EFFECTS OF EFFLUENT DISCHARGED FROM SEABROOK STATION'S SETTLING BASIN ON SURVIVAL OF SELECTED MARINE INVERTEBRATES, SEABROOK ECOLOGICAL STUDIES, 1980

TECHNICAL REPORT XII-4

Prepared for

PUBLIC SERVICE COMPANY
OF NEW HAMPSHIRE
Manchester, New Hampshire

Prepared by

NORMANDEAU ASSOCIATES, INC.
Bedford, New Hampshire

December 1980

TABLE OF CONTENTS

EFFECTS OF EFFLUENT DISCHARGED FROM SEABROOK STATION'S SETTLING BASIN ON SURVIVAL OF SELECTED MARINE INVERTEBRATES, SEABROOK ECOLOGICAL STUDIES, 1980

1.0 INTRODUCTION

Environmental effects of effluent discharged from the Seabrook Station's settling basin have been evaluated by a number of studies since 1978. These programs have included field surveys in 1978 and 1979 plus a laboratory bioassay in 1980 (NAI 1979, 1980) utilizing *Nereis virens* (sand worm), *Crangon septemspinosa* (sand shrimp) and *Mya arenaria* (soft-shell clam). The purpose of this laboratory bioassay was to continue monitoring effluent effects and provide data to support or reject earlier findings. The assay also provided data on the effects of increased dilution (an additional 1 million gallons per day of seawater from the tunnels) on treated sanitary wastes and site surface water runoff. Incidently the assay also provided limited insight into effects of addition of alum to the pond waters as an aid in flocculating suspended particulate matter.

The October 1980 bioassay has been modified, with respect to the previous laboratory study, to provide additional information on chronic exposure and sublethal effects. Periods of exposure for *C. septemspinosa* and *N. virens* were increased from 7 to 14 days to be compatible with *M. arenaria* and to better evaluate chronic exposure effects. To evaluate sublethal effects, data on animal activity and response to external stimulation were recorded. In addition to the three species historically used in the program, a fourth species, *Neomysis americana* (mysid shrimp), was utilized to provide survival data on an additional, sensitive local species. This species is widely used by the EPA and Army Corps of Engineers as an internal standard in NPDES and dredge spoil toxicity evaluation. The program was also designed to

evaluate the impact of diluted effluent as it would be found in the Browns River.

Information provided from these bioassay tests is limited to whether environmental conditions at test sites in question will sustain the test organisms for the test period. While a negative finding (no significant mortality) indicates absence of harmful conditions at the test site during the test period, a positive finding (significant mortality) signals the presence of harmful substance(s), but does not implicate a specific source of environmental disturbance.

2.0 METHODS AND MATERIALS

Procedures used in the January 1980 *in vivo* bioassay followed protocol outlined in "Methods for measuring the acute toxicity of effluents to aquatic organisms" (U.S. EPA, 1978).

Settling pond effluent was pumped from approximately 1 meter below the surface of the settling pond at the discharge weir into a 500 gallon holding tank. Water for the control assays was pumped from the Hampton Harbor at the State Fish Pier and held in a 1000 gallon holding tank. All water was filtered through a 100 micron filter to remove large particulate matter. Filtration at this level had no effect on turbidity of the pond effluent or control water. Most of the suspended particles in the pond effluent are less than 20 microns in size. Water in both primary holding tanks was replenished on a daily basis. Effluent and control water were fed through a series of secondary holding tanks to heat exchangers which heated the effluent to 20°C. The effluent was then pumped to a distribution center which provided diluted effluent (50% V/V) water, undiluted effluent water, and control water at a rate of at least 5 liters per hour (Figure 1). Dilution levels for the mixed effluent (50%) represent a conservative estimate of levels of the pond effluent in the Browns River. Calculations were based on cross-sectional area and average flow rates (river and pond), and assumed a 50% flushing rate for the river on each tidal cycle. Test chambers were 19-liter

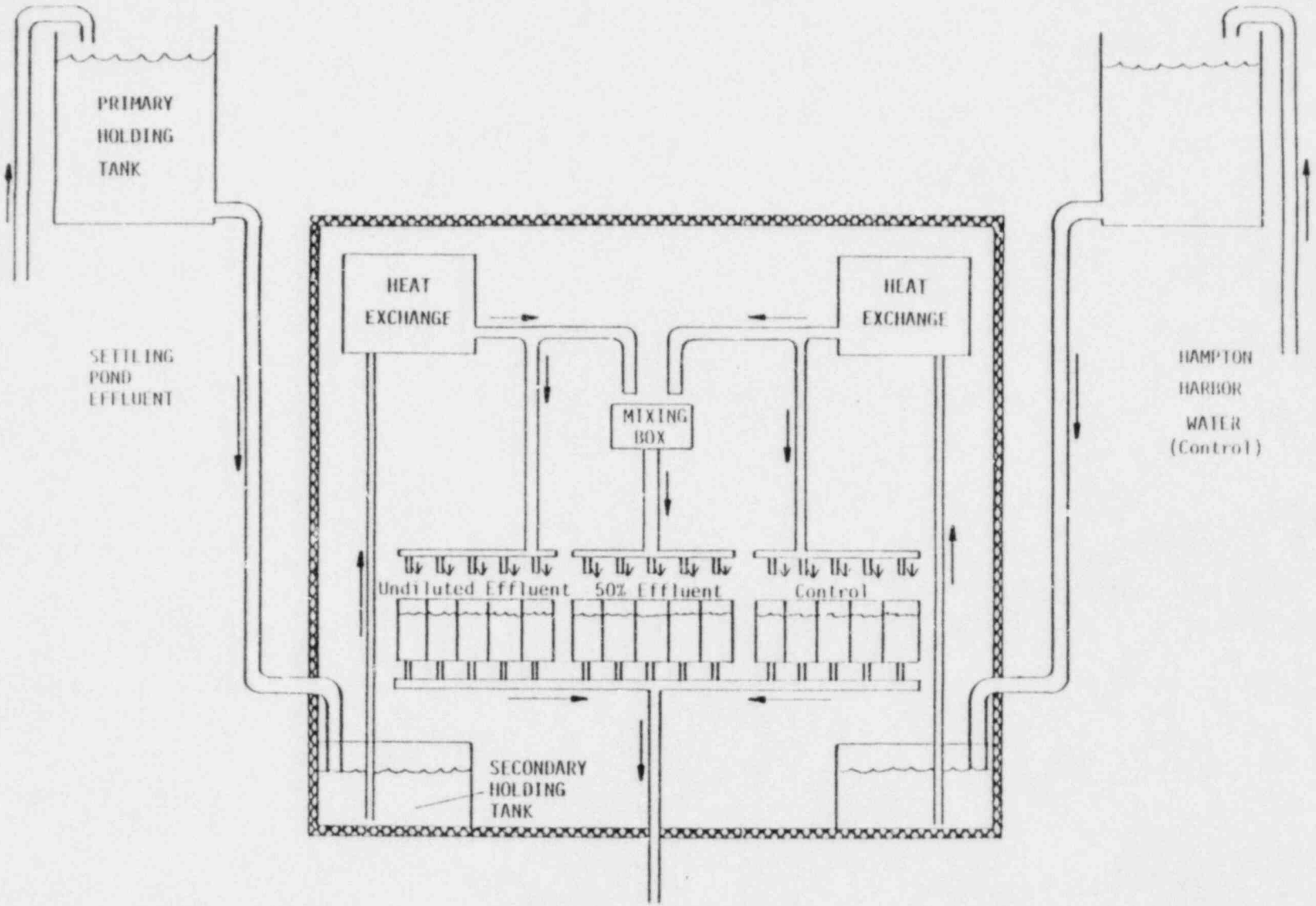

Figure 1. Flow schematic *in vivo* bioassays. Seabrook Settling Pond Effluent Bioassay. Seabrook Ecological Studies, 1979.

glass aquaria measuring 30 cm x 20 cm x 25 cm-deep, filled to a depth of 6 cm with fine sand. Five replicate aquaria were used with each treatment.

Test organisms used in the assay were *Nereis virens* measuring 5-10 cm in length, *Crangon septemspinosa* and *Mya arenaria* (2-3 cm in length), and *Neomysis americana* (14 days old). Numbers of organisms per replicate were as follows: *N. virens*, 10; *C. septemspinosa*, 10; *N. americana*, 10; and *M. arenaria*, 15. Test organisms were from laboratory and field stocks. *Mya arenaria*, *C. septemspinosa*, and *N. virens* were collected from Hampton/Seabrook estuary. *N. americana* were from laboratory populations maintained by Normandeau Associates. Animals were placed in the aquaria 48 hours prior to the start of the assay. Dead animals or those showing obvious (behavioral) signs of stress were removed and replaced. *Mya arenaria*, *N. virens* and *C. septemspinosa* were exposed to the effluent for 14 days, *N. americana* were exposed for a period of 4 days. Animals were fed daily.

Measurements of water temperature, salinity, dissolved oxygen and pH were measured on a daily basis. Test animals were observed daily, numbers of animals alive were recorded, and dead animals removed. Notes on animal behavior and response to external stimulation were noted.

2.1 STATISTICAL ANALYSIS

Counts of live versus dead test organisms in experiments where control survival exceeded 90% were submitted to a 3-factor G-test (Sokal and Rohlf, 1969). This test computed expected chi-square frequency distributions for: 1) condition (live or dead), 2) treatment and 3) replicate, plus tested the independence of the three factors from each other. Of primary concern was comparison of condition with effluent type; finding a significant "dependency" between these two factors would mean that organism deaths were affected by sample treatment.

3.0 RESULTS

Bioassay results, summarized in Table 1, show no significant ($\alpha>0.05$) treatment related mortality for *M. arenaria*, *N. virens* and *N. americana*. Survival in general ranged from 90 to 100% by replicate with the exception of *N. americana* where survival in undiluted effluent averaged 78%. *Crangon septemspinosa* exhibited a significant response to the undiluted effluent with 54% of the test organisms surviving. No significant response was reported for the 50% effluent.

Daily examination of test animals produced no data to indicate the animals to be stressed in any of the treatments. *Mya arenaria* were observed to be filtering water and retracted their siphons when touched with a probe. *Nereis virens* were observed burrowing in the sediment, and animals on the surface retracted into their burrows when proded. The polychaetes also aggressively searched out food (pieces of cut-up clam) during feeding periods. *Crangon septemspinosa* were observed during daily feeding periods and exhibited no evidence of stress. The remainder of the time observations were difficult to obtain as the shrimp were buried in the sediment.

4.0 DISCUSSION

Results of this laboratory assay showed that effluent from the Seabrook Station settling pond had no significant acute effect, in terms of survival or behavioral response, on *M. arenaria*, *N. virens* and *N. americana*. *Crangon septemspinosa* exposed to the undiluted effluent were affected; survival was significantly reduced. These findings are similar to those reported in previous studies on the settling pond effluent (NAI, 1979, 1980).

Mya arenaria, *N. virens* and *N. americana* showed no gross response (i.e. death) or sublethal sign of stress when exposed to either the undiluted or diluted (50% V/V) settling pond effluent. *Mya arenaria* were observed to be actively filtering water during the assay and res-

TABLE 1. SUMMARY OF OCTOBER 1980 *IN-VIVO* BIOASSAY RESULTS. SEABROOK ECOLOGICAL STUDIES, 1980.

SPECIES	DURATION OF TEST	TEMP °C	SAL ‰	D.O. mg/l	pH	NUMBER OF SURVIVORS AT END OF ASSAY				
						REP	UNDILUTED EFFLUENT	50 PERCENT EFFLUENT	CONTROL	G-TEST RESULTS
Mya arenaria	14 days	20°±1°C	E[a]=25.7-28.6	5.2-7.4	6.6-6.8	1	15[b]	15	15	N.S.
						2	14	15	15	
			C =31.3-32.2	5.1-7.3	7.6-7.8	3	14	15	15	
						4	15	15	14	
						5	15	14	15	
Nereis virens	14 days	20°±1°C	E =25.7-28.6	5.2-7.4	6.6-6.8	1	10	8	10	N.S.
						2	10	9	9	
			C =31.3-32.2	5.1-7.3	7.6-7.8	3	9	10	10	
						4	10	9	10	
						5	8	10	10	
Crangon septemspinosa	14 days	20°±1°C	E =25.7-28.6	5.7-6.8	6.6-6.8	1	2	10	10	*** (undiluted effluent vs control)
						2	4	10	8	
			C =31.3-32.2	4.6-6.9	7.6-7.8	3	9	10	10	
						4	6	9	10	
						5	6	9	9	
Neomysis americana	4 days	20°±1°C	E =27.1-27.6	6.2-6.8	6.6-6.8	1	7	9	10	N.S.
						2	9	9	8	
						3	7	9	9	
			C =30.9-31.4	4.6-6.9	7.7-8.0	4	7	10	9	
						5	9	9	9	

[a] E= Settline Pond Effluent C= Hampton Harbor Control Water

[b] Number of animals at start of experiment: *Mya*=15; *Nereis*=10; *Crangon*=10; *Neomysis*=10

***= significant effects at α=.05

N.S. = not significant at α=.05.

ponded as anticipated to external stimuli (gentle prodding of the exposed siphons). *Nereis virens* were active in all treatments; burrows were established and the worms were observed to spend much of the time within the sediment. Animals on the surface quickly retracted into their burrows when prodded. The worms also moved out of their burrows during daily feeding periods and actively searched out food items. No variations in survival or behavioral response were observed between replicates or treatments. The level of activity and condition of the test animals at the conclusion of the test was in sharp contrast to that of survivors from the earlier laboratory assay (NAI 1980). Animals exposed to the pond effluent during the January assay were flaccid and much less active. As no major changes on the composition of the effluent have been reported (except for a five day period during this assay involving the addition of alum to the pond effluent) it would indicate that the improvement in general condition of the animals was in response to the greater dilution of the effluent by greater quantities of sea water seeping into the tunnels (R. Faulkner, PSCo, Pers. Comm.). It is also possible that the presence of readily available food may have had an impact on the general improved physiolcgical condition, but this is unlikely as health of the unfed controls was similar to the fed controls. *Neomysis americana* exhibited no abnormal activity or behavior. *Crangon septemspincsa* exposed to the effluent exhibited no outward sign of stress or unusual behavior in either of the treatments during the assay. Daily observations made during feeding showed animals in the treatments and controls were able to locate food and were aggressive in their search. During other periods, little activity was observed. In general, animals spent most of their time in burrows in the sediment. The October assay results are supported to a limited degree by findings from the earlier assay in which survival of the sand shrimp exposed to undiluted effluent was lower than the controls. The reduced survival of *C. septemspinosa* in the undiluted effluent as compared to the controls and diluted effluent indicates that if estuarine populations were exposed to 100% pond effluent a significant impact could occur to at least one species. This scenerio is an unlikely worse case condition.

The addition of alum to the settling pond water during days 3 to 8 of the assay had no apparent impact on *N. virens*, *M. arenaria* and *C. septemspinosa* (*N. americana* was not exposed to the effluent during this period). During this period animal behavior was "normal" and there was no evidence of any unusual mortality. Water quality during this period showed no evidence of change.

Comparison of undiluted and diluted effluent results showed that dilution of effluent was not required to improve short-term survival of the test species, with the exception of *C. septemspinosa*. In the case of *C. septemspinosa*, dilution of the ponds' effluent to levels comparable with those projected for the Browns River resulted in a significant increase in survival to a point not significantly different from survival in the controls.

5.0 SUMMARY

Results of the October 1980 laboratory bioassay were similar to those from earlier tests. The soft-shell clam, *Mya arenaria*, the sand worm, *Nereis virens*, and the mysid shrimp, *Neomysis americana*, all survived exposure to diluted and undiluted effluent for 14 days with no significant mortalities or effect on their response to external stimuli or modification of behavior. The improved condition (in terms of response to stimuli and physiological condition) of *Nereis virens* as compared to the previous laboratory assay has been attributed to a greater dilution of pond effluent by the addition of another 1 million gallons per day of water from the Seabrook Station's cooling water tunnels. Survival of the sand shrimp, *Crangon septemspinosa*, exposed to the undiluted effluent was significantly lower than that for animals exposed to either the diluted effluent or control water. These results show that the pond's effluent can have an impact on at least one species in the Browns River but that the present dilution of pond effluent in the river is sufficient to reduce effluent levels below an acutely toxic point.

6.0 LITERATURE CITED

Normandeau Associates, Inc. 1980a. Effects of settling pond effluent on survival of selected marine invertebrates: *In situ* Bioassay. Prepared for Public Service Company of New Hampshire. 8 pp.

_____. 1980b. Effects of Seabrook Station's Settling Basin Effluent on Survival of selected marine invertebrates. Technical Report XI-4. Seabrook Ecological Studies, 1979. Prepared for Public Service Company of New Hampshire. 12 pp.

Sokal, R.R. and F.J. Rohlf. 1969. Biometry W.H. Freeman Co., San Francisco. 776 pp.

U.S. Environmental Protection Agency. 1978. Methods for measuring the acute toxicity of effluents to aquatic organisms. EPA-600/4-78-012 U.S. EPA Cincinnati, OH. 51 pp.