Montana Department
of
Fish, Wildlife & Parks

A Field Bioassay of Potential Effects of Champion Pulp Mill Effluents on Brown Trout Egg and Sac Fry Survival in the Clark Fork River

by

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ABSTRACT

A brown trout egg and sac fry survival bioassay is being conducted at three study sites on the Clark Fork River. The bioassay was initiated in November 1984 and will probably terminate about June 1, 1985. The bioassay was designed to aid in evaluating potential effects of year-round discharge of pulp and paper mill effluents on brown trout reproductive success in the river. Bioassay sites are located 4.9 miles upstream from Champion International Corporation's Frenchtown pulp mill, immediately downstream from the effluent diffuser at the mill and 25.6 miles downstream from the mill. Egg and sac fry development and survival are being monitored at the bioassay sites using fiberglass screen egg bags buried in the substrate and fry emergence traps installed over artificially-created redds. A variety of environmental parameters are being monitored at the bioassay sites to evaluate potential correlations with egg and sac fry survival rates.

Description of Study Area

Three locations in the Clark Fork River were chosen for brown trout egg bioassay sites (Table 1). Site 1 was located in a side channel downstream of Council Grove near river mile 343.5. This location served as a control since it is upstream of the Champion outfall but downstream of other point sources of pollution. Site 2 was situated immediately downstream of Champion International's diffuser, which is approximately 4.9 river miles downstream from Site 1. It incorporated two exposure regimes since eggs were placed in both the main river channel, where the pulp mill effluent concentration is primarily regulated by direct surface discharge, and in a side channel adjacent to Champion's settling ponds. The two major factors affecting effluent concentrations in the side channel were uncontrolled groundwater seepage from the settling ponds and flow in the river. The third site was established below the pulp mill effluent mixing zone; it was located about 25.6 river miles below site 2, just upstream from the Cyr bridge.

Table 1. Study site names, river mileage locations and legal descriptions of brown trout bioassay sites.

Site No.	Site Name	Approximate River Mile	Legal Description
1	Council Grove	343.5	T13N,R20W,S06DCC
2	Champion	338.6	T14N,R21W,S14BBB
3	Cyr	313.0	T14N,R23W,S06BCC

Methods

Approximately 10 female brown trout were obtained from the Missouri River below Hauser Dam to provide eggs for use at the bioassay sites. Male brown trout from the Missouri River as well as Mill and Ninemile creeks were used to fertilize the eggs. Hatchery personnel from the Jocko River State Fish Hatchery supervised the egg take. The male/female sex ratio was about 1:3. A greater number of females were spawned than necessary and roughly equal proportions of eggs from each female spawned were used at each bioassay site to minimize any potential parental effects on survival of the eggs. Each group of eggs were water-hardened using water obtained from standpipes at the respective bioassay site. After the eggs were water hardened, a portion of the spawn was transported to Washoe Park State Fish Hatchery. These were allowed to develop to the eyed stage before being planted in the Clark Fork. The other subset of green eggs were immediately planted in the river. The number of green and eyed eggs planted in the river during fall 1984, except those eyed

eggs placed in emergence traps, was measured using a Von Bayer trough (Leitritz 1969) (Table 2).

Two methods were employed to assess egg survival and fry emergence at each study area. The first method involved eggs placed in fiberglass bags that were buried in the substrate; periodically a subsample of bags was removed to monitor development and mortality. Ten groups of green eggs, which consisted of 3 bags each, were placed in the river at each site on 8 November 1984. Eight groups of eyed eggs, which were also composed of 3 bags each, were planted at each study area on 11-12 December, 1984. Each of these bags contained one Von Bayer trough of eggs or 52-56 eggs (Table 2). The second method, which utilized fry emergence traps, didn't allow frequent sampling of eggs during incubation but provided a measure of the cumulative effects of pulp mill effluents on a group of eggs from the green or eyed egg stage through emergence of fry from the gravel. The fry emergence traps used were described by Fraley et al. (accepted for publication) and equipped with fiberglass screen "baskets" that formed an enclosure. The baskets prevented escape of fry from the trap by lateral movement through the gravel. The emergence trap was square and covered approximately 1355 cm^2; its frame was made of strapping iron and had a cross member made from plumbers tape that supported the 1.6 mm delta nylon netting. A netting sock trailed behind the trap which contained a bottle that provided a low velocity holding area to prevent fry mortality. Three fry emergence traps containing green eggs were installed at each bioassay site on 8 November, 1984. Eggs from 3 Von Bayer troughs were placed in each of these traps. Two emergence traps which contained eyed eggs were installed at sites 1 and 2 while only 1 was installed at site 3 on 12-13 December, 1984. A total of 150 eggs were placed in each trap at sites 1 and 2 while only 149 eggs were added to the single eyed-egg trap at site 3 due to a lack of eggs. Fry which emerge from each trap will be collected and preserved to determine fry quality and condition factors.

Table 2. The mean number and range of green and eyed eggs per Von Bayer trough at each study site during fall 1984. Standard deviations are in parentheses.

Study Site	Green Eggs			Eyed Eggs		
	Mean No. eggs/trough	N	Range	Mean No. eggs/trough	N	Range
1	53.5($\pm$1.3)	10	52-55	54.6($\pm$1.1)	10	53-56
2	54.1($\pm$1.1)	10	52-56	54.3($\pm$0.8)	10	53-56
3	53.5	10	--	53.5($\pm$0.5)	10	53-54

Groundwater flow through the substrate, which is also known as apparent velocity, was measured directly with a seepage meter (Lee 1977; Lee and Cherry 1979) rather than by a standpipe (Pollard 1955; Terhune 1958). Previous standpipe methods used to determine apparent velocity were based on an erroneous premise that groundwater flow in the intragravel environment was parallel to the stream bottom when actually it tends to be vertical (Woessner personal communication).

Permeability of the gravel bed at each site was calculated every other month during the incubation period using a Mark VI standpipe as described by Terhune (1958). Three standpipes were positioned adjacent to the sites from which egg bags had recently been removed. All permeabilities were converted to a standard temperature (10 C) to facilitate comparisons of measurements made at various temperatures.

Streambed composition at each bioassay site as well as several redds was determined by obtaining samples using a hollow core McNeil sampler (McNeil and Ahnell 1964). The core tube was inserted 22 cm into the streambottom. Substrate within the core was extracted and placed in the catch basin. Water depth within the sampler was measured to the nearest 0.5 cm and recorded. A 1 liter bottle was filled with sediment-laden water from the corer. The substrate was then removed from the catch basin and placed in a sample bag. Three substrate samples were taken adjacent to the green egg bags at each site shortly after installation of the bags. Another 3 samples were taken parallel to the eyed egg bags. Additionally, one substrate sample will be collected every other month in conjunction with the harvest of egg bags. At the conclusion of the experiment, 3 final samples will be obtained in both the green and eyed egg beds. After fry emergence has occurred, substrate from the fry traps will be sieved to determine the composition.

To date, laboratory analysis of the substrate samples hasn't been completed but substrate samples will be dried, sieved and weighed. The volume of water present in the corer was determined using the appropriate regression equation:

For water depths up to 40.0 cm

$v = 0.241d + -0.001$ $\quad r = 0.99$

For water depths 40.1 to 76.5 cm

$v = 1.26d + -34.42$ $\quad r = 1.0$

Where: d = water depth in cm

v = volume in liters

The water sample from the McNeil corer was later transfered to an Imhoff cone and allowed to settle for a minimum of 25 minutes. The amount of settled material in the Imhoff cone and the total volume of water in the corer were used to determine the volume of sediment that remained suspended. This value was then converted

to the dry weight of material in suspension. The percentage of the total sample weight in each size class will be computed and data will be related to brown trout egg survival using methods described by Tappel and Bjornn (1983).

Water depths, mean velocities and point velocities were measured at 3 locations (the upper edge, middle and lower edge) at the egg bag sites and adjacent to each emergent trap. Depths were measured to the nearest 0.05 ft. Mean velocities were measured at 0.6 the total depth for water 2.5 feet or less while at greater depths two measurements were obtained and averaged; one was taken at 0.2 and another at 0.8 the total depth. Point velocities were measured at the substrate surface.

Water quality in the intragravel environment has been monitored monthly at each study site since egg implantation; parameters measured include pH, alkalinity, hardness, dissolved oxygen, total and volatile suspended solids and ammonia as well as concentrations of copper, cadmium, zinc and iron. Water analysis was performed by the Mt Dept. of Health and Env. Sciences laboratory in Helena, except for measurements of dissolved oxygen, which were determined in the field using the azide modification of the Winkler method. Initially, all intragravel water quality samples were obtained using a metal Mark VI standpipe and a hand-operated rotary pump. However, preliminary results showed distilled water pumped through standpipes contained background concentrations of metals which were high enough to prevent accurate measurements of the water samples. PVC standpipes were then installed at each study site. A proper seal of the substrate surrounding these standpipes was not obtained so their use was discontinued. Seepage meters constructed of PVC were then used to collect water samples but the slow rate of inflow combined with icing and initial flushing problems minimized their usefulness. Currently, a modified PVC seepage meter which would be completely buried within the substrate is being evaluated.

A comparison of dissolved oxygen levels in water from grab samples and those pumped from the same source indicated that no introduction of error occurred when caution was used to avoid the addition of air bubbles (Table 3). A dissolved oxygen meter also tested failed to provide reliable results over a sustained period of time.

Table 3. Comparison of dissolved oxygen concentrations (mg/l) from two collection methods.

Replicate	Grab Sample	Pumped Sample
A	12.6	12.5
B	12.7	12.5

Results

Depths and mean velocities at each site (Table 4) were measured shortly after implantation of the green eggs in November. They were near optimal hydraulic conditions for both brown trout spawning and egg incubation when compared to probability of use curves (Figures 1 and 2) developed by Bovee (1978). A limited number of measurements were made during a period of unseasonably low water levels in December (Table 4). All depths and mean velocities were within Bovee's criteria curves but velocities at site 2 were substantially lower than at the other sites. However, flow conditions at site 2 were not "stagnant", which could cause high embryo mortality (Reiser and White 1981). To date, apparent velocity measurements have been obtained only at site 2 (Table 5) which appear to be relatively low. Permeabilities adjusted to 10 C were approximately 480 and 80 % higher at site 2 than at sites 1 and 3, respectively (Table 6).

Table 4. Physical habitat parameters of brown trout green egg bioassay sites in the Clark Fork River 1984. Standard deviations are in parentheses.

	N	Site 1	Site 2	Site 3
		15-16 November		
Depth (ft)	6	0.94(±0.19)	0.96(±0.05)	1.00(±0.17)
Mean velocity (fps)	6	2.09(±0.60)	2.41(±0.11)	1.98(±0.51)
Point velocity (fps)	6	1.09(±0.41)	1.08(±0.42)	1.17(±0.44)
		8 December		
Depth (ft)	1	0.7	0.55	0.75
Mean velocity (fps)	1	1.15	0.1	0.75
Point velocity (fps)	1	0.45	0.0	0.25

Table 5. Groundwater flow into the Clark Fork River measured by seepage meters at brown trout bioassay sites.

Site	Date	Meter	Apparent Velocity ml/min
2	12/27/84	1	0.3
2	1/4/85	1	0.3
2	1/4/85	2	1.5

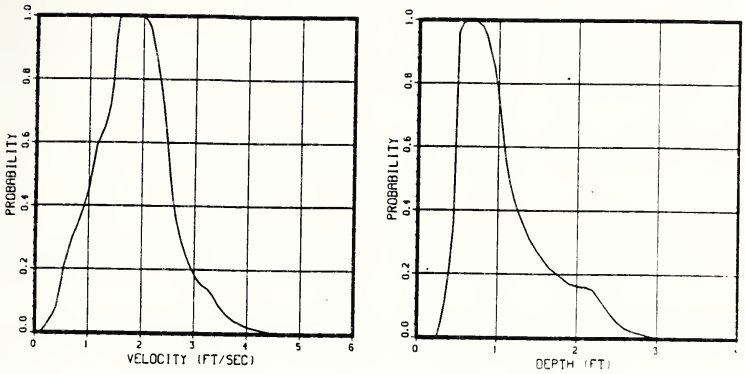


Figure 1. Probability of use criteria curves for brown trout spawning (from Bovee 1978).

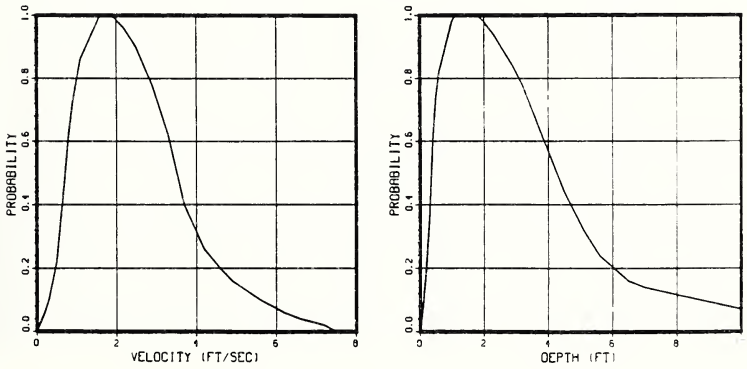


Figure 2. Probability of use criteria curves for brown trout egg incubation (from Bovee 1978).

Table 6. Permeabilities at 10 C from the brown trout bioassay sites on the Clark Fork River.

Site	Date	Number (N)	Permeability cm/min
1	11/20/84	6	0.9[a]
2	11/19/84	6	5.2
3	11/19/84	6	2.8

a - approximate measurement

Two groups of green egg bags were harvested in 1984 (Table 7). The first harvest occurred shortly after the egg plant to determine if handling had caused excessive mortality. Survival was high, varying from 98.8% at site 2 to 95.6% at site 3. The second harvest occurred in early December; survival remained good at all sites. Survival decreased 3.1% at both sites 1 and 3 and 6.8% at site 2 between the first and second harvests.

Table 7. Egg survival from the 3 bioassay sites on the Clark Fork River.

Site	Date	Percent Survival	Eggs Live	Eggs Dead	Stage of Development
1	11/20/84	98.1	159	3	Green
2	11/19/84	98.8	163	2	Green
3	11/19/84	95.6	151	7	Green
1	12/8/84	95.0	151	8	Green
2	12/8/84	92.0	149	13	Green
3	12/8/84	92.5	149	12	Green

Results of the water quality analysis performed in Helena have not been recieved. Only measurements of dissolved oxygen levels near the green egg plants at each site were available (Table 8). All samples from the gravel streambed were obtained using a standpipe. Dissolved oxygen levels were lower in the gravel than those of surface water at all sites. Measurements from the intragravel environment were consistently low at site 2 while they tended to be highest at site 3. Variation in measurements was considerably greater at site 2 than at any other site.

Table 8. Dissolved oxygen concentrations at study sites 1-3 on the Clark Fork River during fall 1984. Standard deviations are in parentheses.

Site	Date	Sample Source	N	D.O. mg/l
1	11/15	Surface	1	11.4
		Gravel	4	10.9(±0.3)
2	11/15	Surface	1	13.5
		Gravel	3	4.9(±3.0)
3	11/16	Surface	1	13.9
		Gravel	4	12.3(±0.51)
2	12/13	Surface	1	13.0
		Gravel	1	3.3
		Gravel-*	1	8.5
1	12/17	Surface	1	11.4
		Gravel	3	10.2(±0.3)
2	12/17	Surface	1	14.1
		Gravel	3	10.3(±2.1)
3	12/14	Surface	1	13.6
		Gravel	3	13.1(±0.3)

*-eyed eggs

Literature Cited

Bovee, K. D. 1978. Probability of use criteria for the family Salmonidae. Instream Flow Information Paper 4. USDI, Fish and Wildlife Service, Office of Biological Services. FWS/OBS-77/63. 80 pp.

Fraley, J. J., M. A. Gaub and J. C. Cavigli. In press. Emergence trap and holding bottle for the capture of salmonid fry in streams. Accepted for publication by the North American Journal of Fisheries Management.

Lee, D. R. 1977. A device for measuring seepage flux in lakes and estuaries. Limnology and Oceanography 22(1):140-147.

Lee, D. R. and J. A. Cherry. 1979. A field excercise on groundwater flow using seepage meters and mini-piezometers. Journal of Geology Education 27:6-10.

Leitritz, E. 1969. Trout and salmon culture. California Department of Fish and Game, Fish Bulletin 107. 169 pp.

McNeil, W. J. and W. H. Ahnell. 1964. Success of pink salmon spawning relative to size of spawning bed materials. USDI, Fish and Wildlife Service. Special Scientific Report: Fisheries No. 469.

Pollard, R. A. 1955. Measuring seepage through salmon spawning gravel. Journal Fisheries Research Board of Canada 12(5): 706-741.

Reiser, D. W. and R. G. White. 1981. Influence of streamflow reductions on salmonid embryo development and fry quality. Research Technical Report, Idaho Water and Energy Resources Institute, Moscow, Idaho. 154 pp.

Tappel, P. D. and T. C . Bjornn. 1983. A new method of relating size of spawning gravel to salmonid embryo survival. North American Journal of Fisheries Management 3(2):123-135.

Terhune, L. D. B. 1958. The mark VI groundwater standpipe for measuring seepage through salmon spawning gravel. Journal Fisheries Research Board of Canada 15:1027-1063.

Update of Intragravel Water Quality Monitoring Methods - 4-15-85

Water quality in the intergravel environment has been monitored monthly at each study site since egg implantation; parameters measured include pH, alkalinity, hardness, dissolved oxygen, total and volatile suspended solids and ammonia as well as the total recoverable concentrations of copper, cadmium, zinc and iron. Water analysis was performed by the Mt Dept. of Health and Env. Sciences laboratory in Helena, except for measurements of dissolved oxygen, which were determined in the field using the azide modification of the Winkler method. Initially, all intergravel water quality samples were obtained using a metal Mark VI standpipe (Terhune 1958) and a hand-operated rotary pump. However, distilled/deionized water pumped through standpipes contained background concentrations of all 4 metals. Although these concentrations were not extremely high, and they tended to be lower than EPA red book criteria standards for aquatic life, they did prevent an accurate assessment of the potential of embryo mortality due to metals toxicity. In order to eliminate use of metal standpipes, PVC standpipes were installed at each study site. A proper seal of the substrate surrounding these standpipes was not obtained; this allowed intrusion of surface water and their use was discontinued. Seepage meters constructed of PVC were then used to collect water samples but the slow rate of inflow combined with icing and initial flushing problems minimized their usefulness. Water samples for metal analysis and common ions are now being obtained from PVC pipes buried within the substrate to a depth of 2-2.5 feet. These standpipes were considered adequately sealed and sampling ground water when water temperatures and/or dissolved oxygen concentrations are substantially different than those of the surface water and the groundwater in the area is known to be recharging the river. Development and testing of the methodology for sampling intergravel water was hampered and slowed by adverse weather conditions and icing associated with the winter field season. The feasibility of the University of Montana's Chemistry Dept. processing the water quality samples is currently being explored. Advantages of switching to the Chemistry Dept. include simplified logistics in transport of the samples to the lab and an increase in the number of common ions which could be analyzed without incurring additional costs. These additional ions would enable calculation of the cation-anion balance and include sodium and sulphate, which may be used to indicate relative concentrations of pulp mill effluent at the study sites. Also, the intensity of the heavy metal sampling program will be reduced if it can been verified that they do not appear to be a significant cause of mortality at the study sites.

Dissolved oxygen samples are being obtained from both PVC and metal standpipes since multiple samples utilizing different sampling equipment provides a better description of the oxygen concentrations at each study site and checks the accuracy of each method.

(April 15, 1985, Updated Table)

Egg survival from three bioassay sites on the Clark Fork River.

Bioassay site	Bioassay type	Date	Survival rate (percentage
Council Grove - control site 4.9 miles above pulp mill	Green eggs	11-20-84	98.1
		12-8-84	95.0
		1-24-85	89.0
		2-22-85	92.0
		3-15-85	86.8
		3-29-85	91.7
	Eyed eggs	1-24-85	91.4
		2-22-85	91.5
		3-15-85	85.3
		3-29-85	83.2
Champion - immediately below mill effluent	Green eggs	11-19-84	98.8
		12-8-84	92.0
		1-23-85	78.8
		2-21-85	0.0*
	Eyed eggs	1-23-85	92.6
		2-21-85	70.8
		3-14-85	66.3
		3-27-85	72.4
Cyr - 25.6 miles below mill effluent	Green eggs	11-19-84	95.6
		12-8-84	92.5
		1-22-85	94.3
		2-25-85	0.0*
	Eyed eggs	1-22-85	92.0
		2-25-85	83.9
		3-14-85	65.8
		4-2-85	48.6

* Green egg bioassay terminated due to winter ice condition.

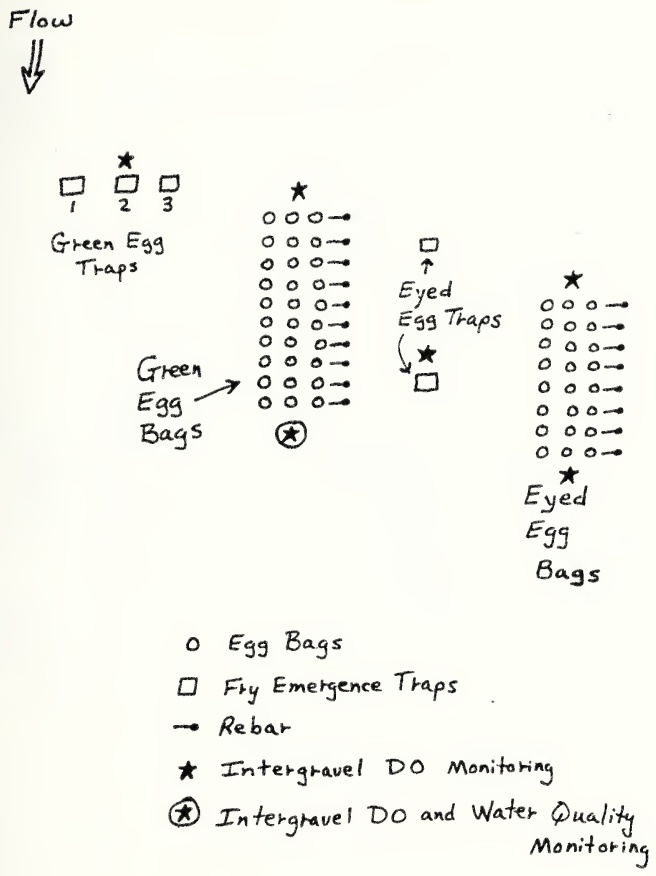
Summary list of environmental parameters monitored at bioassay sites. Correlations of environmental parameters with egg and sac fry survival rates will be evaluated

1. Groundwater flow (apparent velocity) - seepage meters
2. Permeability (K) of the gravel bed - standpipes
3. Substrate composition
4. Water depths, mean velocities and point velocities
5. Water quality parameters:
    a. pH
    b. Alkalinity
    c. Hardness
    d. Dissolved oxygen
    e. Total and volatile suspended solids
    f. Ammonia
    g. Total recoverable concentrations of toxic metals:
        1) Copper
        2) Cadmium
        3) Zinc
        4) Iron

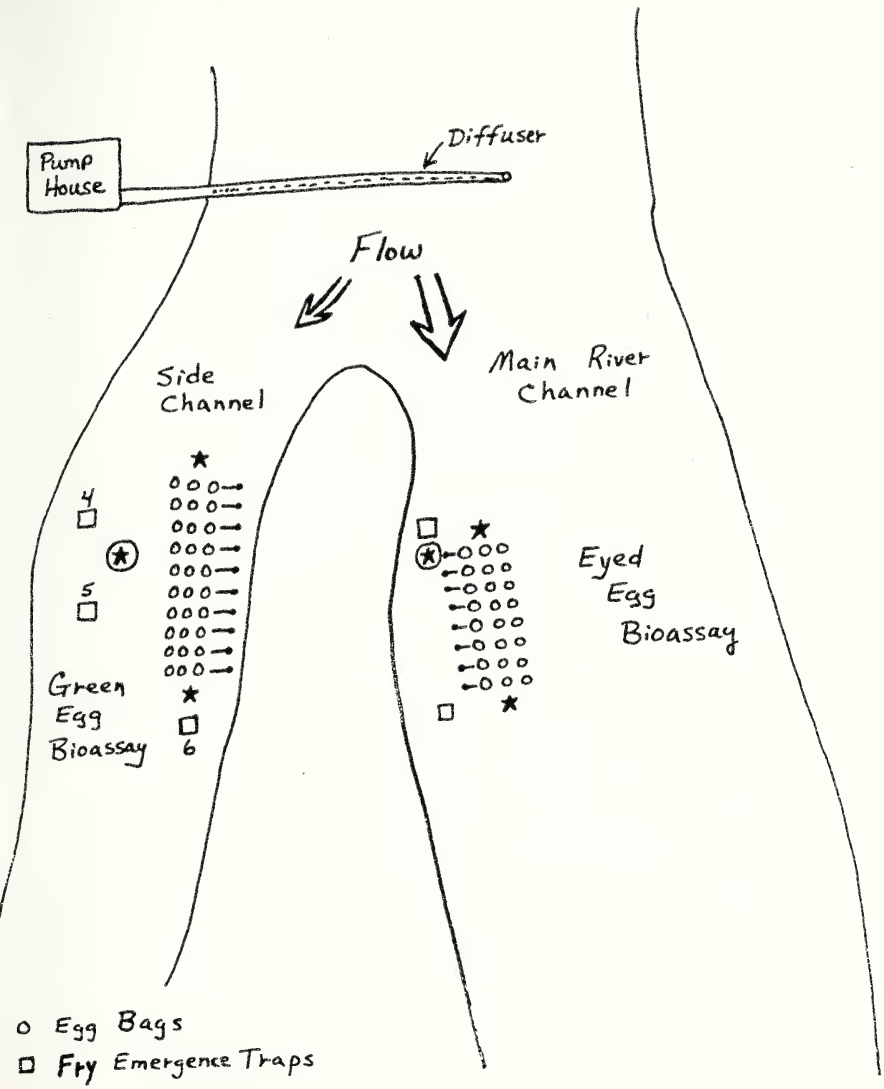
Added in March 1985:

1. Chloride
2. Sulfate
3. Nitrate
4. Phosphate
5. Potassium
6. Total dissolved solids

Council Grove Bioassay Site

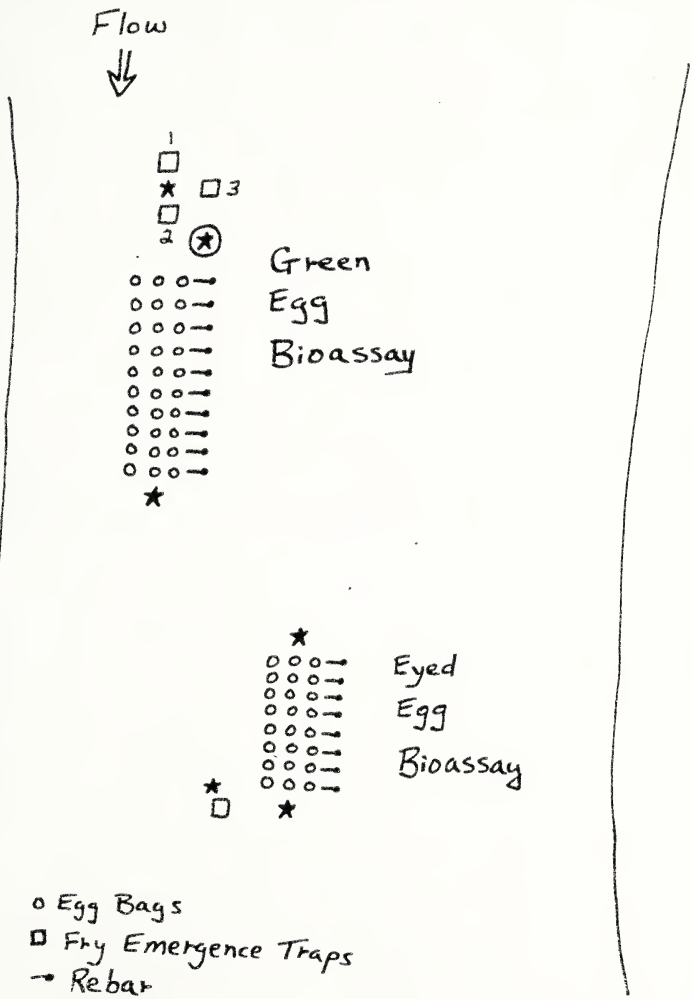


Champion Bioassay Site



o Egg Bags
□ Fry Emergence Traps
— Rebar
★ Intergravel DO Monitoring
⊛ Intergravel DO and Water Quality Monitoring

Cyr Bioassay Site



o Egg Bags

□ Fry Emergence Traps

→ Rebar

★ Intergravel DO Monitoring

⊛ Intergravel DO and Water Quality Monitoring