EFFECTS OF SEABROOK STATION"S SETTLING BASIN EFFLUENT ON SURVIVAL OF SELECTED MARINE INVERTEBRATES

TECHNICAL REPORT XI - 4

SEABROOK ECOLOGICAL STUDIES, 1979

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June 1980

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1.0 INTRODUCTION

In August, September and November, 1978, a series of site specific bioassay experiments were conducted (NAI 1979) to determine *in situ* the acute (7 day) effects of effluent discharged into the Browns River from a one-acre settling pond on the Seabrook Station construction site. The pond includes surface runoff, tunnel drainage and a relatively small quantity of treated sanitary waste. Tests were run to evaluate the discharge effects on the sand worm, *Nereis virens*, the sand shrimp, *Crangon septemspinosa*, and the soft-shell clam, *Mya arenaria*. Field experiments were repeated in August 1979 and a laboratory assay conducted in January 1980 for the purpose of confirming or rejecting initial findings, and evaluating the impact of increased flow from the pond; 0.2 million gallons per day (MGD) in 1978, to 0.9 MGD in 1979 and to 2.2 MGD in 1980. Assays were also modified to provide longer exposure times for *Mya arenaria* and to evaluate the effect of suspended solids.

Information provided from these bioassay tests is limited to whether or not 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

2.1 *IN SITU* BIOASSAY

Procedures employed in the August 1979 bioassay were identical to those used in the 1978 studies (NAI 1979). Adult *Crangon septemspinosa* and juvenile *Mya arenaria* (shell length 25 to 30 mm) were collected in the lower Hampton-Seabrook Estuary within 48 hours of each test. *Nereis virens* were obtained from a local bait dealer, healthy specimens were selected by demonstrating an ability to establish burrows in sand.

Test containers were plastic pans, 25 x 30 x 13 cm, filled to a depth of 8 cm with sand (grain size .05 to .5 mm). The pans were set in wooden frames, with 3 pans for each species, and covered with 1.6 mm mesh plastic screening held in place by a rubber band passed around the circumference of the pan.

On 23 July 1979, frames containing pans with animals in place were set out below MLW in Hampton Harbor. The next day the pans were checked for dead animals before being taken to the test sites. In Browns River, test sites were immediately above and below the settling pond outfall (Figure 1). A new reference or "control" site was located on Mill Creek (Figure 1) which appeared to match environmental conditions (temperature, salinity, D.O.) in Browns River more closely than the Hampton River tributary used in 1978 (cf. Table 1, NAI 1979).

After securing the test frames to the stream bottom at the test sites, water samples were taken daily at low tide and approximately every other day at high tide; samples were analyzed for dissolved oxygen concentration, salinity, and turbidity. Water temperature was measured over the frames at the same time as water samples were being taken. During the test period, screening covering the pans was checked for build up of sediment or detritus and brushed clean as needed. After seven days, test frames were hauled out and *N. virens* and *C. septemspinosa* recovered from the pans by passing the sand through a 6 mm mesh sieve. The

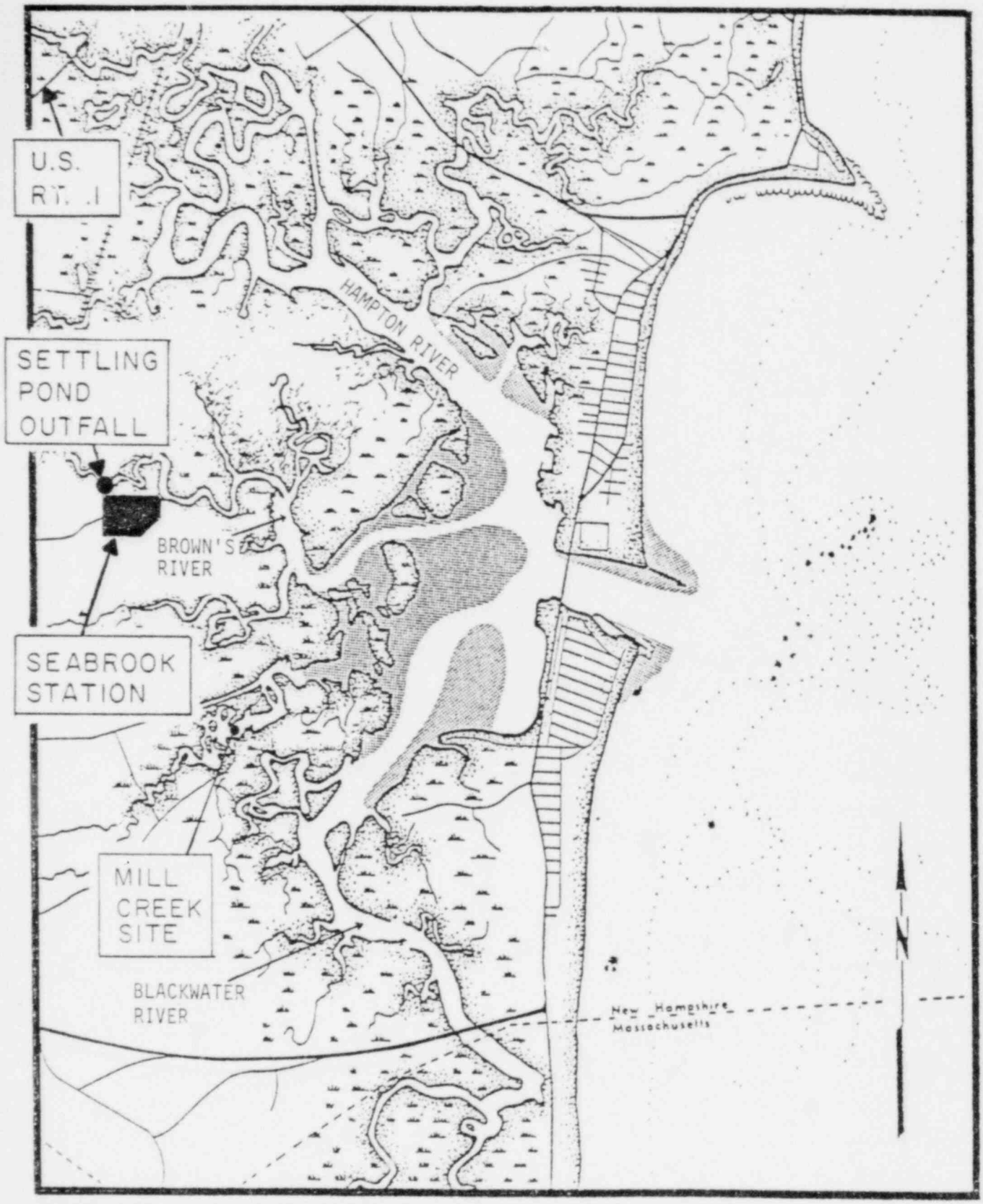

Figure 1. Location of control sites for *in-situ* bioassays. Seabrook Ecological Studies, 1979.

The number of live organisms were then counted and these counts compared against original live counts to determine the total number of dead and missing. After an additional seven days, *M. arenaria* were recovered and data recorded as above.

2.2 *IN VIVO* BIOASSAY

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

Settling pond effluent was pumped from approximately 1 meter below the surface of the settling pond at the discharge weir into a 1000 gallon holding tank. Water for the control assays was pumped from the Browns River approximately 100' downstream of the discharge to a 500 gallon holding tank. Water was collected during the last hour of the flooding tide. 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, heat exchangers and filters (20 micron) to a distribution system to provide filtered and unfiltered effluent, and unfiltered control water at a constant volume (5 liters/hour) and temperature (20°C) (Figure 2). Test chambers were 19-liter 30 cm x 20 cm x 25 cm-deep glass aquaria 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* (5-10 cm), *Crangon septemspinosa* (2-3 cm), and *Mya arenaria* (2-3 cm). Ten representatives of each species were used in each replicate. Test organisms were from laboratory stocks, maintained in the laboratory for approximately 10 weeks prior to the start of the assay. *Mya arenaria* and *C. septemspinosa* were originally collected from the Hampton/Seabrook estuary while *N. virens* were obtained from a local commercial supply house. During the holding period the *M. arenaria* and *N. virens* were maintained at 15°C, while *C. septemspinosa* were kept at ambient temperature (2-10°C). All

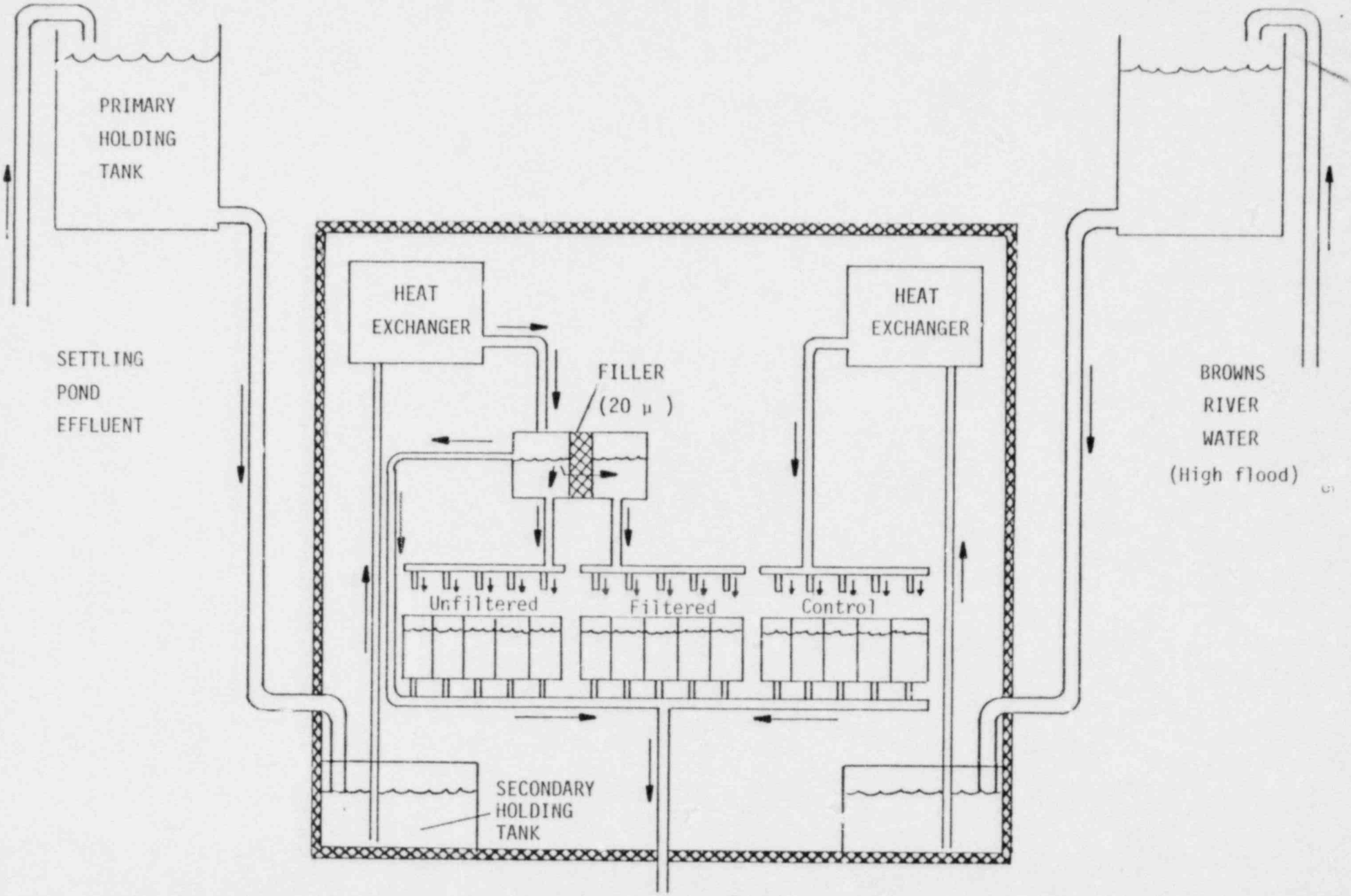

Figure 2. Flow schematic *in-vivo* bioassays. Seabrook Settling Pond Drain Bioassay. Seabrook Ecological Studies, 1979.

animals were acclimitized to the 20°C test temperatures over a period of 5 days (1°C increase per day for *M. arenaria* and *N. virens*, 3-4°C increase per day for *C. septemspinosa*). 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.

Measurements of water temperature, and salinity were made on a daily basis. Dissolved oxygen and pH measurements were measured at the start of the experiment and on days 7 and 14. Test animals were observed daily, numbers of animals alive (for *Mya* and *Crangon*) were recorded and dead animals removed.

2.3 STATISTICAL ANALYSIS

Counts of live versus dead (or missing) 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) test site; and 3) replicates, plus tested the independence of the three factors from one another. Of primary concern was comparison of condition with test site or effluent type; finding a significant "dependency" between these two factors would mean that organism deaths were affected by sample site or treatment.

3.0 RESULTS

3.1 *In Situ* Bioassay

Results of the *in-situ* bioassay, summarized in Table 1, show significant dependency between mortality and test site for *Nereis virens*; that is, significant difference in survival existed between the reference and treatment sites as well as between treatment sites. Data for *Mya arenaria* showed no significant dependencies related to test site or

TABLE 1. SUMMARY OF AUGUST 1979 *IN-SITU* BIOASSAY RESULTS. SEABROOK ECOLOGICAL STUDIES, 1979.

SPECIES	DURATION OF TEST	RANGE OF TEMPERATURE (°C)		RANGE OF SALINITY (‰)		RANGE OF DISSOLVED OXYGEN (mg/l)		RANGE OF TURBIDITY (NTU)		REP	NO. SURVIVORS (MAX. NO. = 20)			
		BROWNS RIVER	MILL CREEK	BROWNS RIVER	MILL CREEK	BROWNS RIVER	MILL CREEK	BROWNS RIVER	MILL CREEK		ABOVE DISCHARGE	BELOW DISCHARGE	MILL CREEK	G-TEST RESULTS
Nereis virens	7 days	Low 20.3	20.5	15.0	15.5	4.8	4.7	1.1	0.4	1	18	6	18	3 factor[a]
		High 26.4	27.6	29.7	31.4	12.4	9.7	6.6	4.4	2	11	9	19	*
										3	15	5	20	
Crangon septemspinosa	7 days	(Same as for *Nereis virens*)								1	10	11	11	
										2	0[b]	15	12	n.a.
										3	14	15	17	
Mya arenaria	14 days	Low 18	16.3	15.0	15.5	4.8	4.7	1.1	0.4	1	20	20	20	3 factor[a]
		High 28.7	27.8	29.9	31.4	12.4	9.7	8.3	4.4	2	20	20	19	n.s.
										3	20	20	19	

* Test results significant at $\alpha = .005$ for station mortality dependency

n.s. Test results not significant at $\alpha = .05$

n.a. Results not statistically analyzed due to survival at reference field control site <90%

b Results suspect as tray was turned upside down before recovery, possibly crushing the organisms.

a condition x site x replicate

replicate. Results of the *Crangon septemspinosa* assays were considered to be inconclusive as mortality in the control samples exceeded 10% and therefore were not analyzed statistically.

3.2 *In-vivo* Bioassay

In-vivo bioassay results summarized in Table 2, show no significant dependency between mortality and treatments (filtered and unfiltered effluent) or between replicates for either *M. arenaria* or *N. virens*. Results of the assay with *C. septemspinosa* show both the unfiltered and filtered effluent having greater mortality than the controls. These results were not tested statistically, however, and do not carry the same weight as those for *M. arenaria* and *N. virens*, as control mortalities exceeded 10% suggesting other sources of stress.

4.0 DISCUSSION

In-situ and *in-vivo* bioassays evaluating the potential acute toxicity of effluent discharged from the Seabrook Station settling pond produced similar findings for *M. arenaria* and *C. septemspinosa* while results of the *N. virens* assays appeared to be ambiguous.

In both field and laboratory assays *M. arenaria* showed no gross response (*i.e.* death) to the settling pond effluents. High survival rates for *M. arenaria* in the 1978 field bioassays had been attributed to the animals ability to respire anerobically for extended periods and reduce or eliminate contact with the effluent. During the *in-vivo* assays at 20°C, clams actively filtered (siphoned) water, exposing themselves to any potential toxicants and indicating that the effluent had no acute adverse impacts. The possibility of physiological impacts such as bioaccumulation, changes in condition indices or reproductive potential, was not addressed.

TABLE 2. SUMMARY OF JANUARY 1980 *IN-VIVO* BIOASSAY RESULTS. SEABROOK ECOLOGICAL STUDIES, 1979.

SPECIES	DURATION OF TEST	TEMP °C	SAL ‰	D.O. mg/l	pH	REP	NUMBER OF SURVIVORS AT END OF ASSAY			G-TEST RESULTS [c]
							UNFILTERED EFFLUENT	FILTERED EFFLUENT	CONTROL	
Mya arenaria	14 days	20°±1°C	E[a] =24.5-27.2	8.0-8.8	7.0-7.5	1	10[b]	10	8	N.S.
						2	10	10	8	
			C =27.0-30.1	8.0-8.3	7.0-7.5	3	10	9	10	
						4	10	10	10	
						5	10	10	10	
Nereis virens	7 days	20°±1°C	E =24.5-27.0	3.0-8.8	7.0-7.5	1	10	9	10	N.S.
						2	9	7	10	
			C =27.0-30.1	8.0-8.8	7.0-7.5	3	9	9	10	
						4	8	9	10	
						5	1	9	9	
Crangon septemspinosa	7 days	20°±1°C	E =24.5-27.0	8.0-8.8	7.0-7.5	1	2	2	1	N.A.
						2	0	3	6	
			C =27.0-30.1	8.0-8.8	7.0-7.5	3	2	3	2	
						4	2	2	6	
						5	1	1	6	

[a] E = Settling Pond Effluent C = Browns River Control

[b] Number of animals at start of experiment = 10 in all experiments

[c]

N.A. = Results not analyzed due to control survival <90%; indicates potential external stress on the test animals

N.S. = significant at $\alpha = .05$.

Treatment mortality was observed for *C. septemspinosa* in both field and laboratory bioassays. However in both sets of bioassays survival in control (or reference) experiments was below the criterion (90%) for a successfully concluded bioassay test. Observations made during the field assay suggested that canabalism might have been a factor in reducing overall survival; although this was not documented. In the laboratory assay no incidences of cannibalism or aggression were noted, meaning that some factor other than cannibalism produced the high mortality in the *in vivo* tests. Potential sources of stress include the rate of temperature change (3-4°C/ day) during acclimitization and the reference (control) water used in the experiment. While the rate of temperature change is higher than the 1°C /day generally used in acclimitizing laboratory stocks, it is within the range experienced by field populations during migrations (Boddeke, 1976). Results suggest that either there is an experimental handling problem with these organisms or that the condition of the estuarine river water used as controls is not of adequate quality for high survival of *Crangon*. These problems will have to be resolved before the effects of the settling pond water on this species can be determined.

Field and laboratory assays with *N. virens* showed some apparent discrepancy in survival rates; field assays showing significant treatment mortality while laboratory assays showed no significant difference in survival between control and treatments. While no significant treatment related mortality was observed in laboratory tests, differences in the physical condition of the animals did exist between controls and treatments. *Nereis virens* recovered from the controls were active and had firm bodies while those recovered from the treatments, both filtered and unfiltered effluent, were sluggish and flaccid. In evaluating the two sets of data (field vs. laboratory) differences in survival of *Nereis* can possibly be attributed to the synergistic effects of the effluent and the different temperature regimes.

Analysis of the filtered and unfiltered effluent showed no significantly different rates of survival for all animals tested. While the assay procedure was designed to remove particulate matter, larger than ≤20 microns, it was estimated that much of the suspended material being discharged during the assay was in the fine silt/clay size range, less than 5 microns.

5.0 SUMMARY

Both field (*in-situ*) and laboratory (*in-vivo*) experiments showed generally similar results with respect to the effects of Seabrook Station's settling pond effluent on selected benthic invertebrates. The soft-shell clam, *Mya arenaria*, survived with no significant mortalities for the maximum test period (14 days) and were observed to be actively filtering in the laboratory tests. The sand worm, *Nereis virens*, showed greater sensitivity. Significant mortalities occurred in the effluent in field experiments at a water temperature of about 25°C while in the laboratory controlled experiments no significant mortalities were noted. *In-vivo* experiments were run at 20°C, however, and the physical condition of *N. virens* were noticeably poorer in the effluent water. Test results on the sand shrimp, *Crangon septemspinosa*, were inconclusive since significant mortality in control tests indicated sources of stress other than experimental; these organisms did appear to be more sensitive than the other two species tested.

Tests results should be considered in light of experimental restrictions. In the field tests, organisms were limited to a confined area and substrate depth and thus could not escape any natural or unnatural environmental conditions by further burrowing or movement; also it was not possible to precisely match conditions at the control site with those at the effluent site. As opposed to field tests, laboratory assays were run with no dilution of the effluent and with no simulation of fluctuating tidal conditions (temperature, salinity, dissolved oxygen), thus simulating a "worst-case" situation. Given these conditions, test results from both these experiments were judged similar.

6.0 LITERATURE CITED

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