# EFFLUENT TOXICITY TESTING AT MILLSTONE NUCLEAR POWER STATION USING THE SHEEPSHEAD MINNOW (*CYPRINODON VARIEGATUS*) DURING 1981 AND 1982

Northeast Utilities Environmental Laboratory

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# INTRODUCTION

The presence of toxic chemicals in the environment has become of increasing national concern. The regulation of toxic chemicals in discharge effluents is being conducted through permit limitations based on chemical analyses and aquatic toxicity testing. Millstone Nuclear Power Station (MNPS) discharges a large volume of effluent (2155 cfs) to Long Island Sound. This discharge is primarily seawater used for condenser cooling. Known potential toxicants added during routine plant operations include chlorine for biofouling control and heavy metals from chemical reactions and erosion in the plant system.

The concentration of known pollutants can be measured with chemical analyses and the potential toxicity assessed based on aquatic toxicity data for the pollutant. But the possible additive and synergistic toxicity of the combination of pollutants or the presences of unknown pollutants can only be monitored with effluent toxicity tests.

Effluent toxicity testing of the discharge from MNPS was begun in 1981 using the sheepshead minnow (Cyprinodon variegatus). The sheepshead minnow has been used extensively in marine toxicity testing and is a recommended effluent test organism by the U.S. Environmental Protection Agency (Peltier 1978). Sheepshead minnow embryo-larval tests (Goodman et al. 1976; Hansen et al. 1977) and life cycle tests (Hansen et al. 1978) have been used to assess chronic toxicity. Generally, the larval stage of fish development is the most sensitive to toxicants (Macek and Sleight 1977). An embryo-larval test will provide much of the same data as an entire life cycle test and can be conducted in a shorter period of time. At MNPS a sheepshead minnow embryo-larval test was started every 2 to 3 months during the period of March 1981 through August 1982. The

embryo-larval test assesses the effects of a toxicant on survival and growth but does not provide information on fecundity and offspring (F1 generation). Therefore, one entire life cycle test was conducted from May 1981 through March 1983 to examine the effects of the effluent on fecundity and the F1 generation. Standardized testing procedures have been modified for long term effluent toxicity testing of the discharge from MNPS.

## MATERIALS AND METHODS

### Testing Facility

Toxicity tests were conducted and stock cultures maintained in a 3 X 3 m insulated environmental room (Fig. 1). Air conditioning was used to maintain an air temperature of 20°C±2. Photoperiod was set at 12 hrs light-12 hrs dark with fluorescent lighting. Unfiltered effluent from the Quarry and control water from Jordan Cove were continuously pumped to separate head tanks (~150 l capacity) in the environmental room. Effluent and control water temperatures were automatically adjusted to 20°C±1 by heating and cooling systems. Water flowed by gravity from the head tanks to flow through stock aquaria and test chambers. All stock populations were maintained in control water. Water temperature and flow rates were measured daily (Monday through Friday). Dissolved oxygen (YSI D.O. meter) and pH (Beckman pH meter) were measured weekly.

Brine shrimp nauplii were used as a food source for sheepshead minnow larvae. Brine shrimp cultures were started on Monday, Wednesday, and Friday in two 20-l glass carboys which were filled with control water, and 6 g of brine shrimp eggs (Aquarium Products, Inc.) were added to each carboy. The carboys were aerated for two days while nauplii hatched from eggs. Then the solution containing egg cases and nauplii was pumped (Masterflex metering pump) to a brine shrimp delivery system (Fig. 2). The delivery system

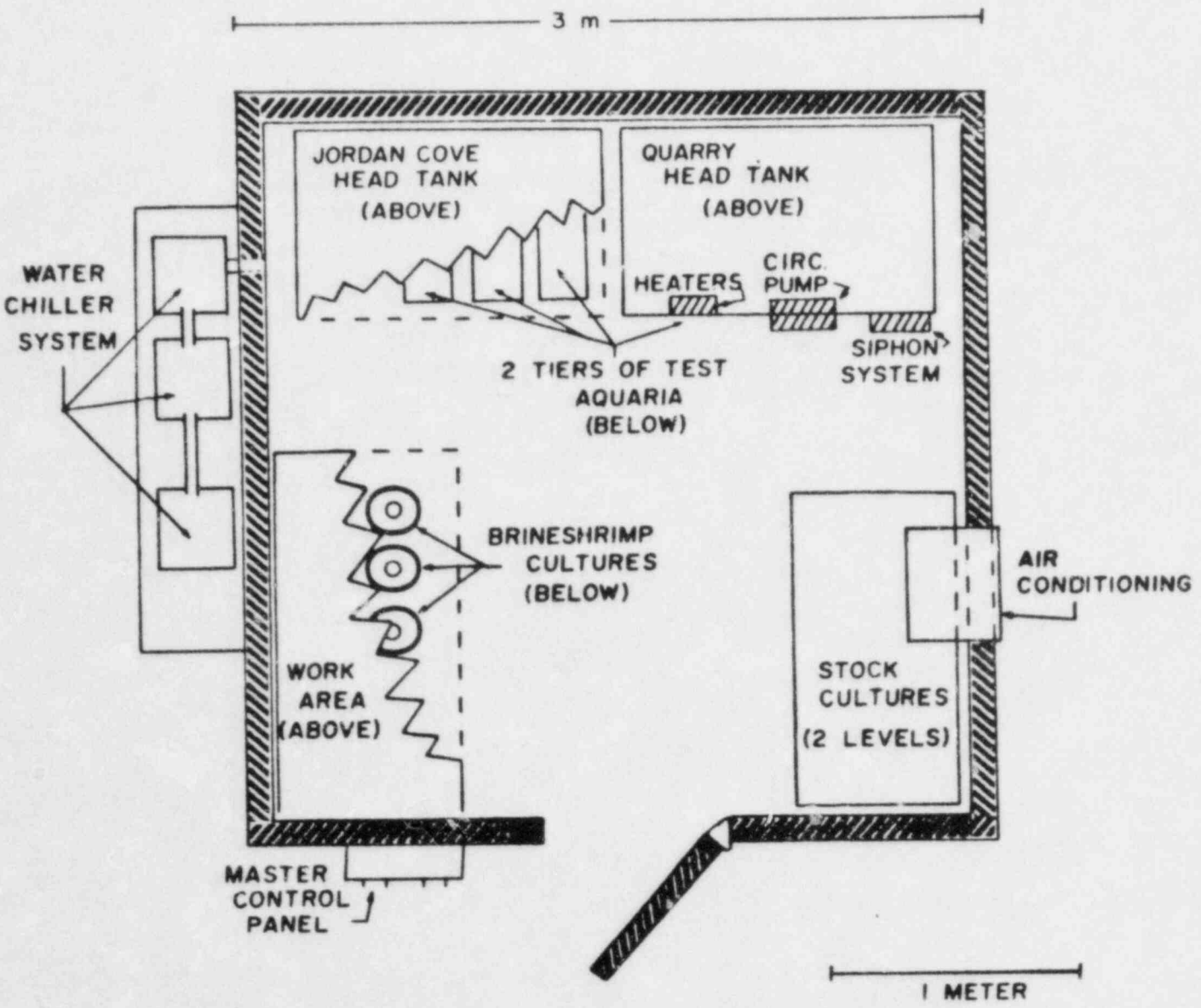


Figure 1. Environmental room used to conduct effluent toxicity tests and to maintain stock cultures.

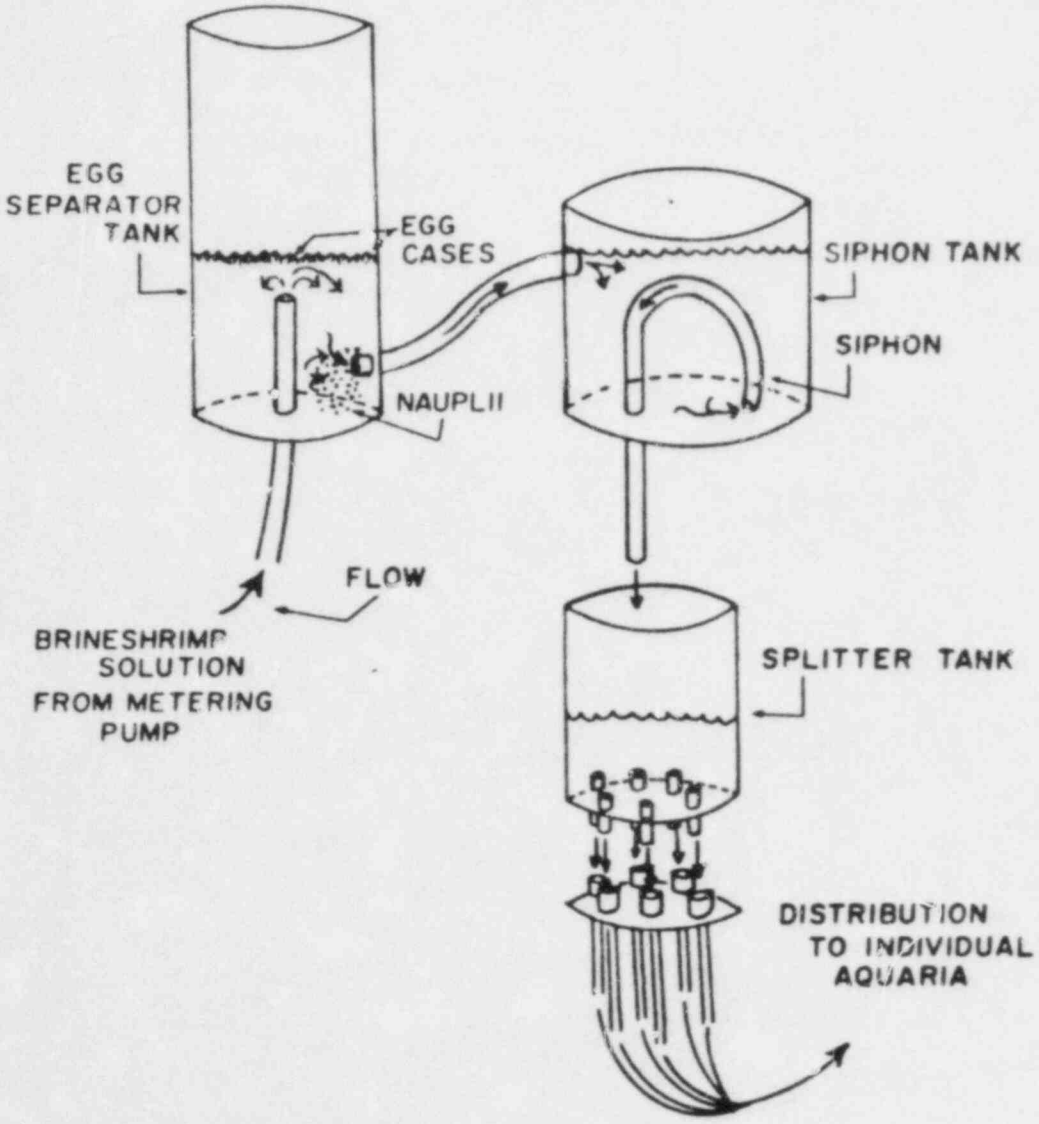


Figure 2. Brine shrimp delivery system.

separated egg cases from nauplii and split the brine shrimp nauplii solution into equal portions. The splits were delivered to larval sheepshead minnow test chambers through tygon tubing. Two 20-l carboys provided sufficient brine shrimp nauplii solution for ~2.5 days of feeding.

Adult Stock

An adult stock of sheepshead minnow was secured commercially (Ichthyological Associates, Middletown, DE). Spawns from the stock were used to conduct the tests. The stock was maintained in 38-l glass aquaria with ~25 individuals per aquarium. The stock was held in flow-through (~150 ml/min) control water at 20°C±1. They were fed daily (Monday through Friday) a commercial flake food (Tetra SM 80) at a rate of ~3 g/aquarium. The fish were not fed on Saturday or Sunday but an additional ration was provided on Friday afternoon (~1530 hrs).

Embryo-Larval Tests

Embryo-larval tests begin during embryological development of the egg and continue through larval development. A large number of eggs (~200) at approximately the same developmental stage are needed to start an embryo-larval test. Two males and five to seven females were placed in a flow-through 38-l spawning tank (flow rate ~150 ml/min). The water temperature was increased to 25°C±1 over a 24 hr period with an immersion heater. Fish were held in the spawning tank at least 10 days prior to collecting eggs. In order to have eggs that were at the same approximate developmental stage, on a Friday afternoon (~1530 hrs) the spawning tank bottom was cleaned by siphoning to remove any eggs that were present. A net (1-cm mesh) was suspended 4-cm from the bottom to prevent the adults from eating the eggs. Monday morning (~0800 hrs) the

fish and net were removed from the spawning tank and the eggs from the weekend spawn were removed by siphoning.

Sheepshead minnow eggs that were collected from the weekend spawn were examined with a dissecting microscope and apparent viable eggs (presence of an embryo or pigmentation) were selected for starting an embryo-larval test. Thirty eggs if possible were placed in each test chamber, except Test 1 had ten eggs per replicate. Test chambers were 600-ml polyethylene containers with a screened outlet (0.505-mm mesh) and maintained a water volume of 500 ml (Fig. 3). Separate triplicate test chambers were provided with flow-through (~90 ml/min) control water and effluent (100%) at 20°C±1. Hatching started in 10 days from spawning and 14 days after the spawn the number of larvae were counted in each test chamber to determine egg viability. The number of larvae were reduced to 10/test chamber. Larval lengths were determined photographically at approximately two week intervals. Mortality and morphological anomalies (if any) were recorded at the time of length measurements. During the test, larvae were fed a continuous supply of 24 to 72 hr old brine shrimp nauplii at a rate of ~60 nauplii/ml and ~2500 ml/treatment/day.

Data collected during the test for comparison of control and effluent treatments included egg viability, larval mortality, morphological anomalies, and growth rate.

## Life Cycle Test

Juveniles (parental generation) from a completed embryo-larval test (Test 2) were used for the life cycle test. The replicates of each treatment (control and effluent) were combined in separate 38-l aquaria. Flow-through (~90 ml/min) control water or effluent was supplied to the respective aquaria. The fish were held for approximately 18 months during which time they matured.

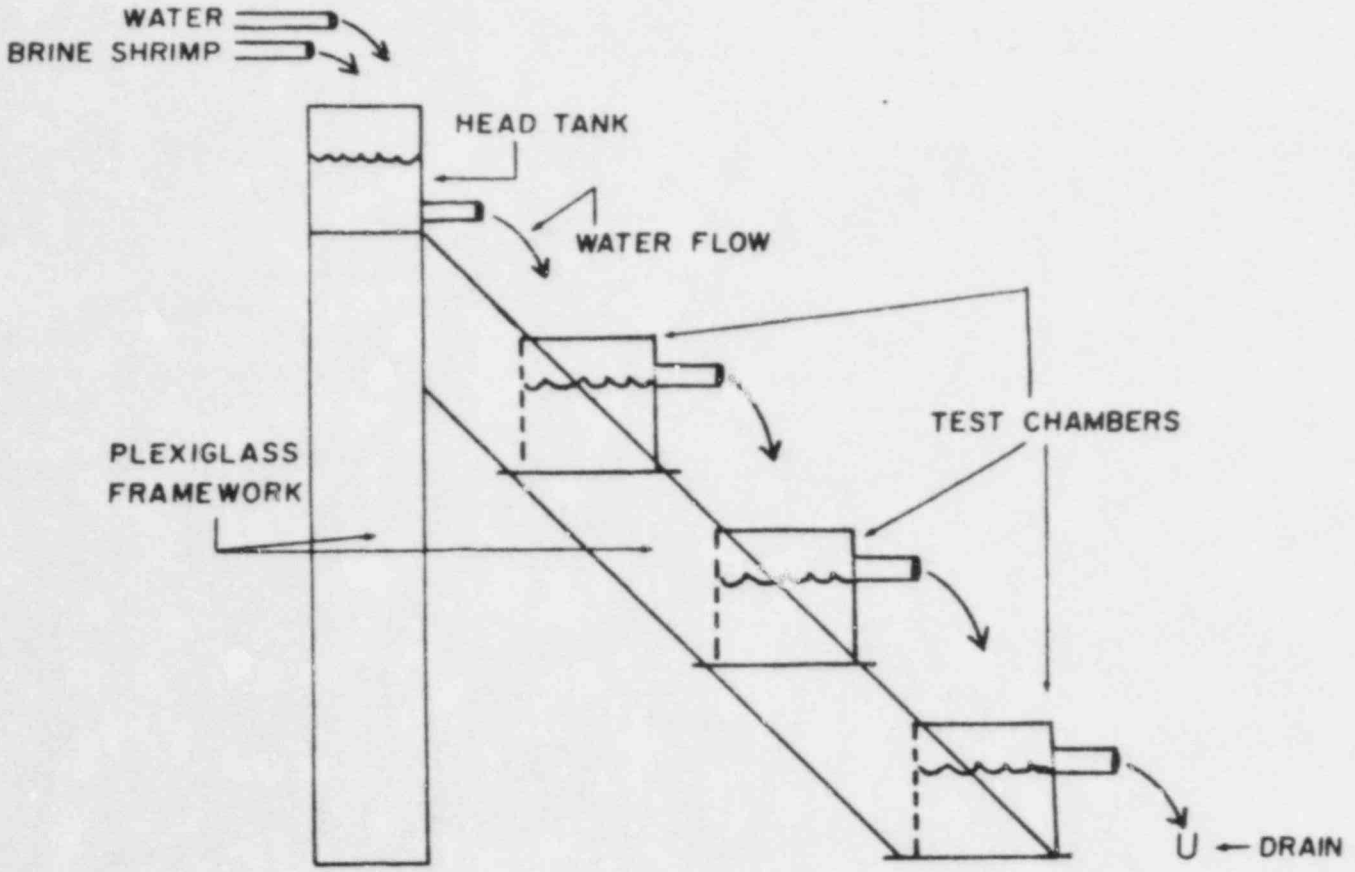


Figure 3. Sheepshead minnow embryo-larval test apparatus.

The water temperature for the reproductively mature individuals was increased to 25°C±1 over a 24-hr period and they were maintained at this temperature for at least one week prior to fecundity measurements. Three male-female pairs for each treatment were randomly selected and placed in individual spawning chambers. Spawning chambers were 5-l aquaria with sloped internal sides to prevent the adults from eating the eggs. The spawning chambers were placed in an incubator at 25°C for two days with light aeration under static conditions. The eggs were then counted in each spawning chamber. The two day spawning period was repeated eight times for random pairs.

Eggs collected from the parental generation spawn were used to examine effects on the F1 generation. The eggs from each treatment from a two day spawning period were combined (replicates) and placed in an embryo-larval test chamber (Fig. 3). The F1 generation portion of the test was similar to that conducted for an embryo-larval test except that 30 eggs were not available per replicate and replicates were not started on the same day.

Data available from the life cycle test included fecundity (parental), egg viability, and F1 larval mortality, growth, and morphological anomalies.

## RESULTS

Seven embryo-larval tests and one life cycle test were conducted from March 1981 through March 1983. One embryo-larval test (Test 4) was terminated early due to system malfunction and only egg viability data collected. Due to the similarity of test results, the data from all embryo-larval tests were summarized together.

## Embryo-Larval Tests

### Egg Viability

Eggs hatched in approximately 10 days from spawning in all 7 tests. Egg viability ranged from 51.7 to 90.0% in controls and 71.7 to 98.9% in effluent treatment (Table 1). This high egg viability maybe due to selecting eggs with developing embryos or pigmentation for testing. Over all, egg viability was lower in controls (73.1%) than effluent (88.9%) treatmeants. A possible cause of this was the heavier siltation load in control water which may have smothered some eggs. Based on the egg viability data, no toxic concentrations of chemicals affecting sheepshead minnow embryological development were present in the effluent.

### Larval Survival

Larval survival data to assess toxicity was restricted to the first 60 days for comparability between tests, although some test organisms were held for up to 200 days. Survival in controls ranged from 73.3 to 100.0% and in the effluent treatment from 40.1 to 100.0% in the six tests completed (Table 2). Generally, survival was 90% or higher. Overall survival was lower in the effluent (86.4%) than in the control (91.8%) treatments but the lower overall survival in effluent treatments can be attributed to Test 7 which had a 40.1% survival. The cause of the increased mortality in Test 7 is not known but the controls in Test 7 also had a lower survival (73.3%) which indicated a problem with experimental conditions rather than a toxic pollutant in the effluent. Except for Test 7, survival was higher in the effluent treatments but did not appreciably differ from the controls.

Table 1. Egg viability for sheepshead minnow embryo-larval tests.

| Test | Treatment | Replicate | Eggs/Replicate | Eggs hatching | % viability |
|---|---|---|---|---|---|
| Test 1 | Control | 1 | 10 | 7 | |
| | | 2 | 10 | 10 | 73.3 |
| | | 3 | 10 | 5 | |
| | Effluent | 1 | 10 | 7 | |
| | | 2 | 10 | 10 | 86.7 |
| | | 3 | 10 | 9 | |
| Test 2 | Control | 1 | 20 | 15 | |
| | | 2 | 20 | 16 | 83.3 |
| | | 3 | 20 | 19 | |
| | Effluent | 1 | 20 | 15 | |
| | | 2 | 20 | 10 | 71.7 |
| | | 3 | 20 | 18 | |
| Test 3 | Control | 1 | 30 | 17 | |
| | | 2 | 30 | 22 | 57.8 |
| | | 3 | 30 | 13 | |
| | Effluent | 1 | 30 | 20 | |
| | | 2 | 30 | 20 | 75.6 |
| | | 3 | 30 | 28 | |
| Test 4 | Control | 1 | 20 | 15 | |
| | | 2 | 20 | 16 | 76.7 |
| | | 3 | 20 | 15 | |
| | Effluent | 1 | 20 | 12 | |
| | | 2 | 20 | 19 | 73.3 |
| | | 3 | 20 | 13 | |
| Test 5 | Control | 1 | 20 | 10 | |
| | | 2 | 20 | 10 | 51.7 |
| | | 3 | 20 | 11 | |
| | Effluent | 1 | 20 | 13 | |
| | | 2 | 20 | 13 | 75.0 |
| | | 3 | 20 | 19 | |
| Test 6 | Control | 1 | 30 | 25 | |
| | | 2 | 30 | 30 | 90.0 |
| | | 3 | 30 | 26 | |
| | Effluent | 1 | 30 | 30 | |
| | | 2 | 30 | 30 | 98.9 |
| | | 3 | 30 | 29 | |
| Test 7 | Control | 1 | 30 | 15 | |
| | | 2 | 30 | 30 | 77.8 |
| | | 3 | 30 | 24 | |
| | Effluent | 1 | 30 | 25 | |
| | | 2 | 30 | 29 | 88.9 |
| | | 3 | 30 | 26 | |

Table 2. Larval survival for sheepshead minnow embryo-larval tests from hatching to 60 days old.

| Test | Treatment | Replicate | Larvae/Replicate | Larvae Surviving | % Survival |
|---|---|---|---|---|---|
| Test 1 | Control | 1 | 7 | 7 | |
| | | 2 | 10 | 10 | 100% |
| | | 3 | 5 | 5 | |
| | Effluent | 1 | 7 | 7 | |
| | | 2 | 10 | 10 | 100% |
| | | 3 | 9 | 9 | |
| Test 2 | Control | 1 | 10 | 10 | |
| | | 2 | 10 | 10 | 96.7% |
| | | 3 | 10 | 9 | |
| | Effluent | 1 | 10 | 10 | |
| | | 2 | 10 | 9 | 96.7% |
| | | 3 | 10 | 10 | |
| Test 3 | Control | 1 | 10 | 8 | |
| | | 2 | 10 | 10 | 90% |
| | | 3 | 10 | 9 | |
| | Effluent | 1 | 10 | 9 | |
| | | 2 | 10 | 8 | 90% |
| | | 3 | 10 | 10 | |
| Test 4 | Terminated due to problems | | | | |
| Test 5 | Control | 1 | 10 | 10 | |
| | | 2 | 10 | 9 | 96.7% |
| | | 3 | 10 | 10 | |
| | Effluent | 1 | 10 | 9 | |
| | | 2 | 10 | 9 | 93.3% |
| | | 3 | 10 | 10 | |
| Test 6 | Control | 1 | 10 | 10 | |
| | | 2 | 10 | 9 | 96.7% |
| | | 3 | 10 | 10 | |
| | Effluent | 1 | 10 | 10 | |
| | | 2 | 10 | 10 | 100% |
| | | 3 | 10 | 10 | |
| Test 7 | Control | 1 | 10 | 6 | |
| | | 2 | 10 | 8 | 73.3% |
| | | 3 | 10 | 8 | |
| | Effluent | 1 | 10 | 1 | |
| | | 2 | 10 | 3 | 40.1% |
| | | 3 | 10 | 8 | |

## Growth

The growth rates in the six tests completed appeared to be linear in both control and effluent treatments (Fig. 4-9). The growth rate per test for controls ranged from 0.207 to 0.252 mm/day and for effluent treatments from 0.213 to 0.275 mm/day (Table 3). There was no apparent difference in growth between control and effluent treatments. Based on the similarity in growth rates between control and effluent treatments no toxic concentations of pollutants were present in the effluent that would effect the growth of larval sheepshead minnow.

## Life Cycle Test

A life cycle test was conducted on individuals from embryo-larval Test 2. The individuals were held under their respective treatments while maturing and tested at an age of 18 months. Fecundity counts on randomly paired males and females showed variability within each treatment (Table 4). There was an apparent decrease in egg counts as the test proceeded, which would indicate problems with experimental conditions. Due to the viability, differences in fecundity were tested with the nonparametric Wilcoxon two-sample test (Sokal and Rohlf 1969) and the last two testing dates excluded because of zero counts. There was no significant difference between fecundity counts from effluent and control treatments (prob. > t=0.89).

Eggs from the fecundity measurements on December 13 and 20 were held for F1 generation observations. A total of 57 eggs were placed in control water and 60 eggs in effluent water. Egg viability for control and effluent treatments was 19.3% and 31.7%, respectively. These egg viabilities were lower than those found in the embryo-larval tests (Table 1). The larval survival was high in both control (100%) and effluent (89.5%) treatments. No morphological

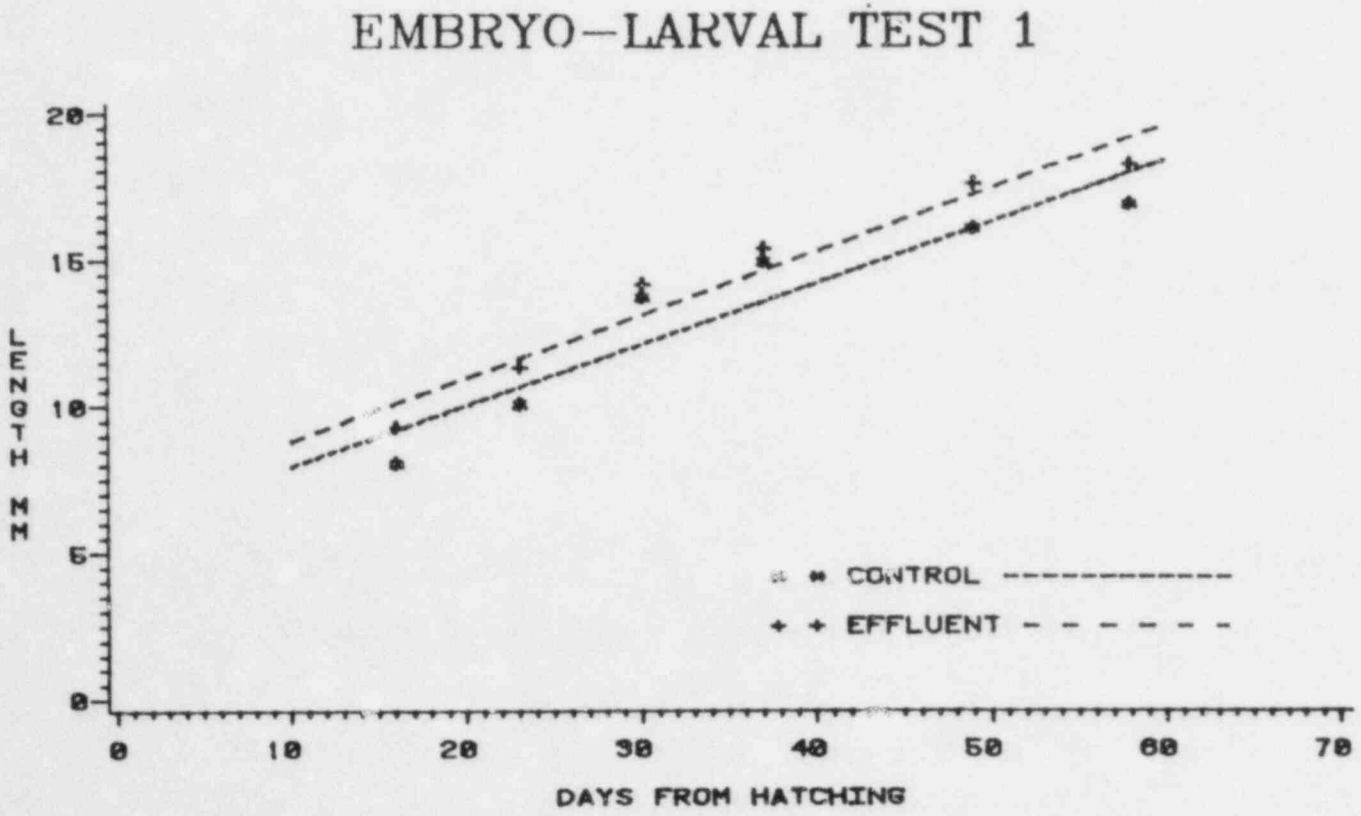


Figure 4. Sheepshead minnow growth for Test 1 with mean length for each measurement date and regression line for each treatment.

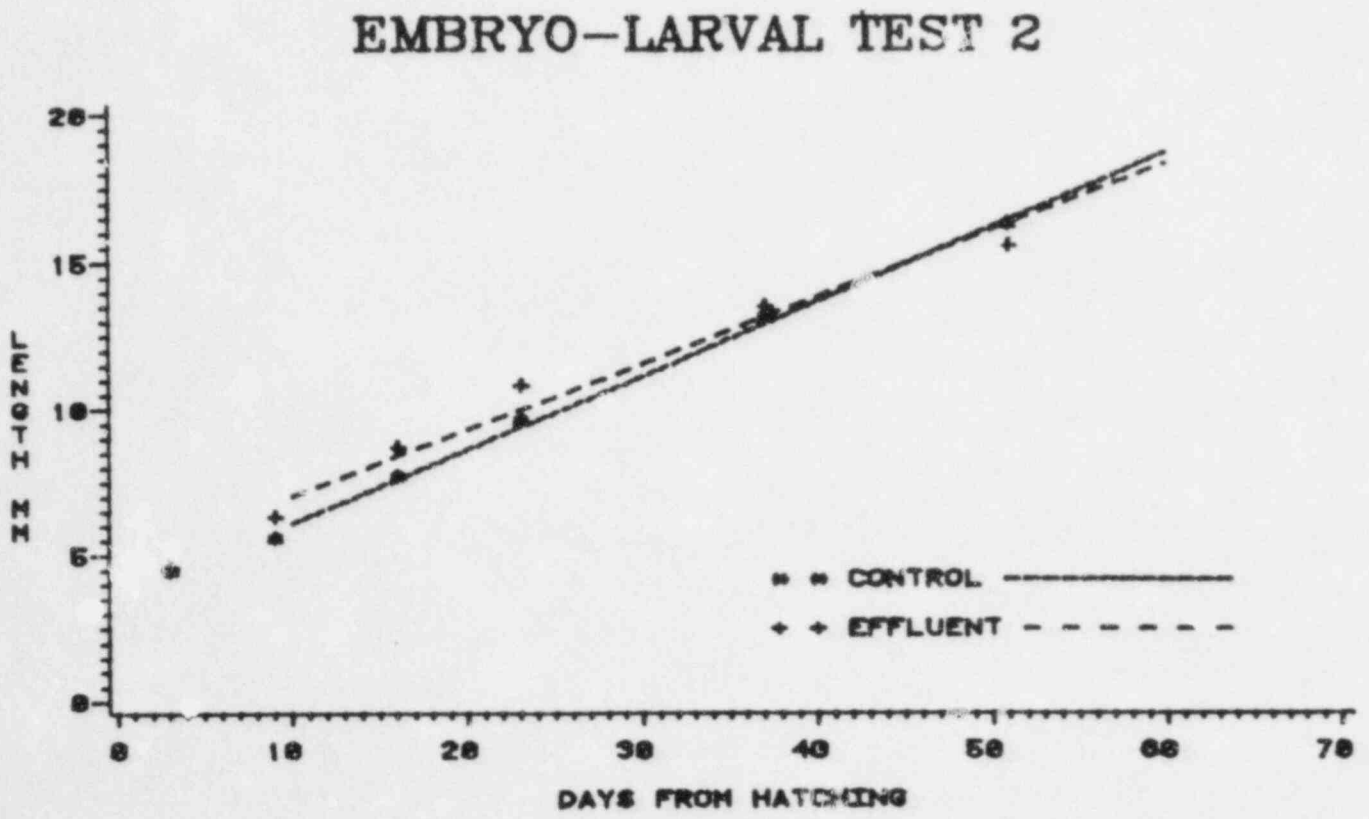


Figure 5. Sheepshead minnow growth for Test 2 with mean length for each measurement date and regression line for each treatment.

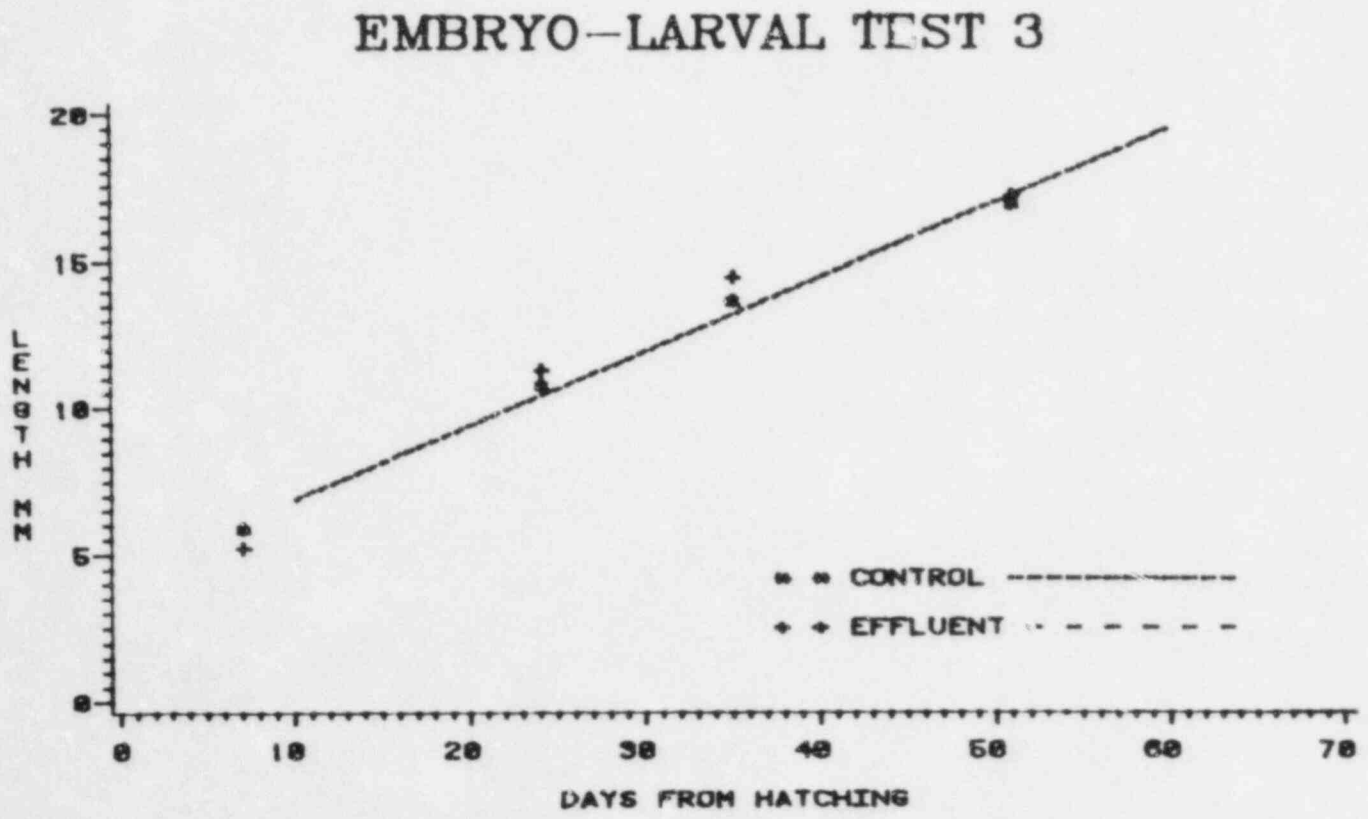


Figure 6. Sheepshead minnow growth for Test 3 with mean length for each measurement date and regression line for each treatment (the two regression lines are plotted as one due to their similarity).

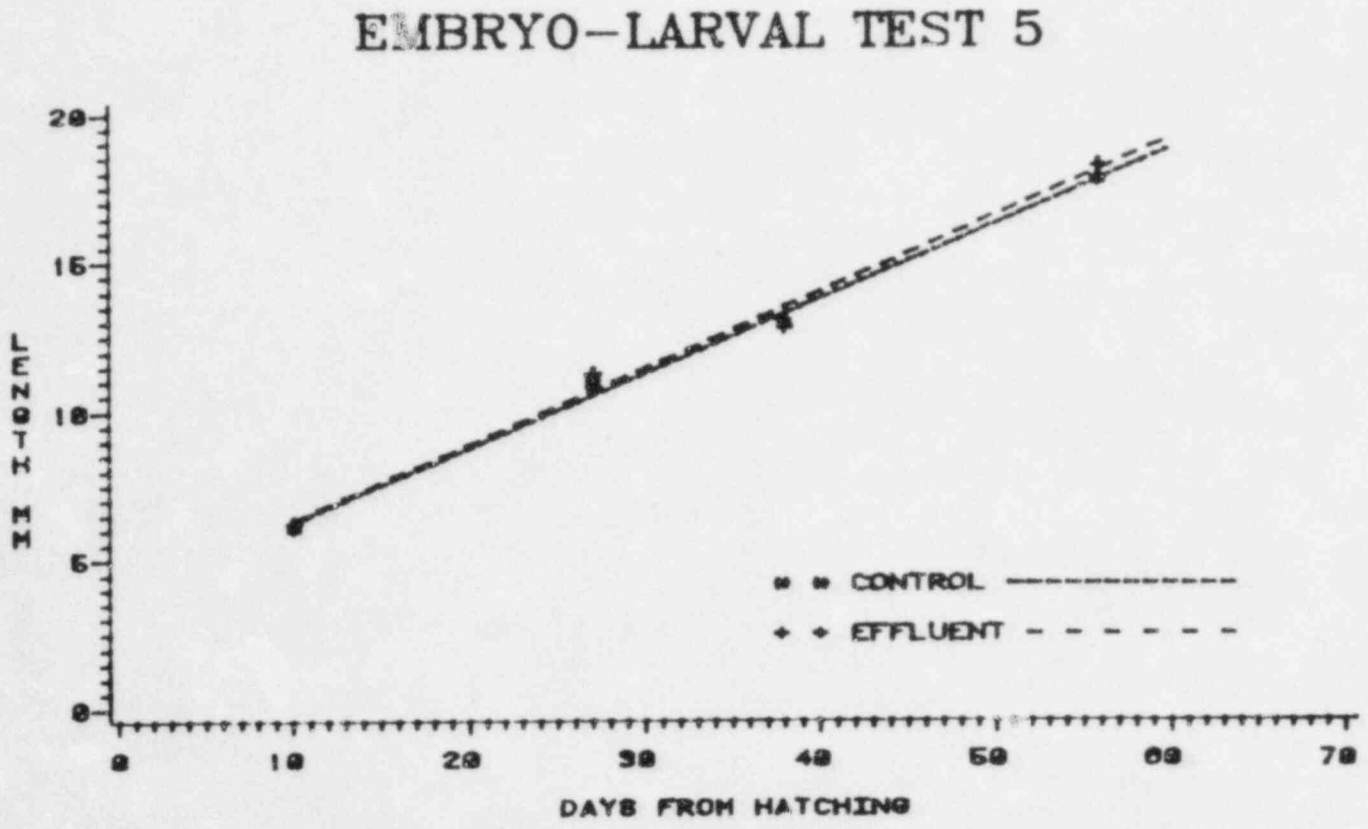


Figure 7. Sheepshead minnow growth for Test 5 with mean length for each measurement date and regression line for each treatment.

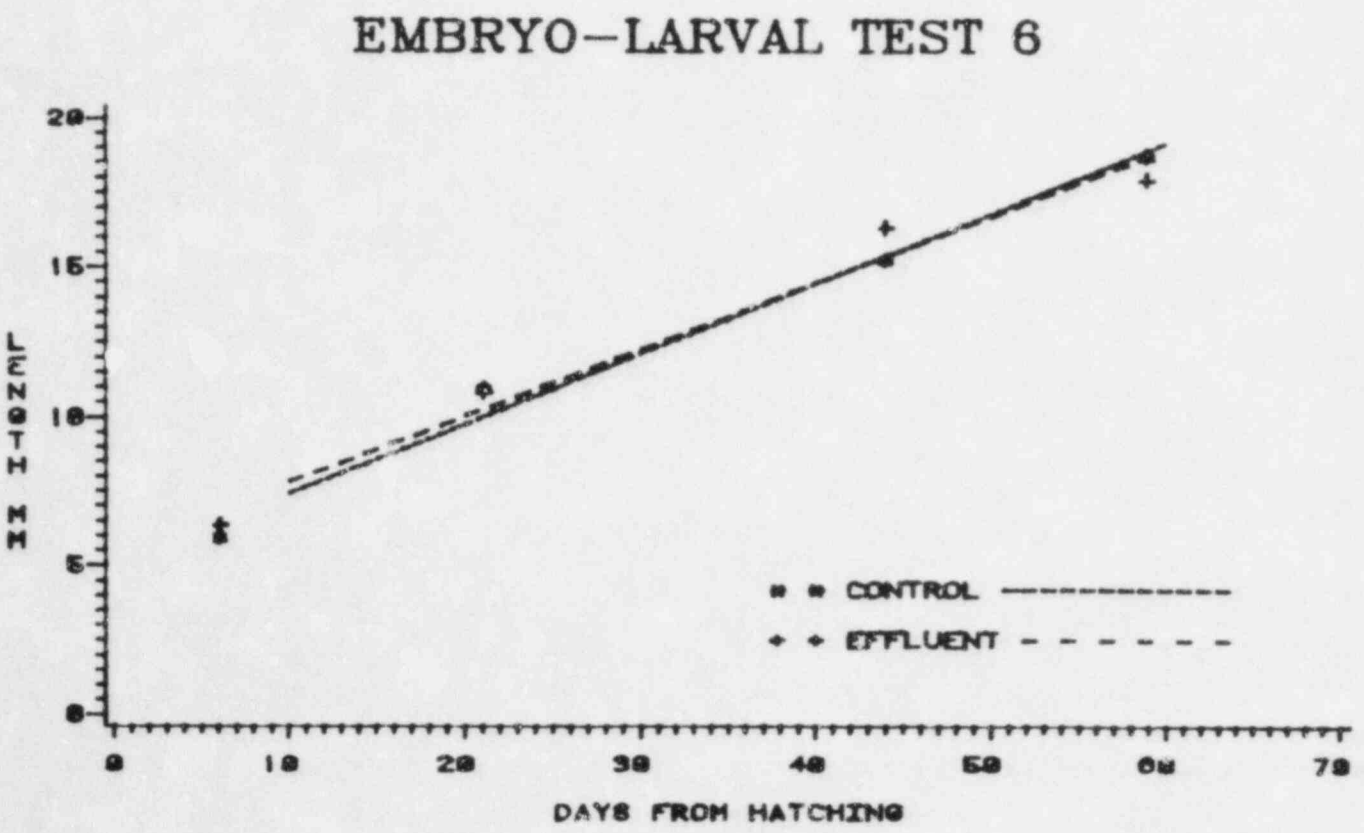


Figure 8. Sheepshead minnow growth for Test 6 with mean length for each measurement date and regression line for each treatment.

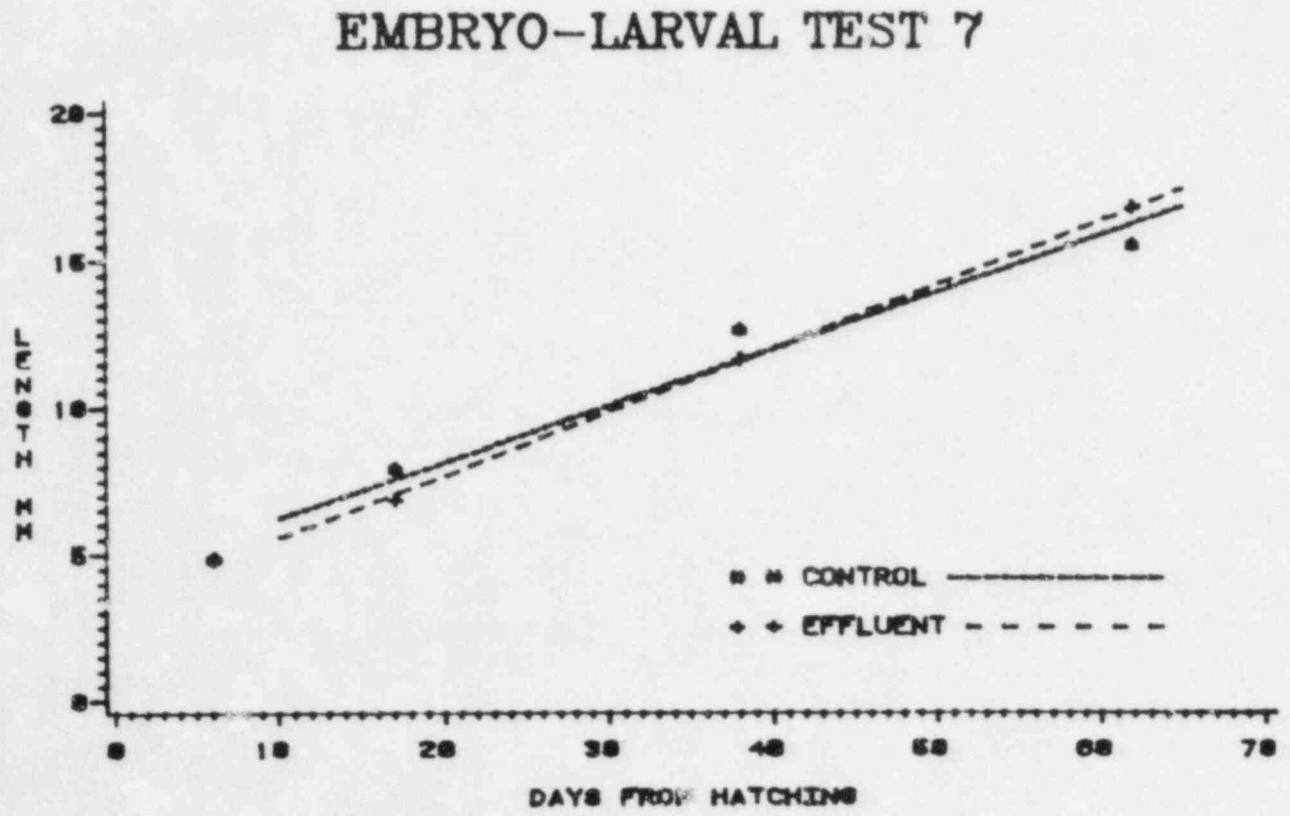


Figure 9. Sheepshead minnow growth for Test 7 with mean length for each measurement date and regression line for each treatment.

Table 3. Linear regression equations of larval sheepshead minnow length (l) in mm versus age (days) from hatching to 60 days for embryo-larval tests.

| Test | Treatment | Equation | $R^2$ |
|---|---|---|---|
| Test 1 | Control | l = 5.93 + 0.207 * days | 0.79 |
| | Effluent | l = 6.99 + 0.214 * days | 0.85 |
| Test 2 | Control | l = 3.63 + 0.252 * days | 0.96 |
| | Effluent | l = 4.77 + 0.226 * days | 0.87 |
| Test 3 | Control | l = 4.42 + 0.251 * days | 0.91 |
| | Effluent | l = 3.95 + 0.274 * days | 0.92 |
| Test 4 | Terminated due to system malfunction | | |
| Test 5 | Control | l = 3.76 + 0.249 * days | 0.94 |
| | Effluent | l = 3.78 + 0.254 * days | 0.94 |
| Test 6 | Control | l = 5.10 + 0.234 * days | 0.92 |
| | Effluent | l = 5.63 + 0.222 * days | 0.92 |
| Test 7 | Control | l = 4.27 + 0.192 * days | 0.85 |
| | Effluent | l = 3.40 + 0.215 * days | 0.89 |

Table 4. Fecundity measurements (2 days) for adults from life cycle test.

| Treatment | Replicates | DATE | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 10/4 | 10/7 | 10/28 | 11/1 | 12/13 | 12/20 | 12/27 | 1/10 |
| Control | 1 | 40 | 51 | 4 | 0 | 0 | 0 | 0 | 0 |
| | 2 | 55 | 24 | 0 | 0 | 23 | 34 | 0 | 0 |
| | 3 | 122 | 246 | 1 | 28 | 0 | 4 | 0 | 0 |
| Effluent | 1 | 3 | 25 | 44 | 13 | 4 | 11 | 0 | 0 |
| | 2 | 33 | 4 | 0 | 2 | 0 | 16 | 0 | 0 |
| | 3 | 103 | 15 | 1 | 0 | 112 | 1 | 0 | 0 |

anomalies were observed in either group. Growth rates over the 90 day period from hatching was similar for control (0.195 mm/day) and effluent (0.175 mm/day) treatments (Fig. 10). These growth rates were lower than those found in the embryo-larval tests.

## DISCUSSION AND CONCLUSIONS

Examination of the aquatic community in the vicinity of the discharge into Long Island Sound would readily identify acute toxicity of the MNPS effluent. The effects of chronic toxicity would be more subtle and difficult to identify from field observations. Due to the possible additive and synergistic chronic toxicity of combinations of chemicals and the presence of unknown pollutants, aquatic toxicity testing of the effluent is necessary to assess the toxicity of the discharge.

Known chemicals that enter the effluent during plant passage of water withdrawn from Niantic Bay include sodium sulfate, boric acid, chlorine and heavy metals. Sodium sulfate occurs from the mixing of sulfuric acid and sodium hydroxide after demineralizer regeneration. Sodium sulfate is a commonly occurring compound in seawater and represents minimal potential toxicity. Boric acid is used in the primary coolant loop of Unit 2 and is routinely discharged. Boric acid would probably form the common salts of sodium borate and calcium borate (Becker and Thatcher 1973) and probably has a low potential toxicity. Of the known routine additions to the effluent, chlorine and heavy metals have the greatest potential toxicity.

Heavy metal concentrations in the effluent have received the most attention as potentially toxic. The concentrations of several heavy metals have been monitored in the effluent since 1971. Copper, zinc, iron, chromium and lead concentrations in the effluent were measured in 1981 and

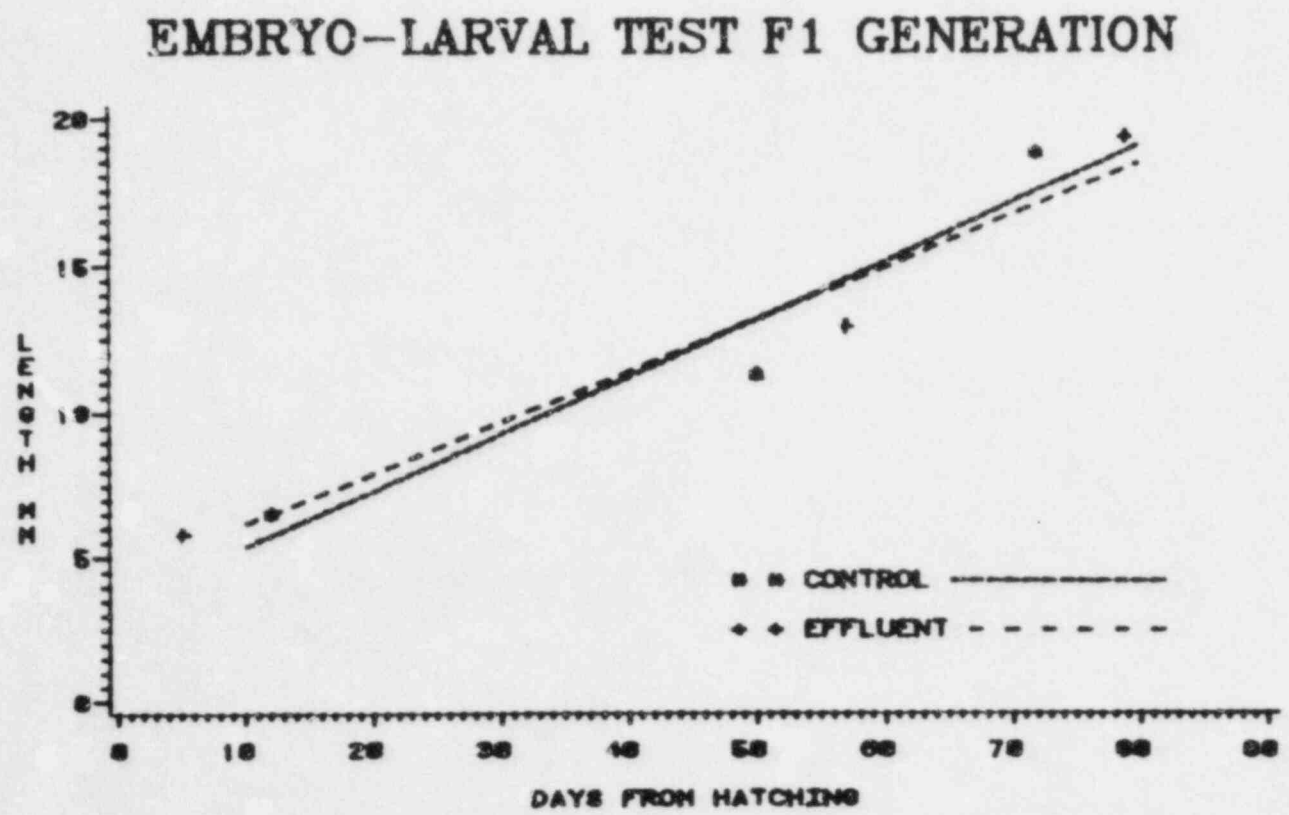


Figure 10. Sheepshead minnow growth for the F1 generation in the life cycle test with mean length for each measurement date and regression line for each treatment.

1982 during five annual sampling periods (Table 5). Water quality criteria for 64 designated toxic pollutants were established in 1980 by the U.S. Environmental Protection Agency (Federal Reg. 1980). Copper, zinc and chromium were among these toxic pollutants and did not exceed the water quality criteria during 1981 and 1982 (Table 5). Nickel was not monitored in effluent but due to the copper-nickel alloy of the condenser tubes could be a potentially toxic pollutant. Waslenchuk (1982) examined nickel concentrations in the MNPS effluent during 1979 and found a range of 2.7 to 4.3 ug/l. The water quality criteria for nickel in saltwater as a 24 hr average is 7.1 ug/l (Federal Reg. 1980).

Chlorine is added to the seawater for biofouling control and is potentially toxic. The toxicity of chlorine to marine fish is dependent on several environmental factors including temperature, exposure time and available forms of chlorine (Capuzzo et al. 1977). Eggs and larvae of white perch, striped bass, blueback herring and eggs of Atlantic silversides had a range of LC50 values of 0.20 to 0.38 mg/l total residual chlorine with 24 to 76 hr exposures (Morgan and Prince 1977). Total mortality of juvenile winter flounder, scup and mummichog occurred after a 30 min exposure to total residual chlorine ranging from 0.25 to 0.65 mg/l (Capuzzo et al. 1977). Discharge limits under the MNPS NPDES Permit are a maximium concentration as free chlorine of 0.25 mg/l at the discharge and 0.1 mg/l at the Quarry Cut. Chlorine concentrations were monitored at least weekly and did not exceed permit limits during 1981 and 1982 (MNPS Chemistry Department, personnal commication).

Based on chemical evaluation and analyses, the discharge of known pollutants attributable to MNPS were not present in toxic concentrations during 1981 and 1982. But toxic conditions could have existed due to additive and synergistic effects with the combination of sub toxic concentrations of several pollutants or the presence of

Table 5. Annual mean and range of heavy metal concentrations (ug/l) in the MNPS effluent and U.S. EPA water quality critera (ug/l) to protect saltwater aquatic life as a 24 hr average.

| Meatal | 1981[a] | 1982[b] | EPA[c] Guideline |
|---|---|---|---|
| Copper | < 3.4 (< 2.0- < 3.9) | 2.1 (1.8-2.5) | 4.0 |
| Zinc | < 14.1 (< 1.6- < 31.7) | 6.1 (2.2-11.8) | 58.0 |
| Iron | < 109.8 (< 50-256) | 53.5 (38.1-74.5) | ---- |
| Chromium | < 2.2 (< 1.3- < 3) | 0.21 (0.15-0.26) | 18.0 |
| Lead | < 3.1 (< 3.0- < 3.3) | 5.1 (3.5-7.4) | ---- |

[a] NUSCo. 1982

[b] NUSCo. 1983

[c] Federal Reg. 1980 - Criterion to protect saltwater aquatic life as a 24 hr average.

unknown pollutants. Therefore, effluent toxicity tests were conducted with sheepshead minnow as a generic effluent toxicity assessment.

Sheepshead minnow have been used extensively in marine toxicity testing. In 96-hr acute toxicity testing, juvenile sheepshead minnow (14-28 days post hatch) were as or more sensitive than Daphnia magna to 28 of 54 organic chemicals tested (Heitmuller et al. 1981). Embryo-larval toxicity test information has been highly correlated to the more extensive chronic life cyle studies (Macek et al. 1978). Data compiled on freshwater embryo-larval tests indicated that they were good predictors of reproductive impairment due to toxicity (McKim 1977). The sheepshead minnow embryo-larval test has been used at the U.S. EPA, Environmental Research Laboratory, Gulf Breeze, Florida to test toxicity of organic compounds (Goodman et al. 1976; Hansen et al. 1977; Hansen and Parrish 1977). Ward and Parrish (1980) reviewed the results of nine embryo-larval tests, eight life cycle tests and one partial life cycle test that used sheepshead minnow. They found that larval mortality was the most sensitive indicator of toxicicty in comparison to egg vialabilty and larval growth. There are indications that sheepshead minnow are less sensitive to heavy metal concentrations than other fish species (eg. larval siversides and winter flounder) used in marine toxicity testing (G. Klein-MacPhee, EPA Naragansett Laboratory, personnel communication).

Toxicity testing at MNPS with sheepshead minnow during 1981 and 1982 indicated no chronic toxicity related to the effluent. No difference between control and effluent treatments were found for egg viability, larval growth and mortality, fecundity and second generation. For the embryo-larval tests, the high egg viability and larval survival in both control and effluent treatments and the remarkably similar growth rates among tests indicated stable

testing conditions. The lower egg viability and growth rates in the life cycle test compared to the embryo-larval tests indicated dissimilar testing conditions but no effluent toxicity since control and effluent treatments were similar. The embryo-larval and life cycle tests were conducted with undiluted effluent which is greatly diluted in adjacent Long Island Sound. Therefore, the test organisms were exposed to much higher concentrations of possible toxicants than aquatic organisms in Long Island Sound. Since no toxic effects were found in undiluted effluent, no toxic concentrations of pollutants would be expected to have occurred in the adjacent waters of Long Island Sound due to the operation of MNPS during 1981 and 1982.

## RECOMMENDATIONS

The present concern about the discharge of toxic chemicals to the environment makes it important to demonstrate whether the large volume of effluent discharged from MNPS is toxic. Due to the possibility of additive and synergistic effects with the combination of chemicals and the possible presence of unknown pollutants, effluent toxic testing is the only feasible method to assess the toxicity of the discharge.

The sheepshead minnow was selected as a test organism because it reproduces under laboratory conditions and can be reared in the laboratory. Since there is some question as to the sensitivity of the sheepshead minnow to heavy metals, Atlantic silverside and winter flounder larvae should be considered as additional test organisms. It is recommended that sheepshead minnow testing be continued until Atlantic silverside laboratory reproduction can be started. Once the test methodology for Atlantic silversides is developed, the sheepshead minnow testing would be terminated. This does not represent an increase in man hours since a majority of

the time requirements is for system maintainance and not actual testing time. The testing with winter flounder larvae would be seasonal based on the natural spawning season. Additional testing facilities would be required for conducting toxicity tests with winter flounder.

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