Development of Long Term Effluent Toxicity Testing Procedure with the Bay Mysid (*Mysidopsis bahia*) and Preliminary Testing Results at Millstone Nuclear Power Station.

Northeast Utilities Environmental Lab
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INTRODUCTION

Millstone Nuclear Power Station presently discharges 5×10^6 m^3 of cooling water daily, this volume will double once Unit 3 is operational. This water contains low concentrations of chemicals and heavy metals. Chemicals discharged include chlorine, sodium sulfate, boric acid, sodium phosphate, oils and greases. Heavy metal concentrations primarily arise from gradual erosion or corrosion of the condenser tubes (a copper - nickle alloy) and zinc which is used as a corrosion inhibitor throughout the cooling system. Federal regulation of industrial effluents began in 1972 with passage of the Federal Water Pollution Control Act which set limitations for various point source effluents. Subsequently, U.S. Environmental Protection Agency (EPA) guidelines were developed for conducting aquatic toxicity tests. Standardized acute and chronic testing procedures were developed for several organisms where known chemicals were being tested. Guidelines were not developed for long term effluent testing.

Chemical analysis of effluents can determine known pollutant concentrations and the potential toxicity can be assessed using aquatic toxicity data. However, the possible additive and synergistic toxicity of combinations of pollutants or the presence of unknown pollutants can only be monitored with long term effluent toxicity tests.

Northeast Utilities Environmental Laboratory (NUEL) began testing with the bay mysid (*Mysidopsis bahia*) in 1981. The objective of testing was the development of a test procedure using the bay mysid to assess long term effluent toxicity.

Concurrent with procedure development the toxicity of the effluent was assessed.

Mysids are widely used in aquatic toxicity testing due to their sensitivity to toxicants, ability to reproduce under laboratory conditions and their short generation time (Nimmo and Hamaker 1982). They are also a recommended EPA effluent test organism (Peltier 1978). Of six commonly used toxicity test organisms (mysid shrimp, bluegill sunfish, sheepshead minnow, daphnid, Selenatrum capricornutum a freshwater alga and Skeletonema costatum a marine alga), the mysid shrimp was found to be the most sensitive organism to the 86 priority pollutants tested (Nimmo and Hamker 1982).

Two procedures were used during testing from September 1981 to March 1983. The first using the standardized procedures developed for specific chemical toxicity testing (ASTM 1983), which provided data on growth rates, generation time and survival rates. The second procedure provided data on brood size which was used as an indicator of the mysids ability to reproduce successfully. Brood size is defined as the number of eggs or juveniles in a female brood. This modified procedure was based on two premises: (1) that mysid brood size has been found to be a sensitive indicator of toxicity (ASTM 1983) and (2) that the traditional criteria for acceptable control water has been the ability of the test organism to survive and reproduce in it (ASTM 1983). Therefore, if a mysid population can be maintained in 100% effluent the effluent would not be considered toxic.

MATERIALS AND METHODS

Testing Facility

Toxicity tests were conducted and stock cultures were maintained in a 3 x 3 m insulated environmental room (Fig. 1). An air temperature of 20°C ± 2 and a 12 hr light and a 12 hr dark photoperiod was maintained. Unfiltered control water from Jordan Cove and undiluted effluent from the Quarry were continuously pumped to separate head tanks (~150 l) where water temperatures were maintained at 20°C ± 1. Effluent and control water flowed by gravity to stock aquaria and test chambers. Stock cultures were maintained in control water at 20°C ± 1. Dissolved oxygen and ph were measured weekly. Water temperature and flow rates were determined daily.

Stock cultures were fed 24-72 hr old brine shrimp nauplii (Artemia salina) twice daily in equal portions to all aquaria at sufficient concentrations to assure against starvation and cannibalism of young by adult mysids. Mysids used during testing were fed 24-72 hr old brine shrimp nauplii pumped at a rate of approximately 60 nauplii/ml and approximately 2500 ml/day/treatment (Fig. 2).

Testing Procedures

Two testing methodologies were analysed 9/81 to 3/83 for development of long term testing procedures to optimize testing efficiency (Table 1).

The first of which used the standardized toxicity test procedures used for known chemicals (ASTM 1983). The second which modified the standardized procedures to obtain population stability data.

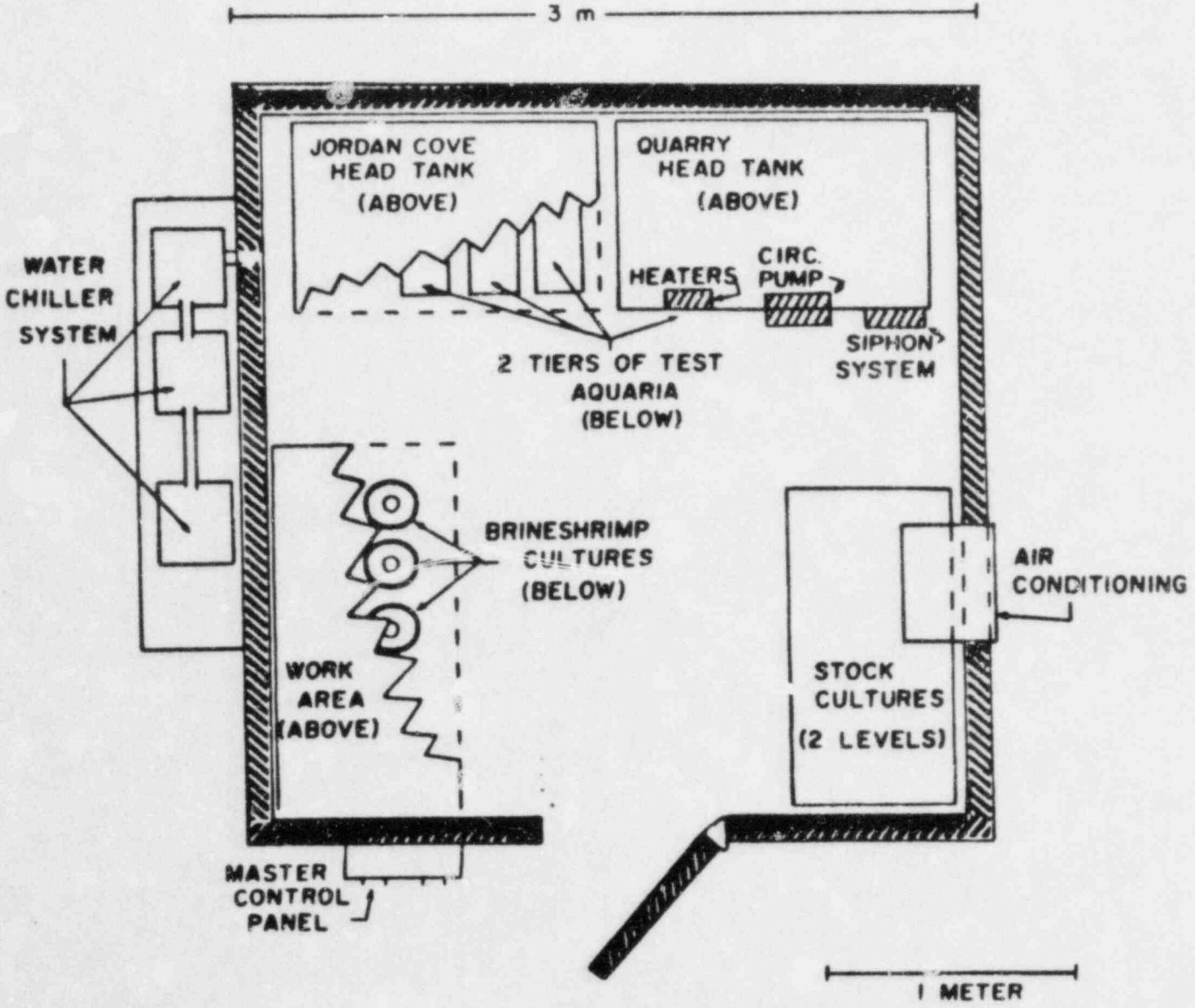

Figure 1. Environment room used for conducting toxicity tests and maintaining stock cultures.

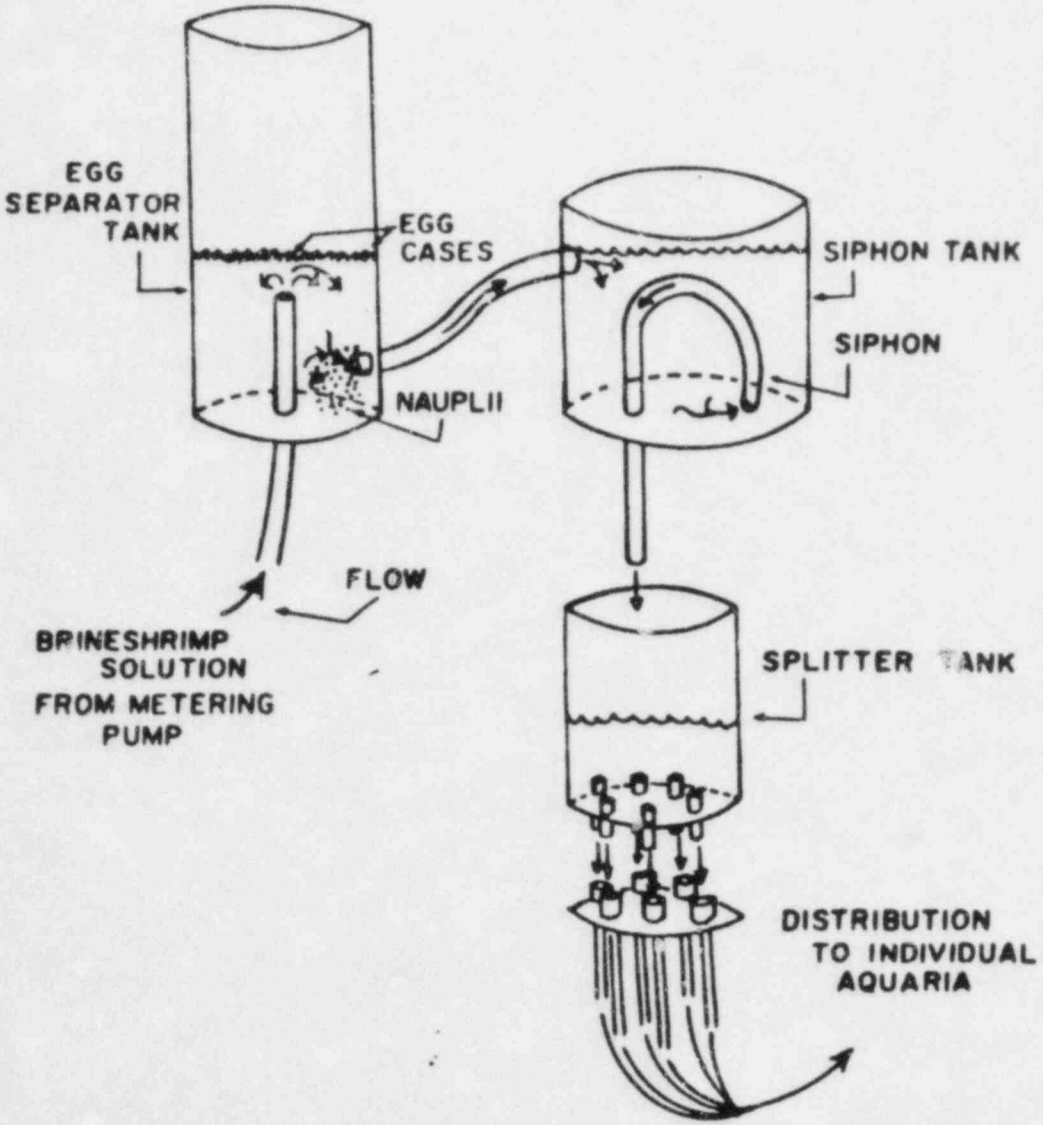

Figure 2. Brine Shrimp delivery system.

Initial Testing Procedure (Test 1)

Bay mysid shrimp testing began at NUEL using standardized chronic toxicity testing procedures (Nimmo et al 1978 and ASTM 1983).

Procedures consisted of isolating gravid females from stock cultures to secure day old juveniles. Ten juveniles were placed in each of three effluent and control test chambers (Fig. 3). Test chambers were polyethyelene containers (~300 ml) with outlets screened with 0.333 mm mesh to retain the test organisms. Photographs were taken biweekly for determination of growth rates. Mysids were measured to the nearest one-tenth of a mm. Young released (F_1) from the first generation (Parental) were separated from adults to prevent cannibalism and for continuation of testing. The F_1 generation test was conducted in the same manner as the parental test. Parental and F_1 tests were conducted for 30 and 19 days respectively.

Data obtained included growth rates, generation time and survival rates. This data was useful in assessing effluent toxicity but a procedure was needed which would assess the long term effects of the effluent and be more time efficient.

Modified Procedures (Test 2 through 5)

Juveniles and adults were placed in aquaria under effluent and control conditions. Various initial populations were used during Test 2 through Test 5 (Table 1). Test 2 used 10 juveniles and 15 adults in one 38 liter aquaria under effluent and control treatments. Test 3 had initial populations of 5 adults and 10 juveniles. Test 3 through 5 used triplicate test chambers to measure the variability between populations. Test 4 had

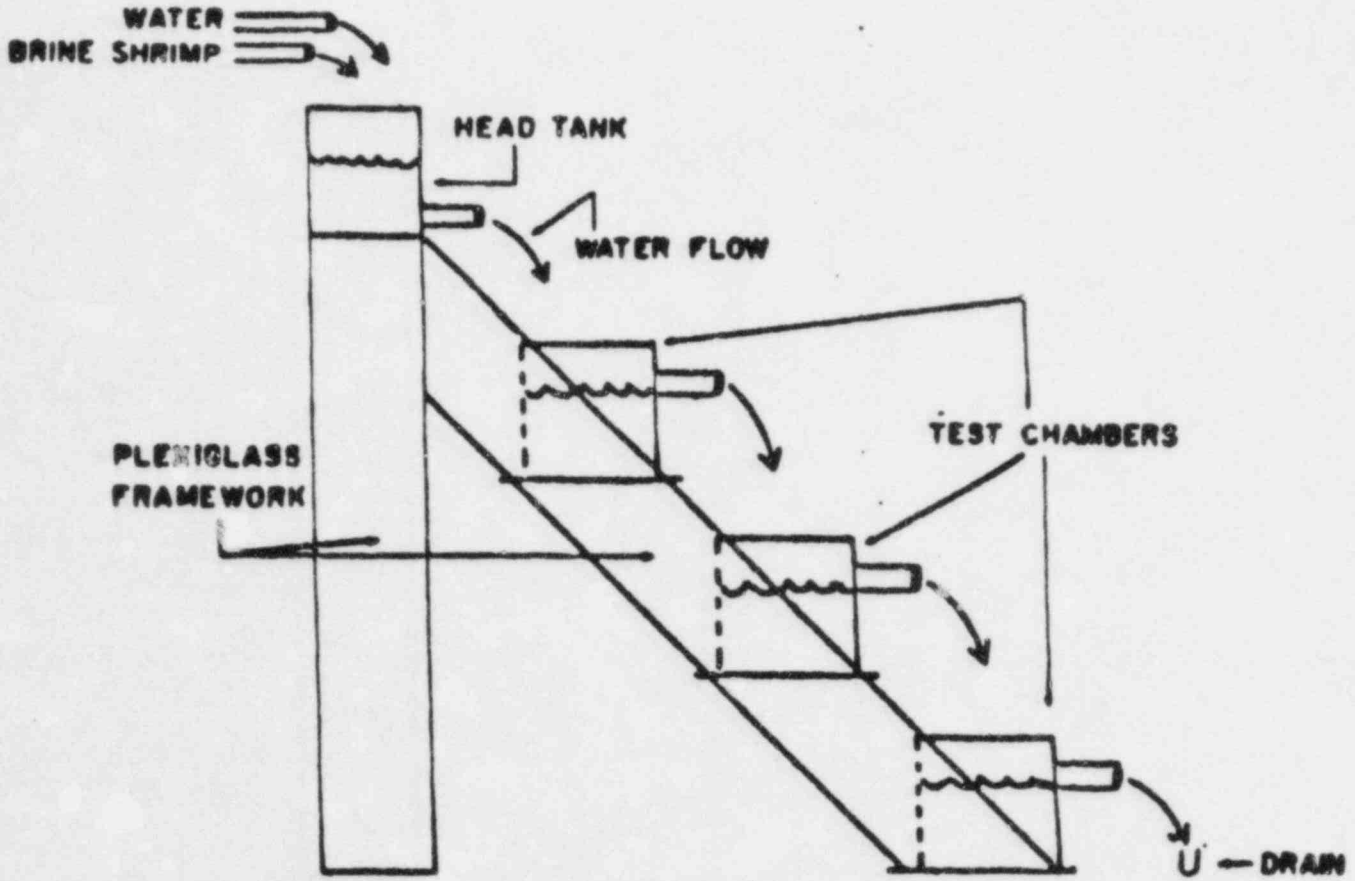

Figure 3. Mysidopsis bahia test apparatus used for Test 1.

Table 1. Summary of bay mysid testing procedures with test chamber type, initial population size, duration of test and parameters measured.

Test	Testing chamber type, number per treatment	Initial population count	Duration of test	Parameters measured
1	300 ml polyetheylene 3 replicates.	10 juveniles	Parental - 30 days F_1 - 19 days	Growth rate, survival rate, brood count, length data
2	38 l aquaria 1 each treatment	10 juveniles 15 adults	74 days	Brood count, length data
3	3 l aquaria 3 replicates	10 juveniles 5 adults	96 days	Brood count, length data, final population count for effluent
4	3 l aquaria 3 replicates	5 adults	46 days	Brood count, length data, population counts for both treatments
5	3 l aquaria 3 replicates	10 juveniles 10 adults	58 days	Brood count length data population counts for both treatments.

initial counts of 5 adults due to low stock population densities. Test 5 began using an initial population of 10 adults and 10 juveniles in each aquaria.

Brood counts were obtained during Test 2 through 5 by removing a gravid female biweekly from each aquaria.

The duration of testing varied from 46 to 96 days (Table 1). Due to the extended period of testing several system problems were encountered which caused the termination of tests. Test 2 was terminated after 74 days of testing due to a failure in the water distribution system. Test 3 was terminated after 96 days due to system malfunctions with the addition of brine shrimp which caused mortality in control aquaria. Test 4 was terminated after 46 days of testing due to low populations in control aquaria. Problems with the testing system were corrected prior to initiation of the next test.

Data obtained from these tests consisted of brood counts and length of females with broods. Total population counts were obtained in Tests 4 and 5 upon completion of testing. Test 3 total counts were obtained in effluent aquaria only. No total counts were obtained from either treatments in Test 2.

Analytical Methods

Instantaneous morality rates and instantaneous population growth rates were calculated using the following equation (Ricker 1975):

$$r = \log N_1 - \log N_o$$

r = rate of instantaneous mortality or population growth

N_1 = final population count

N_o = initial population count

A positive r value indicates an increase while a negative value indicates a decrease.

Differences in instantaneous mortality or population growth rates between control and effluent treatments were tested with a T-test. The parental generation in Test 1 was tested for differences in rates of mortality, as this test provided data on the initial and final population of a single generation of mysids. Instantaneous population growth rates were calculated for Test 4 and Test 5. These tests provided data on the population density of several generations over an extended time period.

RESULTS AND DISCUSSION

Although Test 2 through Test 4 were conducted to determine the feasibility of their use in long term effluent toxicity testing, data were collected which were useful in assessing the toxicity of MNPS effluent. Standardized procedures used in Test 1 proved to be more time consuming than the procedures used in later tests.

Results collected in Test 1 allowed calculations to be made of mysid growth rates, generation time and survival rates. Data collected from Test 2 through 5 focused primarily on brood size and population stability (Table 1).

Test 1

Test 1 indicated a mysid generation time from 30 to 36 days at 20°C in both test and effluent treatments. Reitsema and Neff (1980) found that mysids maintained at 25°C had a shorter life cycle of 14 to 21 days. Test 1 analysis indicated no significant ($\alpha = 0.05$) difference between growth rates under effluent and control treatments (Fig. 5 and Table 2). Mysids which were 24 hrs old (average size of 2.04 mm) grew an average of 3.7 mm in 30 days.

A key indicator of population stability and whether or not toxic pollutants are present in effluent waters is mortality rates. Instantaneous mortality rates (as the rate of change for the duration of the test) were calculated for the parental generation in Test 1 (Table 3). No significant ($\alpha = 0.05$) difference in mortality rates between control (mean of $-0.69 \pm .45$ S.D.) and effluent (mean of $-0.46 \pm .42$ S.D.) treatments were detected.

Mortality rate and growth rate data indicated no toxic concentrations of pollutants were present in MNPS effluent. But the testing procedure was very time consuming.

Test 2 through 5

Data collected from Test 2 through 5 allowed calculations of mysid brood size, and population growth to be made. Data were used as indicators of population stability and the ability of the mysids to reproduce (ASTM 1983).

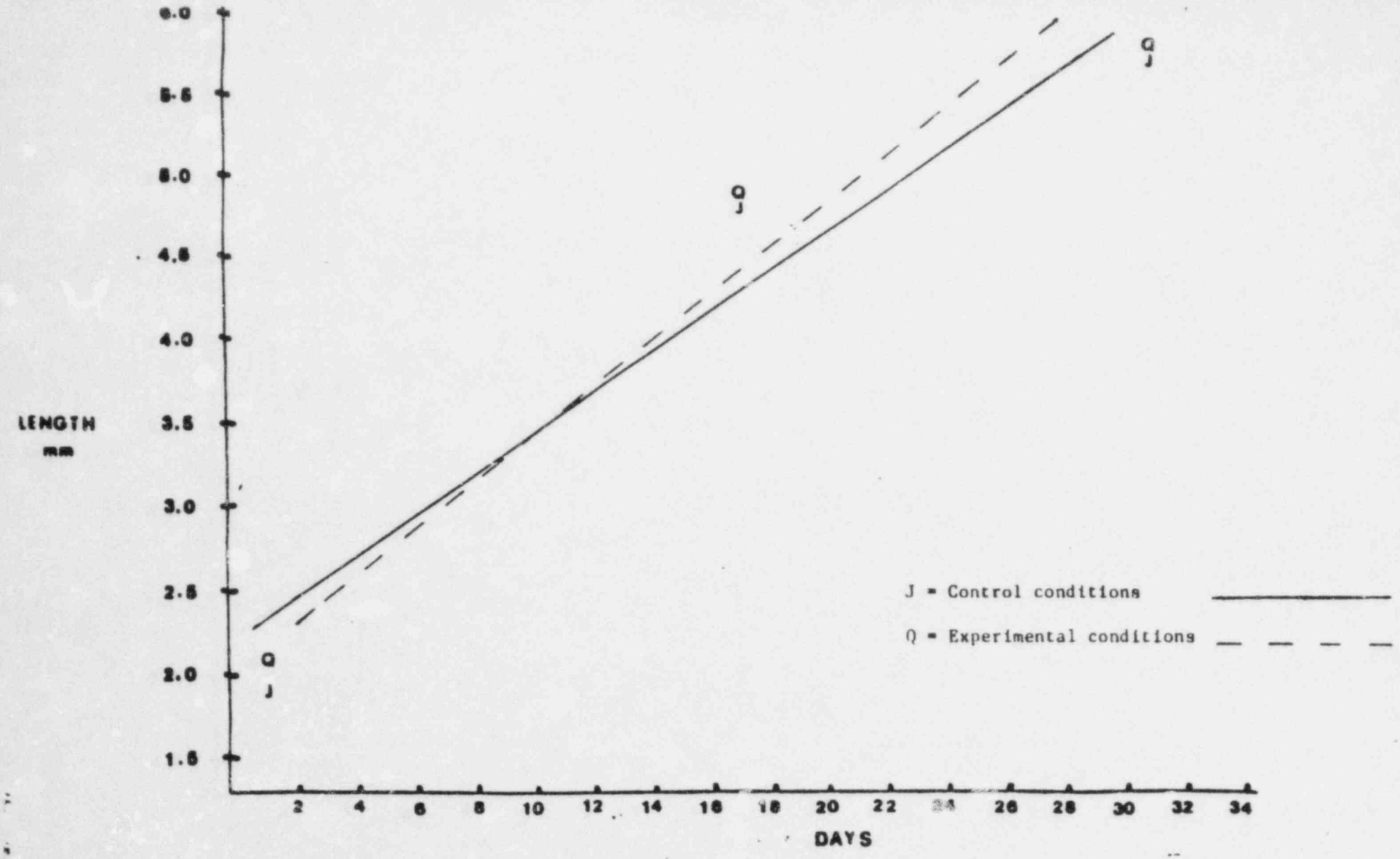

Figure 5. Mysid growth for parental generation, Test 1, with mean length for each measurement date and regression line for each treatment.

Table 2. Linear regression equations for mysid shrimp length (L) in mm versus age (days) for Test 1 parental and F_1 generations for control and effluent treatments.

Generation	Treatment	Equation	R^2
Parental	control	L = 2.01 + 0.130 * age	0.89
	effluent	L = 2.18 + 0.126 * age	0.89
F_1	control	L = 0.545 + 0.087 * age	0.95
	effluent	L = 1.056 + 0.095 * age	0.78

Table 3. Instantaneous mortality rates (as rate of change for the duration of the test - 30 days) for parental generation Test 1 for control and effluent treatments.

	Control	Effluent
Rep 1	-0.511	-0.916
Rep 2	-0.357	-0.105
Rep 3	-1.204	-0.357

Brood size ranged from 1-35 (mean of 14.24) in control and 1-26 (mean of 12.64) for effluent treatments during Tests 2 through 5. Nimmo and Hamaker (1982) found a reduction in mysid brood size was indicative of toxic concentrations of the pesticide, Dimilin. Lengths of gravid females ranged from 4.96 to 8.96 mm (mean of 7.03 mm) in control specimens and 5.2 to 9.20 mm (mean of 7.06 mm) in effluent treatments. A significant relationship exisited between female length and brood counts in both effluent and control treatments (Fig. 6 and 7). Analysis of covariance (with length as the covariate) showed no significant ($\alpha = 0.05$) difference between brood size in control and effluent treatments.

Instantaneous population growth rates (as the rate of change for the duration of the test) were calculated for Test 4 and 5 (Table 4). Although the rate of population growth was apparently greater in effluent (mean of 0.98 ± 1.35 S.D) than in control (mean of 0.47 ± 1.32 S.D.) analysis by T-test indicted no significant ($\alpha = .05$) difference between control and effluent treatments.

Both the effluent and control populations retained the ability of self-maintenance during Test 2 through 5, apparently unimpaired by potential effluent toxicity. With a generation time of 30 to 36 days (at 20°C) at least two generations in Test 2, 3 and 5 were exposed to effluent in a period up to 3 months with no detectable effect.

Heavy Metals

Concentrations of various chemicals occurring in the MNPS effluent were monitored 5 times a year (NUSCo 1982 and 1983). The potential toxicity of the MNPS effluent due to heavy metals may be of concern in the MNPS area. Heavy metals leaching from the MNPS cooling system are primarily

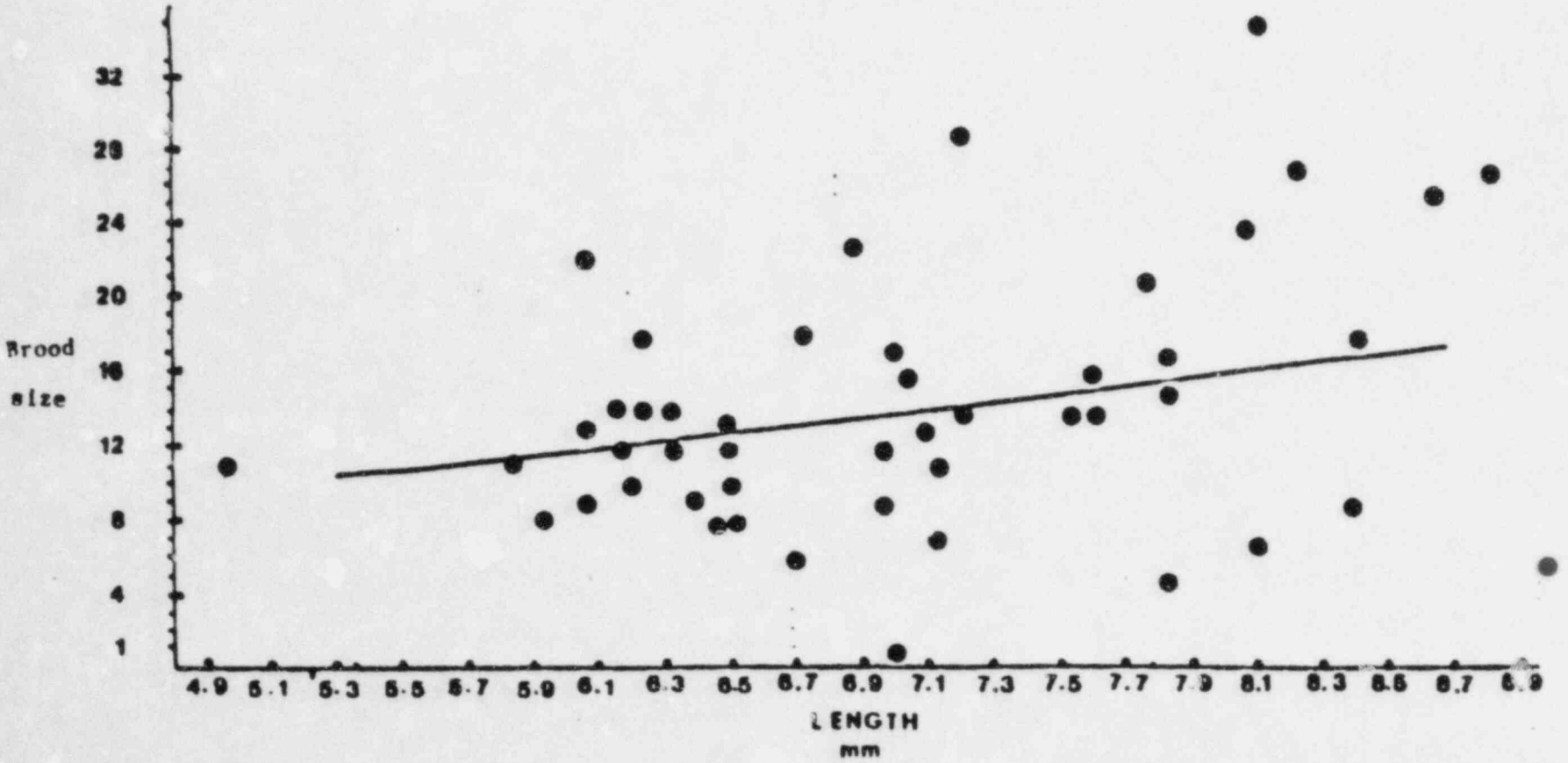

Figure 6. Brood size versus female length (mm) for control treatments.

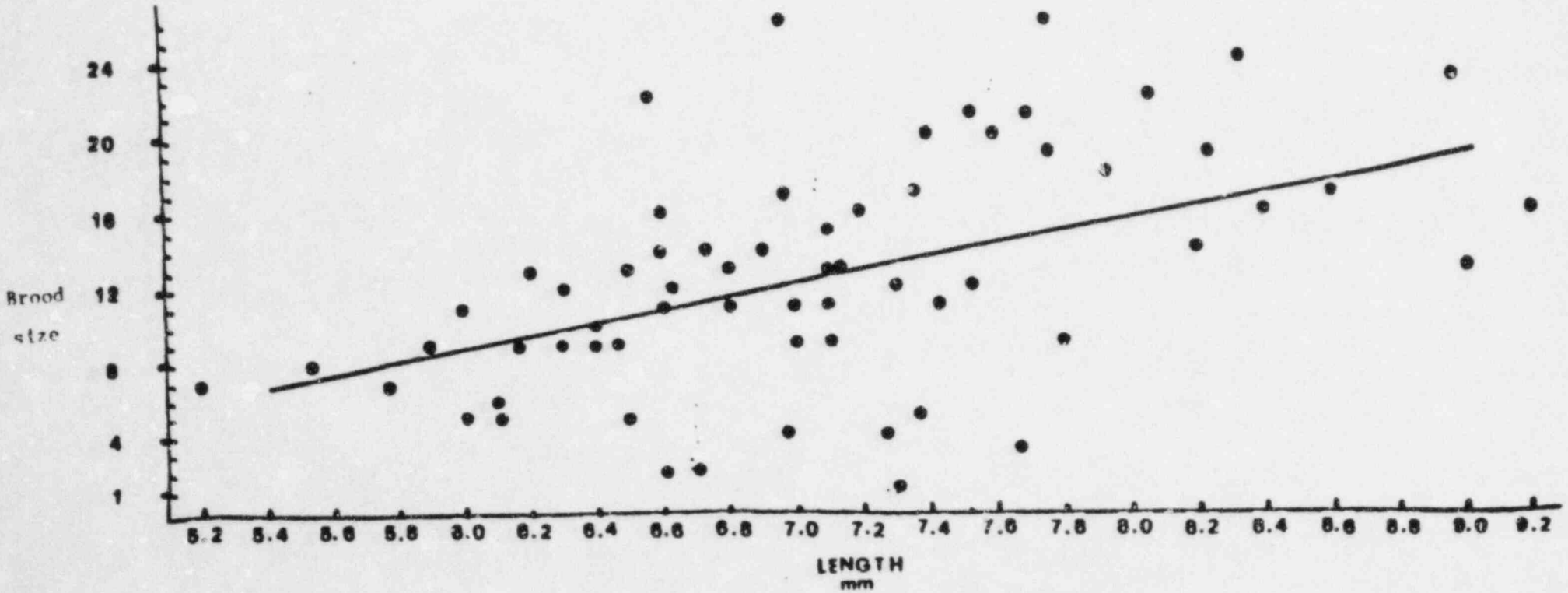

Figure 7. Brood size versus female length (mm) for effluent treatments.

Table 4. Instantaneous population growth rates (as rate of change for the duration of the test) for Test 4 (46 days) and Test 5 (58 days) for control and effluent treatments.

		Control	Effluent
Test 4	Rep 1	0.470	2.708
	Rep 2	1.030	0.470
	Rep 3	1.974	2.695
Test 5	Rep 1	0.140	-0.105
	Rep 2	-1.897	-0.105
	Rep 3	1.131	0.223

copper, zinc and nickel. The concentrations of these heavy metals necessary to induce both chronic and acute effects in the bay mysid is well above those levels measured during plant operation at MNPS (Table 5). Thus the potential toxicity of the effluent during toxicity testing due to heavy metal concentrations was minimal.

CONCLUSION

Effluent toxicity testing at NUEL with the bay mysid was conducted with two objectives: (1) develop procedures for long term toxicity testing of the effluent and (2) collect data to assess the toxicity of the MNPS effluent.

Procedures were developed and modified from the standardized procedures to optimize the efficiency and reliability of assessing the toxicity of MNPS effluent. The testing procedures used in Test 1 and those developed later were evaluated for reliability of the testing system and the usefulness of data in assessing the toxicity of the MNPS effluent. Once tests are begun, using the modified testing procedure, daily inspections of the test systems and populations are accomplished at a level which is more time efficient than the standardized test procedure used in Test 1. Data collection every two weeks requires minimal effort and enables reliable conclusions to be drawn as to the long term toxicity of MNPS effluent.

Data collected during testing of the effluent indicated no toxic effects to the bay mysid. No detectable differences between control and effluent treatments were found for the mysid shrimp growth rates, survival rates, brood size and population mortality rates.

Table 5. Mean annual soluble trace metals occurring in MNPS discharge waters, EPA water criterion standards, and acute and chronic toxicity levels for Mysidopsis bahia.

Element	Concentration in µg/l Seawater Occurring in Quarry Water (± standard deviation)	[2] Water Criterion Standards	[4] Mysidopsis bahia Acute Value	Chronic Value
[1] Copper	1981: 2.4±0.9; 1982: 1.9±0.3	4.0 µg/l over a 24 hr. period	181 µg/l	54 µg/l
[1] Zinc	1981: 13.8±11.4; 1982: 5.8±4.4	58 µg/l 24 hr. period Not to exceed 170 g/l at any time	173 µg/l	166 µg/l
[2] Nickel	1979: 2.6 - 4.3 µg/l above ambient level of 2.4 µg/l.	7.1 µg/l for 24 hr. period Not to exceed 149 µg/l at any time.	152 µg/l	92. µg/l

1. NUSCo 1981; NUSCo 1982.
2. Waslenchuck 1982.
3. Federal Register 1980.
4. EPA 1980.

Considering dilution of the Millstone cooling waters by Long Island Sound receiving waters (Waslenchuk 1982), and that no toxic effects were found in undiluted effluent, no toxic concentrations of pollutants would be expected to have occurred in the waters adjacent to MNPS during the test period.

RECOMMENDATIONS

With passage of the Clean Water Act of 1977 bioassays are becoming increasingly important. Industries are required to demonstrate that effluents fall within limitations set by both State and Federal Agencies and do not harm indigenous populations. Under the Clean Water Act of 1977, effluent toxicity testing can be required in the NPDES permit. Bioassays at Millstone are an essential tool in answering questions as they arise as to the potential toxicity of the effluent.

Using the procedures described for Test 5 we propose to test continuously starting ten adults and ten juvenile mysids in each of three replicate aquaria in both effluent and control treatments. Biweekly subsampling of brooding females for length and brood counts will coincide with photographic estimates of populations. Tests will be conducted for 60-70 days inorder that at least 2 generations of mysids will be exposed during each test. Brood size and length data will be collected in combination with total population composition data upon completion of testing.

Although no biological response to effluent concentrations of chemicals was found for MNPS operations during the test period we recommend continuation of testing at the present level. With Millstone Unit 3 scheduled for commercial operation in 1986, continued effluent toxicity testing will

enable future comparisons to be made with a reasonable degree of certainty, not only with regards to the present two units operating but in addition to prestart-up, start up and post operational Unit 3.

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