EFFECTS OF SETTLING BASIN EFFLUENT ON SURVIVAL OF SELECTED MARINE INVERTEBRATES: *IN SITU* BIOASSAY

For

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1.0 INTRODUCTION

A series of site specific bioassay experiments were conducted to determine, *in situ*, the short-term (7-day) effects of effluent from Seabrook Station settling pond on survival of selected estuarine invertebrates. The one-acre settling pond receives influent from site dewatering, stormwater runoff, and tertiary treated sanitary wastewater while Seabrook Station is under construction. Some settling pond water is lost through evaporation and below ground seepage, but an average of approximately 0.2 million gallon per day flows into the Brown's River.

Bioassay tests were intended to show if any (unspecified) substances harmful to Hampton-Seabrook estuarine organisms were present in the vicinity of the settling pond outfall (Figure 1) during selected testing periods. Chemical identification of potentially hazardous substances, or speculation as to their origin, was beyond the scope of this study.

2.0 METHODS AND MATERIALS

2.1 SELECTION OF ORGANISMS

Crangon septemspinosus (sand shrimp) and *Nereis virens* (clam worm) were chosen because these species were known from previous experience to be among the most sensitive to environmental stress, particularly lack of oxygen (NAI, 1973). *Mya arenaria* (soft-shell clam) was included because of wide spread concern for the welfare of nearby Hampton Harbor stocks. All organisms were collected in the lower Hampton-Seabrook Estuary by hand within 48 hours of each test.

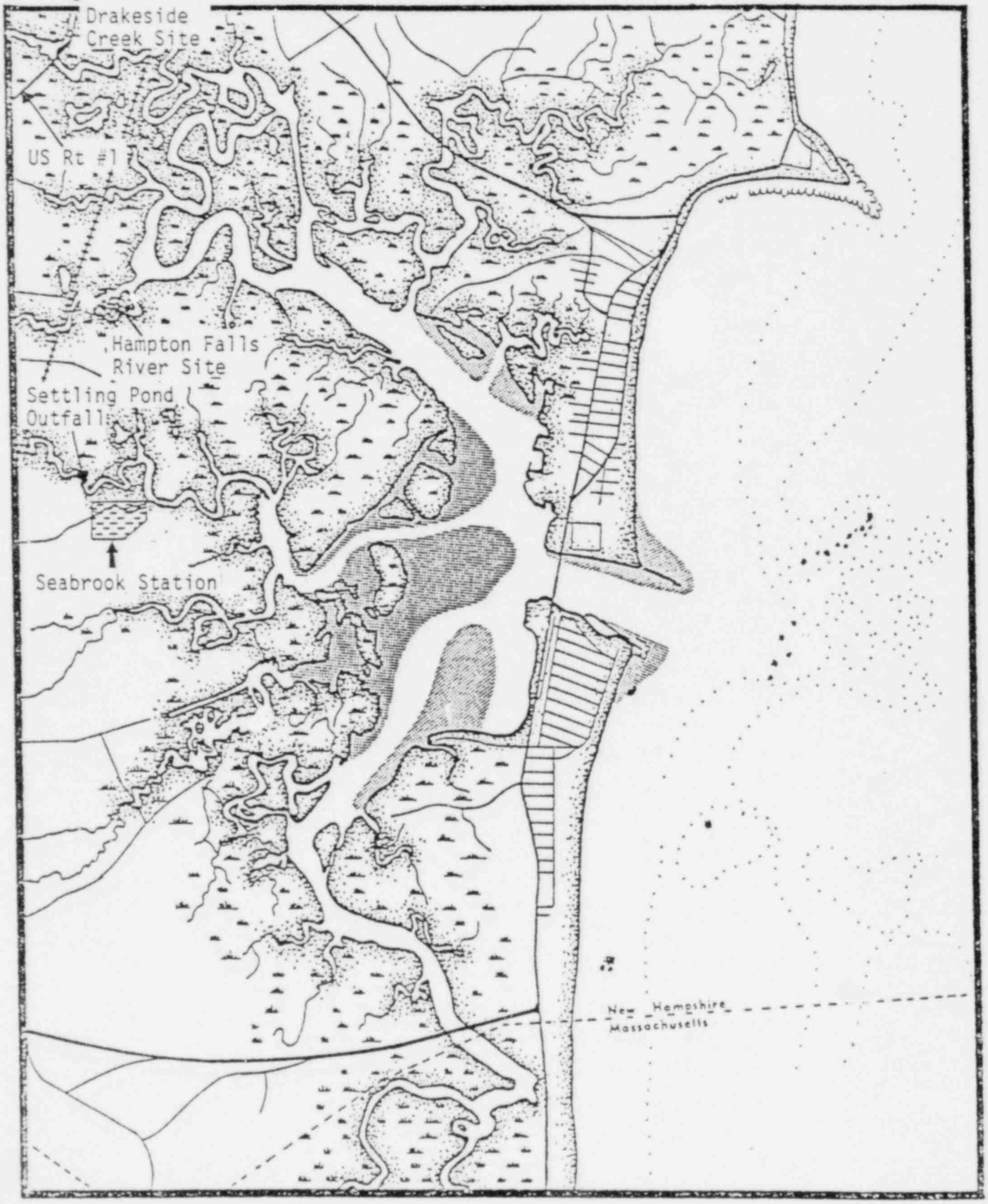

Figure 1. Settling pond bioassay test sites.

2.2 FIELD PROCEDURES

Test containers used were plastic pans, 25 x 30 x 13 cm, filled 8 cm deep with sand <0.505 mm. The pans were set in wooden frames, six pans to a frame, and covered with 1.6 mm mesh plastic screening held in place by a rubber band passed around the circumference of the pan.

On the day before the beginning of a test, frames, each containing six 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 Brown's River, test sites were immediately above and below the settling pond outfall (Figure 1). For the initial tests (15-22 August 1978), a far-field reference site on the Hampton Falls River was used; subsequently (12-19 September; 6-13 November 1978), a creek (Drakeside) flowing into the Hampton River was selected as a reference site because the salinity range more nearly matched conditions near the settling pond outfall (Table 1). The 12-19 September experiment was run due to a greater than 10% mortality of *N. virens* at the control site in the earlier (12-22 August) run.

After securing the test frames to the stream bottom, water samples were taken on several succeeding high and low tides and the samples analyzed for dissolved oxygen concentration, salinity, and turbidity. Water temperature was also measured over the frames on high and low tides. Screening covering the pans was brushed clean each day during low tide.

At the end of seven days, test frames were hauled out and organisms recovered from the pans by passing the sand through a 6 mm mesh sieve. 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. Results were statistically analyzed only when mortality of organisms at the reference site was less than 10%.

TABLE 1. RESULTS OF SETTLING POND BIOASSAY. SEABROOK ECOLOGICAL STUDIES, 1978.

	NO. LIVE INDIVIDUALS PER CONTAINER @ START	TEMP (°C) RANGE		SALINITY ($^0/oo$) RANGE		DISSOLVED OXYGEN (mg/l) RANGE		TURBIDITY (FTU) RANGE		PERCENT MORTALITY			G-TEST RESULTS
		BROWNS RIVER	REFERENCE SITE	BROWNS RIVER	REFERENCE SITE	BROWNS RIVER	REFERENCE SITE	BROWNS RIVER	REFERENCE SITE	ABOVE DISCHARGE	BELOW DISCHARGE	REFERENCE SITE	
				15 - 22 AUGUST									
M. arenaria	29-30	18.3- 26.5	16.8- 26.9	19.6- 31.8	30.8- 31.8	3.5- 8.6	[illegible].2- 10.5	1.3- 2.9	1.4- 5.6	0 6.7	0 0	0 0	3-factor n.s.
N. virens	30									53 43	63 47	10 33	n.a.
				12 - 19 SEPTEMBER									
C. septemspi[illegible]sus	20	12.9- 18.4	13.5- 17.8	16.1- 31.9	22.4- 31.7	4.7- 9.0	4.4- 8.4	1.2- 3.7	1.1- 3.2	25 0 10	25 0 15	0 35 10	n.a
N. virens	20-22									5 5 14	0 5 0	0 10 0	3-factor n.s.
				6 - 14 NOVEMBER									
C. septemspinosus	20	6.4- 10.9	8.1- 10.8	16.3- 32.1	24.0- 32.1	8.8- 11.8	8.5- 10.7	0.5- 3.5	0.8- 2.9	10 15 20	15 10 10	30 20 15	n.a.
M. arenaria	20									0 0 5	0 0 0	0 5 *	2-factor[a] n.s.
N. virens	20-21									0 0 5	0 5 10	0 0 0	3-factor n.s.

n.s. = test results not significant at α = .05 (see appendix for details)

n.a. = results not statistically analyzed due to mortalities at reference (field control) site in excess of 10%.

[a] replicates lumped because one pan (*) was tipped over on recovery

2.3 STATISTICAL ANALYSIS

Generally, counts of live versus dead (or missing) *Mya arenaria* and *Nereis virens* 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) pans (replicates), and tested the independence of the three factors from one another. Of primary concern was comparison of condition with test site; finding a significant dependency between these two factors would mean that organism deaths depended on where the frames were placed with respect to the settling pond discharge (this was the treatment effect being tested).

3.0 RESULTS AND DISCUSSION

From the *M. arenaria* or *N. virens* test results statistically analyzed, no dependency could be demonstrated between mortality and site (Table 1, Appendix Table 1). However, *N. virens* results were not statistically tested when water temperatures were highest (18.3-26.5°C at Brown's River) due to mortalities in excess of 10% at the reference site. Average mortalities at the experimental sites were more than twice those of the reference site during August.

Crangon septemspinosus results were not analyzed statistically because deaths at the reference site could not be reduced below an average of 15%. Observations suggested that aggressiveness among individuals within each pan may have largely contributed to the mortalities at each site. *Crangon* were not tested at maximum summer temperatures nor at dissolved oxygen concentrations below 4 mg/l (Table 1).

As expected, *Mya arenaria* were minimally affected in these brief tests. Like other bivalve molluscs, soft-shell clams can resist unfavorable conditions by remaining dormant. With little or no oxygen supply, *M. arenaria* can survive for up to 8 days at 19°C (Collip, 1921) using a form of anaerobic respiration unique to certain invertebrates

(Hammen, 1969). November mortalities for *M. arenaria* were analyzed by a 2-way rather than a 3-way test of dependency because some of the specimens were accidentally lost on recovery causing inequality of test populations.

These bioassay results suggest that: 1) seven days may not be sufficient to test the physiological tolerance of a facultative aerobe, such as *M. arenaria*, and 2) testing at temperatures in excess of 18°C may be necessary to provide credible evidence of no appreciable harm to obligate aerobes, such as *C. septemspinosus* and *N. virens*. Perspective provided by the reference sites (Figure 1) was constrained by inability to duplicate (even after considerable search) the physical (particularly salinity) environmental regime at Brown's River.

Conclusions, drawn from the results (Table 1) show that no harmful substances are suspected to emanate from Seabrook Station settling pond, however, these must be considered tentative and only applicable to the cooler months of fall 1978. Any additional *in situ* bioassays, should attempt to mitigate some of the problems encountered in the 1978 tests; specifically: 1) all three organisms used in 1978 should be tested at maximum 1979 summer temperatures, 2) *C. septemspinosus* test specimens should be separated from one another to eliminate the possibility of injury due to aggression, and 3) *M. arenaria* tests should run for a minimum of 14 days.

4.0 LITERATURE CITED

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APPENDIX TABLE 1. SEABROOK SETTLING POND: BIOASSAY G-TEST STATISTICS.

NEREIS VIRENS: SEPTEMBER 19, 1978

3-WAY G-TEST

HYPOTHESIS TESTED	DF	G
COND x REPS x STAT IND	12	11.543
REPS x STAT IND	4	0.062
COND x STAT IND	2	3.087
COND x REPS IND	2	1.967
COND x REPS x STAT INT	4	6.426

NEREIS VIRENS: NOVEMBER 13, 1978

3-WAY G-TEST

HYPOTHESIS TESTED	DF	G
REPS x STAT x MORT IND	12	9.623
STAT x MORT IND	2	4.324
REPS x MORT IND	2	4.426
REPS x STAT IND	4	0.054
REPS x STAT x MORT INT	4	0.818

MYA ARENARIA: AUGUST 22, 1978

3-WAY G-TEST

HYPOTHESIS TESTED	DF	G
REPS x STAT x MORT IND	7	7.270
STAT x MORT IND	2	4.417
REPS x MORT IND	1	2.773
REPS x STAT IND	2	0.011
REPS x STAT x MORT INT	2	0.069

MYA ARENARIA: NOVEMBER 13, 1978

2-WAY G-TEST

HYPOTHESIS TESTED	DF	G
STAT x MORT IND	2	1.979