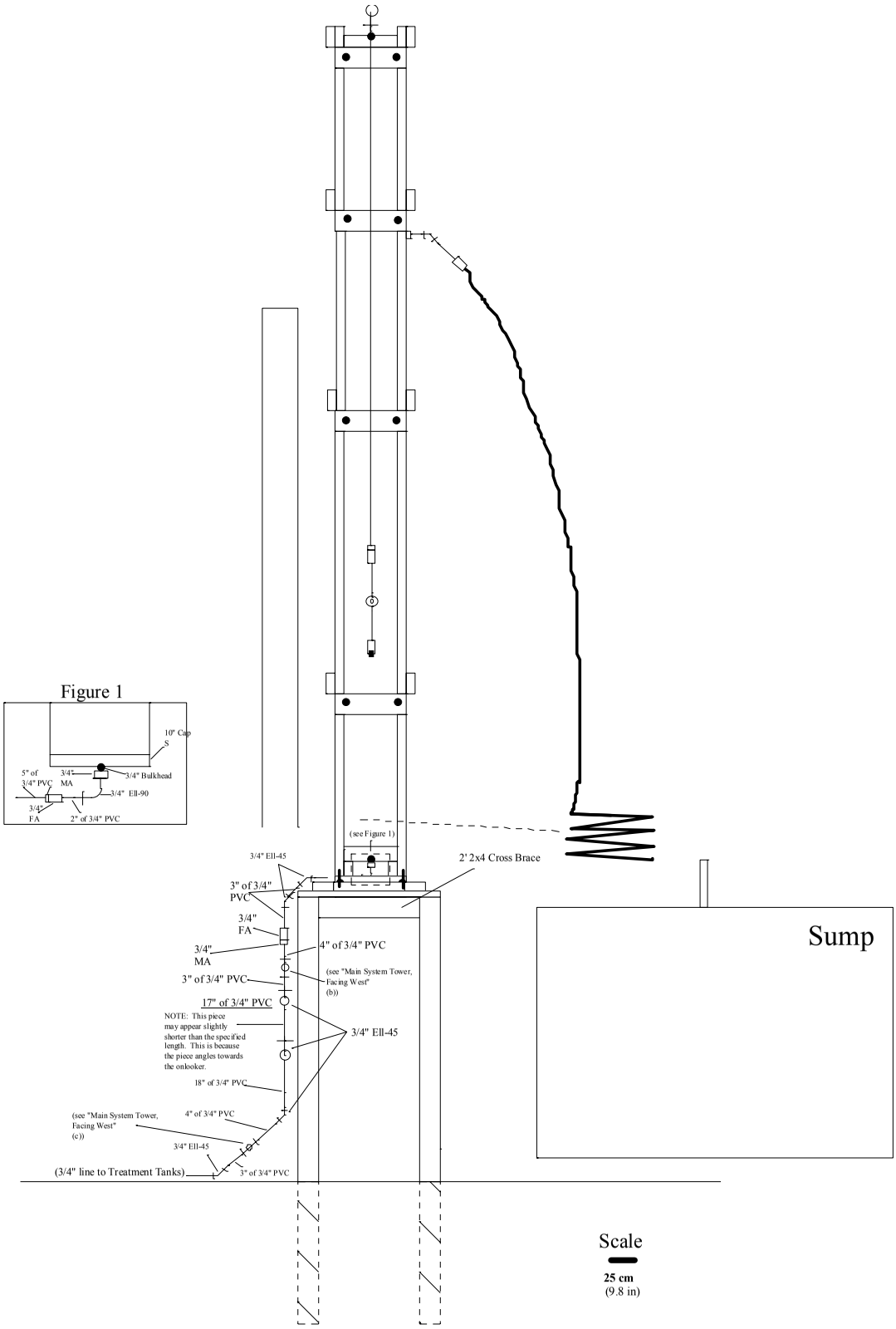
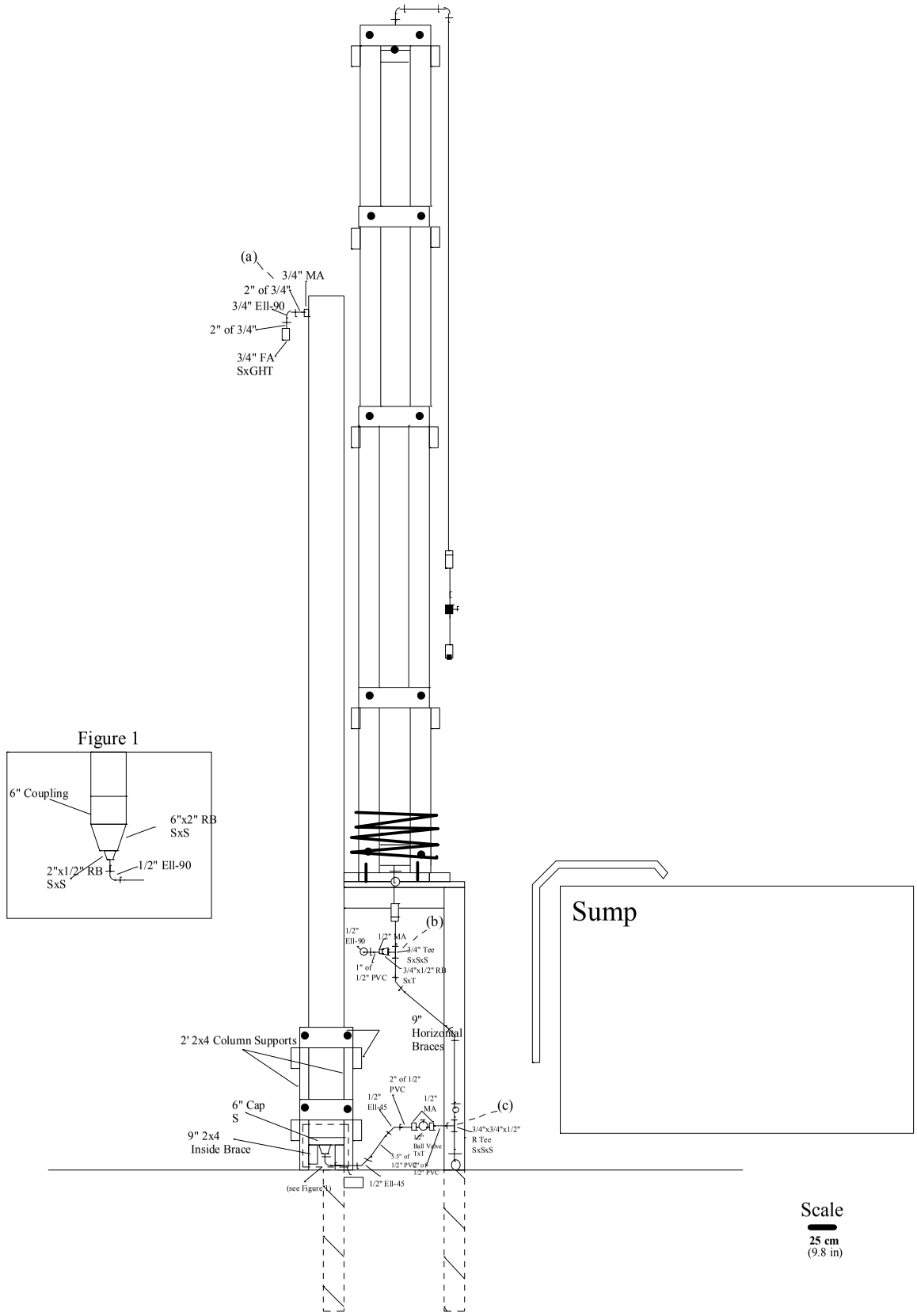
Figure A-5. West-facing view of tower of dissolved gas system built at Little White Salmon NFH.