



**CERATOMYXA SHASTA EXPOSURE TRIALS  
AT OXBOW FISH HATCHERY 2005**

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## ABSTRACT

Seven day exposure trials were conducted from April 2005 into June 2005 at the Snake River water intake at Oxbow Fish Hatchery as a continuation of the *Ceratomyxa shasta* exposure trials of 2004. The results from the 2004 trials were reported in the Idaho Department of Fish and Game Fishery Research Report 04-48. The intent of the 2005 study was to refine and to focus the conclusions acquired in 2004, especially emergence of the parasite into the Snake River. The first two trials in April, and the first trial in May 2005, did not produce infections of *C. shasta* in sentinel fish. Once infection commenced in the fourth trial, prevalence of infection increased in sentinel fish as Snake River water temperatures warmed. The last three trials, which occurred in May, June and July of 2005 had prevalence of infection of 20%, 51%, and 79% respectively. No significant relationships were found between prevalence and water chemistry parameters.

We maintain the recommended release of fall Chinook salmon sub-yearlings from Oxbow Fish Hatchery during early May to reduce exposure to the infective stage of *C. shasta*.

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## INTRODUCTION

The Idaho Department of Fish and Game (IDFG) has operated the Oxbow Fish Hatchery (OFH) since its construction in 1961. This facility serves primarily as an egg taking station that provides steelhead *Oncorhynchus mykiss* eggs for Niagara Springs Fish Hatchery. Adult steelhead are trapped below Hells Canyon Dam, transported to OFH, and held until spawning. Spring Chinook salmon *Oncorhynchus tshawytscha* are trapped below Hells Canyon Dam, transported to Rapid River Hatchery, and held until spawned. The OFH is funded by Idaho Power Company (IPC) as a part of the mitigation for the hydroelectric system of the Hells Canyon Complex.

The Hells Canyon Settlement Agreement (HCSA) of 1980 specified propagation of fall Chinook salmon as a mitigation requirement for IPC. Early attempts to satisfy this requirement failed because of poor water quality parameters such as elevated water temperatures during holding and spawning of adults, or low water temperatures during egg incubation and early rearing. In December 2000, IDFG began to evaluate the rearing of fall Chinook salmon using well water during early rearing, and a mixture of well water and water from the Snake River to complete rearing. The water from the Snake River contains the infective actinospore stage of *Ceratomyxa shasta* (Munson et al 2005).

*Ceratomyxa shasta* is a myxosporean that infects freshwater salmonids (Bartholomew et al. 1989) and has a distribution of the Pacific Northwest which includes California, Idaho, Oregon, Washington, British Columbia, and Alaska. The alternate host is the polychaete worm *Manayunkia speciosa* (Bartholomew et al. 1997). The infective actinospore is released from the worm host, attaches to the salmonid host, and invades. Neither horizontal nor vertical transmissions in the salmonid host have been documented. Diagnosis is usually accomplished by microscopic detection of spores from intestinal scrapings or polymerase chain reaction (PCR). Infectivity of *C. shasta* seems to be enhanced by warmer water temperatures, low water flows, and high infective actinospore numbers (PacifiCorp 2002).

Avoidance of the parasite is the only effective management practice since there is no treatment for the infection. Although treatment of hatchery water supplies through sand filtration, ozonation, ultraviolet irradiation, and chlorination have been successful in decreasing infections in production facilities, these methods cannot completely eliminate infection. The first exposure trials at OFH in 2004 estimated *C. shasta* emergence in the Snake River in early May of 2004 and prevalence of infection reaching 91% in mid-June of 2004 (Munson et al. 2005).

## STUDY SITE

This study was conducted at OFH, which is located on the Oregon side of the Snake River in Baker County, at river mile 270 (602 river miles or 969 km from the Pacific Ocean). The hatchery pump station is located approximately 200 meters downriver of Oxbow Dam Hydroelectric Plant (UTM 11T 511485E 4979901N). The hatchery rearing protocol is to use well water for early rearing of fall Chinook salmon, while a combination of well and Snake River water is used to complete the rearing of sub-yearling salmon prior to release. The same exposure site used in the 2004 trials was selected for the 2005 exposure trials at OFH (Figure 2) (Munson et al 2005). The live-box was placed in the Snake River at the hatchery's pump station, where river water is acquired for fish culture.

## OBJECTIVES

We initiated this study to support data collected during the original exposure trials performed in 2004, which attempted to determine the onset of *C. shasta* parasitic exposure to fall Chinook salmon reared at OFH. Furthermore, we continued to investigate the prevalence of infection after a 7-day exposure using sentinel fish. If appropriate, recommendations will be made to alter current rearing protocol of fall Chinook salmon to reduce the impacts of *C. shasta*.

## METHODS

Tripliod rainbow trout *Oncorhynchus mykiss* eggs were obtained from Hayspur Hatchery and reared in well water at the Eagle Fish Health Laboratory (EFHL). These rainbow trout (RBT) ranged in size from 0.55 to 1.57 g/fish during the exposure trials (See Table 1). Each exposure trial consisted of 50 RBT placed in a cylindrical aluminum box or experimental unit (EU) that measured 47 cm in length x 30.3 cm in diameter. Limited holding space in the EFHL wet lab restricted the number of EUs to one per trial. The RBT were transported to and from OFH in plastic bags with water and oxygen. These fish were challenged by exposure to Snake River water for 7 d, returned to the wet laboratory at EFHL, and held in 13°C well water for 60 d; approximately 650 Celsius temperature units (CTU's). Each EU was equipped with a STOWAWAY XTI temperature logger to monitor water temperatures. Flow data was acquired from the Idaho Power Company website by subtracting the daily flow at the Pine Creek Gaging Site from the average daily flow at the Hells Canyon Gaging Site. This approximated the average daily flow at OFH (See Table 1). An YSI meter (model 556 MPS) was used to determine pH, temperature, dissolved oxygen, percent dissolved oxygen, and conductivity. Turbidity was measured with a Hanna Industries HI 93703 turbidimeter. Mortalities were recorded daily and wet mounts of intestinal fluid from these fish were examined microscopically for *C. shasta* spores. Fish mortalities that were not positive by microscopic examination were stored frozen and tested for CS by polymerase chain reaction (PCR). The combined data was used to determine prevalence.

Once the holding period was completed, the remaining fish were euthanized with tricaine methane sulphonate (MS 222) and the last third of the large intestine removed for examination. Initial examination of intestinal wet mounts followed the protocol established by the American Fisheries Society Blue Book, 4<sup>th</sup> edition (Bartholomew 2001). The same section of gut, from fish that were not positive by microscopic examination, was placed in alcohol for confirmation through PCR diagnostic assay (Palenzuela et al. 1999).

All data analysis was performed using SYSTAT 11 (SYSTAT Incorporated, Chicago, Illinois). Potential explanatory variables for the prevalence of *C. shasta* were examined using Pearson and Spearman correlation matrices. Correlations of explanatory variables and prevalence were assessed. Explanatory variables that were significantly correlated ( $R > 0.5$  and  $P < 0.05$ ) with each other were not used in the same model. Prevalence was arc sine transformed for regression assumptions. Three separate multiple regression analyses were conducted of potential explanatory variables due to missing information and small sample size.

## RESULTS

Results from the first three exposure trials conducted from (1) 4 April, 2005 until 11 April, 2005, (2) 18 April, 2005 until 26 April, 2005, and (3) 4 May, 2005 until 11 May, 2005 demonstrated that the infection rates of *C. shasta* were below levels of detection. Mortality due to *C. shasta* was not detected during holding at the EFHL wet laboratory and final sampling did not detect *C. shasta* from intestinal wet mounts or through PCR examination (Table 3). The mean water temperature was 8.16°C, 9.15°C, 11.97°C for the three trials, respectively (Table 1). The mean daily flow at OFH during the trials was 14,481 cubic feet per second (cfs), 10,740 cfs, and 16,768 cfs respectively (Table 1). Water chemistry parameters for all exposure trials are presented in Table 2.

Results from the fourth exposure trial at OFH that was conducted from 17 May 2005 to 24 May 2005 demonstrated the first positive *C. shasta* observations. Prevalence of infection was measured at 20%, and it took 41 days to produce the initial mortality attributed to *C. shasta* in these sentinel fish (Table 3). The mean days to death was 44.5 days. The mean water temperature during this trial was 13.78°C, while the daily flow averaged 31,223 cfs (Table 1).

The fifth exposure trial at OFH was from 1 June 2005 until 8 June 2005. Twenty-three fish were found to be *C. shasta* positive out of 45 fish examined for a prevalence of infection of 51% (Table 3). The first mortality due to *C. shasta* in these sentinel fish was 31 days post exposure and the mean days to death was 40.1 days. The mean water temperature was 15.5°C and the mean daily flow was 20,220 cfs (Table 1).

Results from the sixth exposure trial at OFH of 1 July, 2005 to 8 July, 2005 demonstrated the peak of infectivity of *C. shasta* during these trials. Twenty-two fish were positive for *C. shasta* of the 28 fish examined, for a prevalence of 79% (Table 3). The onset of mortality was 25 days into holding while the mean days to death decreased to 34 days. The mean water temperature was measured at 18.57°C and the mean daily flow was at 15,887 cfs (Table 1).

### **Statistical Comparisons between Exposure Trials**

No significant correlations were observed with potential explanatory variables and prevalence in Spearman or Pearson correlations. Multiple regression analysis was performed to examine relationships of Mean Temperature and Mean Discharge resulted in insufficient evidence for relationships. Analysis of potential relationships of pH, conductivity, and other water quality parameters taken at the beginning and end of each exposure trial did not result in statistically significant relationships.

## DISCUSSION

The onset of *C. shasta* infectivity in the Snake River, at the OFH water intake, was observed in the fourth exposure trial (Table 1). Lower water temperatures and elevated Snake River flows in 2005 explain differences of commencement and prevalence of infection (Table 1) when compared to the trials at OFH in 2004. Uday et al. (1975) showed that water temperatures produced differences in initial time of infection. Despite lower water temperatures and higher river flows, onset of infection in the 2005 trials was only two weeks later. This difference could possibly have been further reduced if the 2005 fourth trial timing was advanced by a week. As in 2004, once *C. shasta* infectivity began in the Snake River, it continued throughout the duration of the investigation.

Upon retrieval of the live-box after completion of the sixth trial, we noticed a hole in the screen of the live-box. Mortalities were not noticed in the live-box, thus it is assumed that many of the sentinel fish escaped. This offers an explanation of why a low number of fish were examined during the last exposure trial.

The target size of release for fall Chinook salmon at OFH is 43 fish per pound (FPP) (10.6 g/fish). This target size is usually achieved at OFH by early May. The results from this study and the study conducted in 2004, strongly suggest the release of fall Chinook salmon from OFH should continue to be in early May to avoid *C. shasta* impacts on survival after release. Fall Chinook salmon reared at OFH on Snake River water have not demonstrated *C. shasta* infection when sampled prior to release (IDFG database 2006). It has not been determined whether this is due to 1) higher susceptibility of rainbow trout to this parasite, 2) mechanical injury to the infective actinospore, or 3) parasite distribution in the Snake River water column not allowing ready uptake into the hatchery water supply. To minimize *C. shasta* exposure and to maximize survival, fall Chinook should be released in early May. This would allow OFH fall Chinook salmon to reach size of release and to avoid this parasite once discharged into the Snake River for migration to the ocean.

## RECOMMENDATIONS

The HCSA (1980) stated that IPC will provide a facility capable of producing 1,000,000 subyearling fall Chinook salmon. The Oxbow Fish Hatchery Conceptual Design Report (Fishpro 2004) details facility renovations to accommodate HCSA production goals, including well water enhancement. We recommend that OFH continue to release fall Chinook salmon subyearlings in early May to allow sufficient growth and to avoid *C. shasta* infection. These strategies should maximize survival after release into the Snake River.

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Table 1. Oxbow Exposure Trial 2005 Data.

<b>Exposure Dates</b>	<b>Daily Mean</b>	<b>Mean</b>	Minimum	Maximum	<b>Trout Mean</b>	N	<b>CS+</b>	<b>%+</b>
<b>Dates</b>	<b>Flows (cfs)</b>	<b>Temp</b>	<b>Temp</b>	<b>Temp</b>	Weight			
				(C°)				
4/4/05-4/11/05	14,481	8.16	7.7	8.78	1.3 g	50	0	0
II	10,740	9.15	8.36	9.91	1.04 g	45	0	0
4/18/05-4/26/05								
III	16,768	11.97	11.66	12.44	1.57 g	42	0	0
5/4/05-5/11/05								
IV	31,223	13.78	13.58	14.04	0.55 g	49	10	20
5/17/05-5/24/05								
V	20,220	15.5	15.14	15.93	1.45 g	45	23	51
6/1/05-6/8/05								
VI	15,887	18.57	17.84	19.47	1.0 g	28	22	79
7/1/05-7/8/05								

Table 2. Oxbow Water Chemistry Parameters.

<b>Trial</b>	<b>Time</b>	<b>Temp.</b>	<b>al</b>	<b>DO</b>	<b>Turbidity</b>	<b>Conductivity</b>
	<b>Of Day</b>			<b>mq/l</b>	<b>FTU</b>	<b>mS/cm</b>
	10:00	7.44	8.35	10.39	1.74	0.458
	10:35	8.4	8.62	9.22	2.37	0.444
II	12:00	9.03	8.41	7.75	2.12	0.438
	12:15	9.95	8.51	10.1	2.67	0.41
III	11:30	10.86	8.8	9.02	3.44	0.386
	12:00	12.07	8.78	6.69	4.69	0.374
IV	12:15	14.03	8.43	6	4.17	0.337
	11:30	14.88	8.6	8.11	3.89	0.332
V	10:30	15.13	8.2	8.22	3.53	0.335
	11:50	15.69	8.08	5.77	2.95	0.306
VI	12:30	18.15	8.39	4.68	2.63	0.287
	12:30	19.1	8.26	5.69	3.14	0.303



Table 3. Oxbow II trial infectivity data

<b>Trial</b>	<b>C. shasta + Wet Mount</b>	<b>C. shasta + PCR</b>	<b>Total C. shasta +</b>	<b>Prevalence</b>
	0/50	0/50	0/50	0
	0/45	0/45	0/45	0
III	0/42	0/42	0/42	0
IV	10/49	0/39	10/49	20%
V	23/45	0/22	23/45	51%
VI	15/28	7/13	22/28	79%

		<b>Mean days to death</b>
<b>Trial I</b>	ND	ND
<b>Trial II</b>	ND	ND
<b>Trial III</b>	ND	ND
<b>Trial IV</b>	41 days to onset to mortality	44.5
<b>Trial V</b>	31 days to onset to mortality	40.1
<b>Trial VI</b>	25 days to onset to mortality	34

ND=No data



Figure 1. IDFG Hatcheries

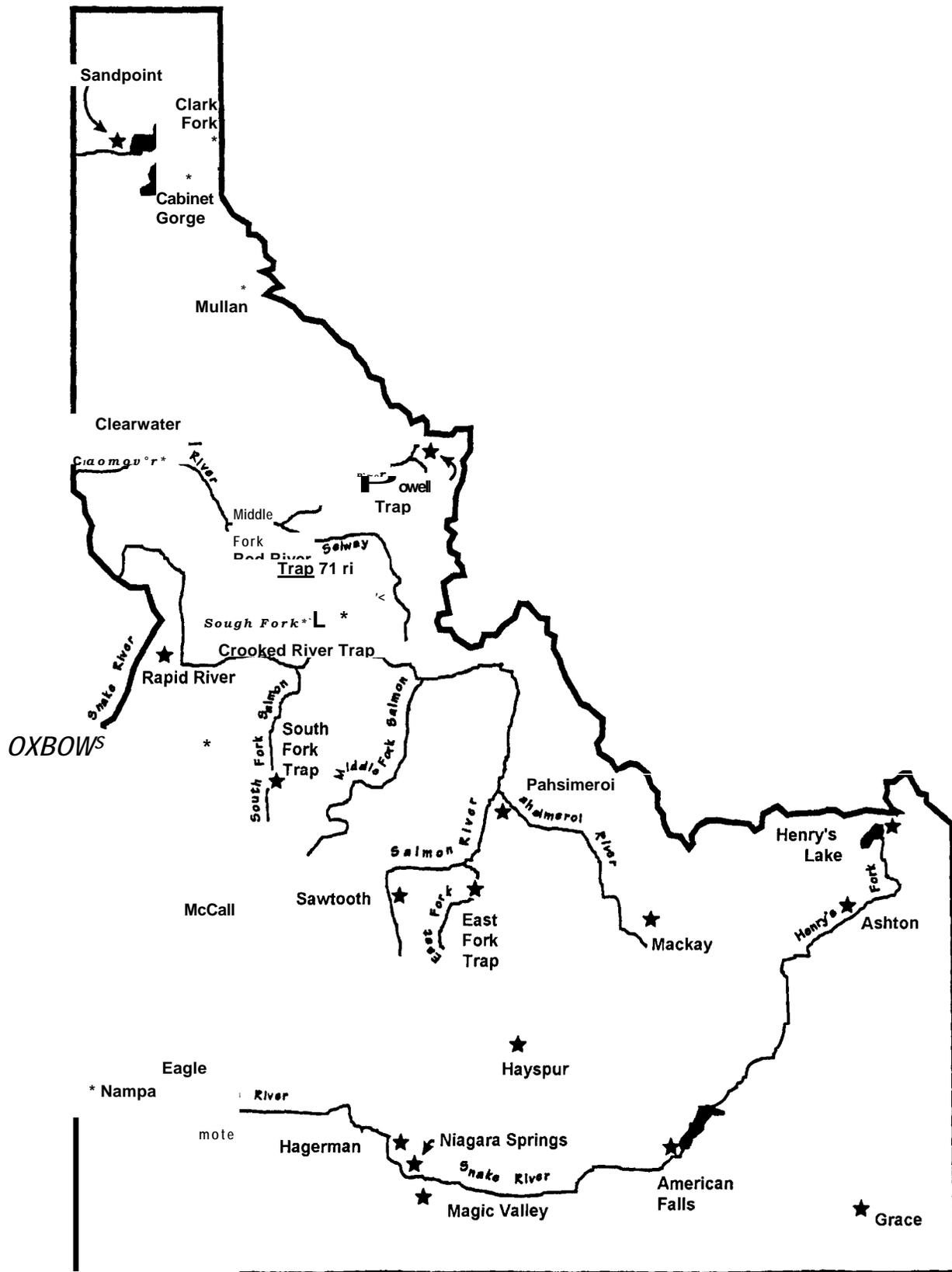
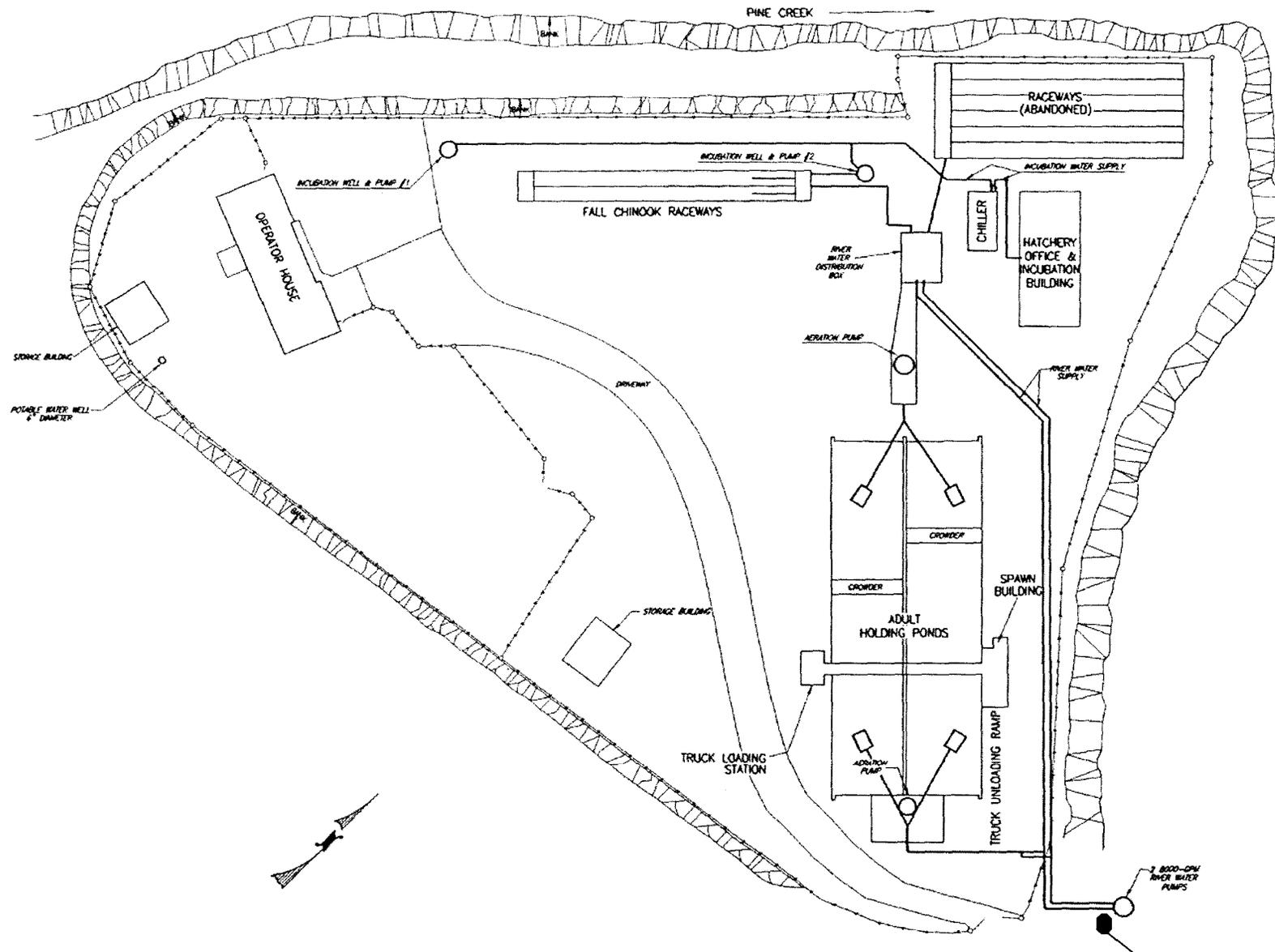


Figure 2. Exposure site at Oxbow Fish Hatchery.



2005 Exposure

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A handwritten signature in black ink, appearing to read "Bill Hutchinson", written over a horizontal line.

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