

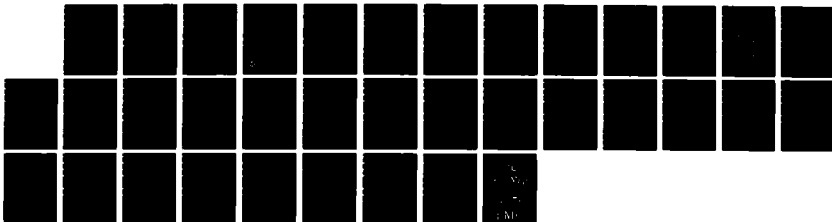
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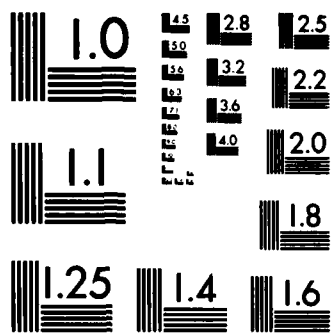
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APPLIED MARINE RESEARCH LABORATORY
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NORFOLK, VIRGINIA

TOXICITY TESTS OF THE SEDIMENTS
FROM THE PORT OF HAMPTON ROADS:
SUBLETHAL EFFECTS

By

Raymond W. Alden, III, Ph.D., Principal Investigator
Robert Young, Co-Investigator, and
Suzanne S. Jackman

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Supplemental Contract Report
For the period ending September 1984

Prepared for the
Department of the Army
Norfolk District, Corps of Engineers
Fort Norfolk, 803 Front Street
Norfolk, Virginia 23510

Under
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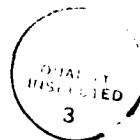
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TOXICITY TESTS OF THE SEDIMENTS FROM
THE PORT OF HAMPTON ROADS: SUBLETHAL EFFECTS

By
Raymond W. Alden, III*
Robert Young and Suzanne Jackman**

INTRODUCTION

The periodic dredging of navigational channels is vital to the maintenance of seaport systems. Unfortunately, the dredged materials from urban estuaries are often highly contaminated. Pollutants introduced directly or indirectly into the waters of these ecosystems are generally partitioned into, and concentrated in the sediments. Therefore, a problem of major concern to port cities is how potentially toxic dredged materials can be disposed with minimal ecological damage.

Recently, a great deal of attention has been focused upon the feasibility of open ocean disposal of dredged materials (Pequegnat et al., 1978). The U.S. Environmental Protection Agency (EPA) and the U.S. Army Corps of Engineers (COE), responsible for disposal permitting have developed specific criteria for ocean disposal. An implementation manual (EPA/COE, 1978) sets the technical guidelines by which the ecological effects of dredged materials must be evaluated before an open ocean disposal site may be permitted. The guidelines describe a series of lethal bioassay

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experiments which are designed to evaluate the acute toxicity of sediments in order to minimize or prevent severe damage to open ocean ecosystems.

The use of bioassays in the assessment of dredged material toxicity is far more ecologically sound than previous criteria which were based solely upon bulk chemical analysis of sediments. However, these experiments do not indicate any potential subtle or chronic effects on the health of communities indigenous to the waters surrounding an ocean disposal site. Therefore, some investigators have expressed concern over sublethal effects of dredged material disposal (Pequegnat et al., 1978). Nonetheless, few studies have addressed this important research issue.

The present study represents an assessment of the potential sublethal effects of ocean disposal of sediments dredged from a highly industrialized estuary. A series of sublethal bioassays were conducted to determine the effects of sediment fractions from various areas within the Port of Hampton Roads, Virginia on the physiological responses of the grass shrimp Palaemonetes pugio, and the sheepshead minnow Cyprinodont variegatus. Respiration and osmoregulation capacity were selected as two condition indices for assessing the overall health of the test organisms following exposure to the suspended solid fractions of sediments from the various dredge sites.

METHODS AND MATERIALS

Study Area

The Port of Hampton Roads, Virginia, is one of the largest natural harbors in the world. Hampton Roads and the surrounding estuarine systems of Tidewater Virginia provide the setting for one of the most highly industrialized coastal areas on the east coast of the United States that includes the cities of Norfolk, Virginia Beach, Chesapeake, Portsmouth, Newport News, and Hampton (Figure 1a). The harbor also supports the largest military port in the world. International trade involving bulk transport of goods dominate the Port's activities and create a demand for channels deep enough to handle large deep-draft vessels (e.g. coal colliers, grain freighters, oil tankers). The Norfolk District COE is responsible for maintaining the navigational channels of this seaport system in order to insure the safe passage of military and commercial vessels. On the average, $4.1 \times 10^6 \text{ m}^3$ of sediments are dredged annually by the Norfolk District in order to maintain the channels at a depth of approximately 13m. In addition, plans are currently being made to deepen the channels to 17m, an operation which would greatly increase disposal requirements.

The collection stations were selected to geographically cover the major channels of the Port. The sediments for the sublethal bioassays were collected at Stations KK, D, E, F, H, I, J, J/K, (between Stations J and K), N, N/O (between Stations N and O), O,

Figure 1a. The study area for the sublethal effects of sediments from the Port of Hampton Roads, Virginia.

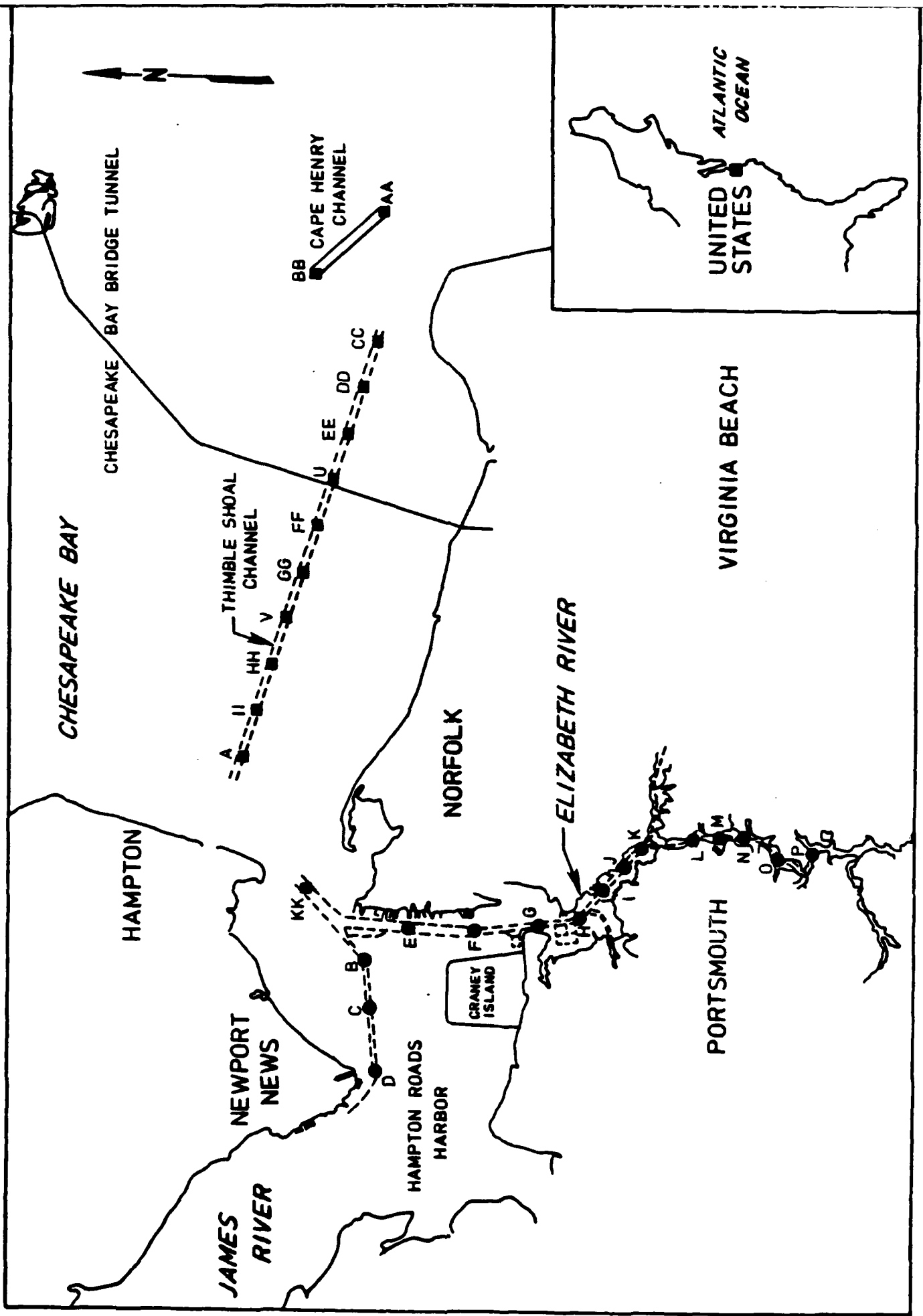
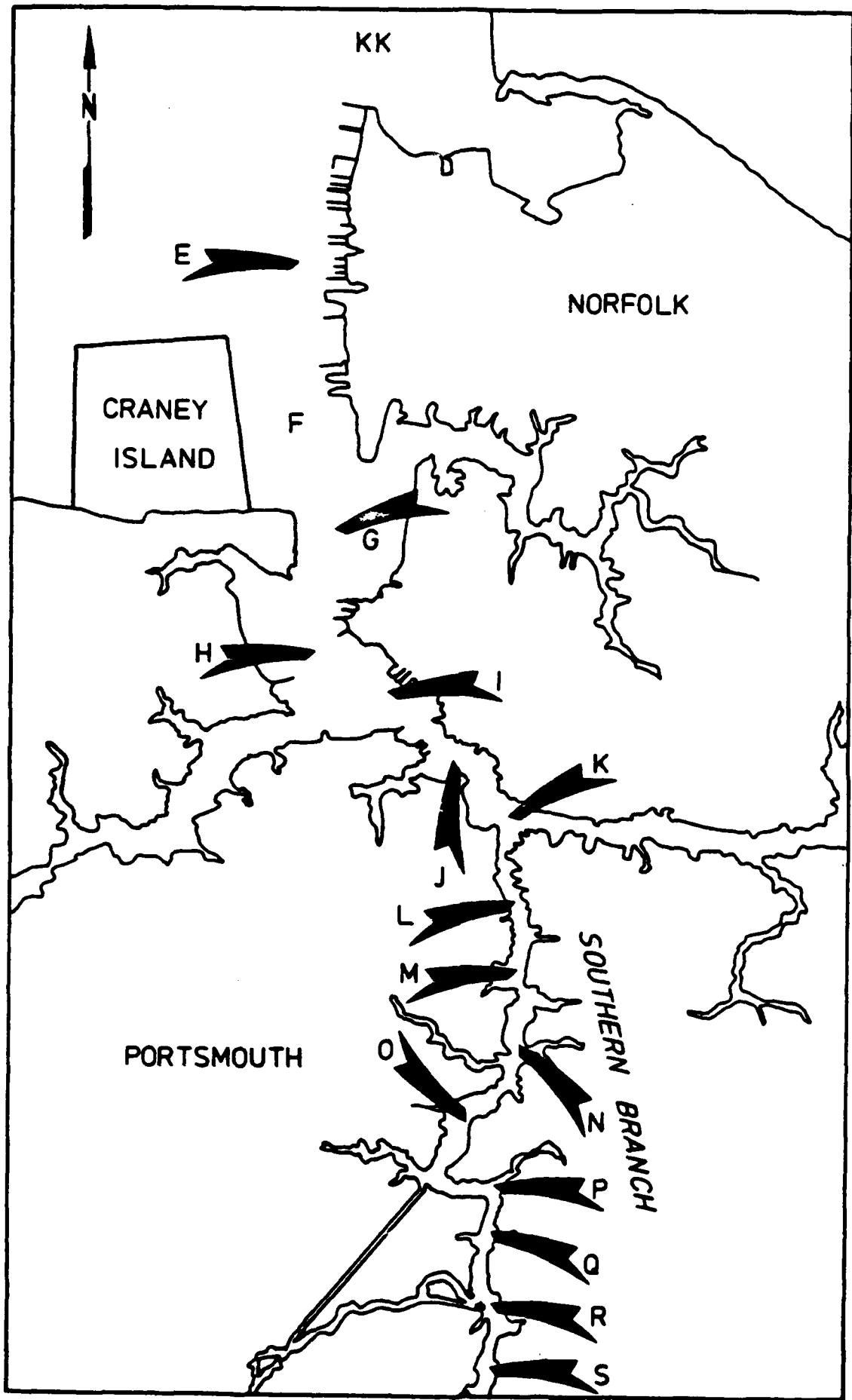


Figure 1b. Study area in the Southern Branch of
the Elizabeth River, Virginia.



O/P (between Stations O and P), Q and R (Figure 1b). Stations KK, D and E were sampled in Hampton Roads Harbor, while Stations F, H, I, J and J/K were taken in the main stem of the Elizabeth River. The remainder of the stations were located in the Southern Branch of the Elizabeth River. In addition, these experiments supplement lethal and sublethal bioassays conducted in 1980, and directly correspond to lethal toxicity tests conducted in 1982 and 1983.

Surface sediments from Stations D, E, H, K, M and P were evaluated for lethal and sublethal effects on P. pugio in previous studies (Alden et al., 1981; Alden and Young, 1982). It was found that sediments from Stations D, E, H and P produced minimal effects on the shrimp. Bioassays in the present study were conducted to confirm that the full range of sediments potentially taken from these stations would be nontoxic during future deepening operations. The sediments from these stations were tested as a composite taken to a depth of 3m (10 ft.) to evaluate the potential sublethal effects of dredged materials produced by Harbor deepening.

Significant lethal and sublethal effects were observed in the 1980 studies for sediment fractions taken from Stations K and M. In order to delineate the region of toxic sediments, a series of "screening" suspended solid bioassays (i.e. tests employing only 100% concentrations of the elutriates; see Alden and Young, 1984) and the associated sublethal experiments were conducted at

numerous stations in the region (I, J, J/K, N, O N/O, O/P, Q, R). The remaining stations (KK, D, E, F, H, O and J) were selected to complement the geographic coverage of Hampton Roads Harbor and the main stem of the Elizabeth River.

Sediments were collected at each of the stations by a large clamshell grab (0.76 m³) or a 20 l stainless steel Pearce bucket dredge. The sediments in the central portion of the grab were scooped into washed, 18 l polyethylene snap-top containers and stored at 40C until the experiments were conducted.

Bioassay Methods

Respiration and osmoregulation experiments were conducted on the grass shrimp. This species is widely employed as a standard estuarine test organism in toxicity and physiological studies, and strong arguments have been made for its use in dredged material assessments (Lee et al., 1977). The sheepshead minnow is another species commonly used in such studies (EPA/COE, 1978). The fish were used in respiration experiments in 1983 to confirm the quality of outer harbor sediments.

The suspended solid fraction of the sediments were tested in the sublethal physiological experiments. Elutriation of the dredged materials was begun by vigorously mixing a 1:4 (v/v) mixture of the sediments in artificial seawater with compressed air. The resulting suspension was allowed to settle for one hour, and the supernatant was pumped into the test chambers.

Flow-through respiration monitoring systems were developed to allow the periodic monitoring of shrimp respiration rates during the bioassays. The respiration system employed a peristaltic pump to cycle water from 30 l reservoir tanks through 0.5 l respiration chambers, and back to the reservoirs. A valving system in the head of the respiration chamber allowed monitoring of water either entering or leaving the chambers with an oxygen analyzer. The differences between input and output oxygen levels, along with flow rate data, allowed the calculation of respiration rates of the animals in the chambers.

The test organisms were either collected from nonindustrial estuaries or were obtained from commercial hatcheries specializing in the culturing of nonstressed populations of bioassay organisms. The test organisms were allowed to acclimate gradually to a 20°C temperature and 30 ppt salinity regime. No animals were acclimated for less than four days or more than two weeks prior to testing. Ten small shrimp or five larger shrimp of approximately equal adult size were placed in each respiration chamber. Triplicate chambers were set up for each elutriate concentration and respiration readings were taken at 4, 24, 48, 72 and 96 hours after initial exposure.

All metabolic data were biomass standardized by dividing the respiration rates by the estimated dry weight of the organisms in the chambers at the time of the readings. In order to estimate the biomass of the organisms in the chambers at any point in time

during the experiments, the dry weights of the animals surviving 96 hours were measured. The total biomass in each chamber was then divided by the number of animals in order to provide a mean dry weight estimate per animal. These values were then multiplied by the number of organisms alive in each chamber during the sampling period to obtain an estimate of the amount of biomass. The estimated biomass values were further corrected for the metabolic loss of biomass estimated to have occurred between each sampling period. The metabolic weight loss correction factor was calculated by taking a running average of respiration rates measured for any two periods, and multiplying it by the number of hours between the readings. This value was multiplied by a factor of 0.87 g dry wt/g O₂ and adding this value to the biomass estimate. The 0.87 g dry wt./g O₂ factor was based upon calculations employing empirical data reported by Welsh (1975) for the caloric content and oxycalorific equivalents of value P. pugio at 20°C as follows:

$$0.87\text{g dry wt/g O}_x = 0.217\text{g dry wt/Kcal} \times 4.0\text{ Kcal/g O}_2 \quad (1)$$

Since similar data could not be found for the sheepshead minnow and the ultimate effect of metabolic loss on the total biomass was fairly small, the same weight per unit oxygen ratio was used for the respiration rate calculations for the fish. In order to account for any oxygen which may be been consumed by a high Biochemical Oxygen Demand (BOD) of the elutriates within the respiration chambers, water from the respiration chambers containing the test organisms was pumped through empty chambers

while monitoring the oxygen. The amount of oxygen loss in the chambers without animals was used as a correction factor in respiration rate calculations to account for the BOD of the elutriate. The amount of oxygen attributed to BOD was always a small fraction (i.e. less than 10%) of the oxygen consumed by the animals.

Osmoregulation capacity of P. pugio was employed as a second condition index to evaluate the relative health of test organisms following the 96 hour suspended solid bioassays. Numerous physiological and bioassay experiments have been conducted on estuarine species in the past in order to determine which environmental conditions (natural or introduced by man) affect the ability of an organism to regulate its internal osmotic pressure across a salinity range. Most of the studies have concentrated upon decapod crustaceans renowned for their osmoregulation capacities. Bioassays of heavy metals (Thurberg et al., 1973), chlorinated hydrocarbons (Caldwell, 1974; Nimmo and Bahner, 1974; and Roeijodi et al., 1976), and petrohydrocarbons (Anderson et al., 1974) have been conducted in order to determine the concentrations of the particular toxins that produce significant sublethal effects on the complex systems that allow test organisms to osmoregulate.

Following the suspended solid phase bioassays, the grass shrimp from each of the control and experimental tanks were randomly divided into two groups which were exposed to water of either 10

or 35 ppt salinity for 24 hours. Grass shrimp hyperregulate at the low salinities and hyporegulate at the higher salinities (Roelijodi et al., 1976; Alden et al., 1982). The internal osmotic pressure of each shrimp was determined by exposing the hemocoel, saturating a small absorbent filter pad with body fluids, and measuring the osmolality on a vapor pressure osmometer.

Statistical Analysis

A linear regression analysis was conducted with the respiration data to determine whether the respiration rates of shrimp significantly changed over the course of the experiment. Those data which showed a significant correlation with time were further evaluated through an Analysis of Covariance (ANCOVA) program. Time was the covariate, with the station effects as dummy variables, and the appropriate station-time interactions. Kim and Kohout (1975) describe the use of multiple regression techniques employing dummy variables to produce ANCOVA statistical models.

For those experiments which exhibited no significant relationship between respiration rates and time, trends in autocorrelation were evaluated with a Durbin-Watson test. If a relationship was not significant, all the readings for any given treatment were considered replicates for further analysis. These data were then evaluated by ANOVA, followed by a Duncan's Multiple Range test to determine which sediments produced respiration rates significantly different from control values.

The data from the osmoregulation experiments were statistically evaluated for each salinity regime with an ANOVA of the osmolality readings among shrimp exposed to sediments from the various stations in any given bioassay set. A Duncan's Multiple Range test was conducted for each significant ANOVA to determine which stations contained sediments that produced osmolality readings in the test populations which were significantly different from control values for the same salinity.

RESULTS

The results of the sublethal bioassays on the grass shrimp are presented in Table 1. The station designations correspond to those presented in Figure 1. Four sets of sublethal experiments were conducted during suspended solid phase bioassays of 1982 and 1983. The first set of experiments evaluated sediments composited to a depth of 3m at representative stations evaluated during the 1980 bioassays (Alden et al., 1981; Alden and Young 1982). None of the experimental conditions during this first set produced significant effects on the respiration or osmoregulation capacity of the test animals.

The second and third experiments were conducted during the screening suspended solid bioassays which were designed to better define the region of sediment toxicity in the Elizabeth River. The respiration values and osmoregulation capacity of the shrimp are not significantly affected at Station I in the main stem of the Elizabeth River. However, as the Southern Branch is approached, the hyporegulation capacity (Station J and J/K), and the respiration rates (Station J/K) are affected. This pattern seems to confirm the fairly dramatic respiration effects which were observed for Station K sediment fractions in the 1980 experiments (Alden et al., 1981). The maximum absolute effects were observed for sediments from Stations N, N/O and O in the Southern Branch: respiration rates were depressed to approximately 60% of the control values; and hyporegulation capacity at the high salinities was also affected. The tests run on Station O/P sediments

TABLE 1

Results of Sublethal Experiments: mean values of respiration rates, osmolality at 10 ppt., and osmolality at 35 ppt for Palaemonetes pugio are indicated (standard errors in parenthesis; n=15). The asterisks indicate values are significantly different from controls ($\alpha=0.05$)

Experiment #	Date	Station	Respiration Rate (mgO ₂ g.dry wt ⁻¹ hr. ⁻¹)	Osmolality (mOsm kg ⁻¹) at 10 ppt	Osmolality (mOsm kg ⁻¹) at 35 ppt
1	3/82	Control	2.28 (0.13)	629.17 (5.37)	714.50 (12.81)
		D (3m)	1.92 (0.13)	625.75 (13.77)	715.71 (9.02)
		E (3m)	1.65 (0.13)	647.57 (16.10)	720.86 (12.16)
		H (3m)	1.94 (0.33)	636.22 (13.83)	749.00 (13.96)
		P (3m)	1.74 (0.11)	659.00 (11.38)	720.25 (13.00)
2	3/82	Control	2.21 (0.07)	655.57 (17.03)	691.44 (16.57)
		I	2.10 (0.06)	662.20 (22.65)	733.20 (21.43)
		J	1.94 (0.11)	641.70 (10.24)	754.90 (19.79)*
		N	1.35 (0.10)*	619.88 (9.72)	777.10 (17.06)*
		O	1.27 (0.08)*	617.57 (12.17)	776.63 (11.79)*
3	5/82	Control	2.64 (0.19)	609.29 (5.76)	691.71 (11.65)
		J/K	1.52 (0.12)*	639.70 (14.10)	795.71 (28.18)*
		N/O	1.61 (0.12)*	618.50 (18.36)	757.75 (14.62)*
		O/P	2.32 (0.13)	634.63 (26.95)	756.00 (20.64)*
		Q	1.85 (0.25)*	643.89 (47.17)	780.40 (21.35)*
		R	2.03 (0.17)*	644.43 (14.17)	790.13 (12.57)*
4	4/83	Control	0.92 (0.11)	531.60 (19.15)	788.30 (12.33)
		F	1.16 (0.11)	530.78 (22.78)	821.30 (9.79)
		KK	1.18 (0.13)	536.80 (28.89)	818.80 (16.96)

Note: (3m) = Experimental sediments composited to a depth of 3m.

indicated that respiration was not significantly affected, but the hyporegulation capacity was affected, although not as severely as most of the other stations in the region. These findings parallel those of the 1980 respiration experiments for Station P sediments which produced little or no effects on the metabolism of the test populations. Sediments from Stations Q and R produced significant, but more moderate, respiration effects (i.e. depression to 70% and 77% of control rates, respectively). The hyporegulation capacity was also significantly affected by the sediment fractions from these stations. It should be noted that the hyperregulatory capacity of the shrimp, exposure to low salinities did not appear to be affected by any of the experimental conditions.

The tests on shrimp exposed to sediments from the Hampton Roads Stations F and KK showed no significant sublethal effects. It is interesting to note that the values of the physiological parameters for the shrimp during the 1983 experiments were different from those observed in the 1982 experiment. Respiration rates were lower and the apparent osmoregulation capacity was reduced. The populations of shrimp for the 1983 experiments were taken from a different location (the Eastern Shore) from the previously used collection site (the Lynnhaven), possibly accounting for the metabolic differences.

The experiments with the fish were associated with the suspended solid bioassays designed to confirm the quality of sediments of

Hampton Roads Harbor and the main stem of the Elizabeth River (Table 2). No significant differences were observed between the respiration rates of experimental and control populations.

TABLE 2

Results of respiration experiments on Cyprinodont variegatus.
 Membranes are indicated (standard errors in parenthesis; n=15).
 None of the experimental values were significantly different
 from those of the controls ($\alpha = 0.05$).

Experiment #	Date	Station	Respiration Rate (mg O ₂ g. dry wt. ⁻¹ hr. ⁻¹)
1	3/83	Control	1.14 (0.10)
		F	1.02 (0.07)
		D	0.90 (0.06)
		KK	1.28 (0.11)
2	4/83	Control	1.12 (0.10)
		E	1.30 (0.16)
		H	1.15 (0.17)
		J	1.34 (0.28)

DISCUSSION

Despite an overall concern of investigators over the sublethal impact of dredged material disposal (Pequegnat et al., 1978), few studies have been conducted on the sublethal effects of sediment fractions. DeCoursey and Vernberg (1975) examined the effects of waters taken from active dredge sites and contained disposal areas in Charleston Harbor, South Carolina on the physiology of larval zooplankton. These investigators reported that the respiratory rates of the cladoceran Daphnia pulex, and the larvae of the polyphaete Polydora sp., were significantly depressed for sites which eventually produced significant lethal effects in long-term studies of up to 20 days. Therefore, the physiological effects were seen as indicators of potential long-term chronic effects.

In another such study, Shuba, Tatem and Carroll (1978) reported that elutriates of contaminated sediments significantly retarded the growth of Palaemonetes pugio larvae. Until the 1980 and 1981 sublethal experiments of the Hampton Roads program (Alden et al., 1981; Alden et al., 1982), these two previous investigations appear to be the only ones in which the sublethal effects of dredged materials fractions have been examined.

Respiration and osmoregulation capacity are two physiological parameters which are often used as condition indices to the overall health of marine test species in sublethal bioassays.

Anderson (1977, 1979), for example, reviewed the use of these physiological functions in sublethal bioassays of petrohydrocarbons and other organic toxins, while Waldichuk (1974) reviewed the effects of heavy metals on these processes. Katz (1979) discussed a theory of toxicity of pollutants suggesting that many chemicals disrupt membrane permeability across the gills, integument, and gut of aquatic organisms. According to this theory, physiological functions such as respiration and osmoregulation which are associated with these exchange surfaces are often the first processes to be disrupted, with lethality resulting if the pollution stress and biological damage are severe enough.

The present study indicated that there were significant sublethal effects associated with exposure of the test organisms to elutriates from certain stations in the Southern Branch of the Elizabeth River. As with previous studies (DeCoursey and Vernberg, 1975; Alden et al., 1981), the respiration rates of test organisms were depressed dramatically (60% of control values) upon exposure to the elutriates of sediments producing the greatest lethal effects. Likewise, the hyporegulation capacity of shrimp exposed to high salinities declined following exposure to the elutriates from the same stations. Thus, these two physiological functions are seen as sensitive indicators of sediment toxicity in the Port. In fact, the sensitivity of these tests was demonstrated by the fact that the sublethal experiments indicated significant

effects for the stations in the Southern Branch even when the apparent toxicity was moderately low in concurrent lethal bioassays. The low levels of mortalities was apparently due to the removal of contaminated sediments by maintenance dredging operations 4-6 months prior to the tests. Lethal effects of the sediments were observed to return during tests 18 months following dredging (Alden and Young, 1984), so it appears that the sublethal tests were capable of detecting even a reduced level of toxicity.

The delineation of the regions containing sediments capable of producing sublethal effects is quite apparent. The sediments from the Hampton Roads Harbor and the main stem of the Elizabeth River do not produce significant effects. However, the region between Stations J and O in the Southern Branch appears to produce significant physiological effects on test populations.

The sublethal and lethal effects of the sediments between Stations O and P appear to be negligible (Alden et al., 1981). In this region of the Southern Branch, the river makes a right angle turn and the sediments are somewhat sandy. The stations further upstream (Stations Q and R) produced moderate sublethal effects, but only low levels of mortalities (Alden and Young, 1984), so the relative quality of the sediments in this reach is debatable.

The relative quality of the sediments which would be dredged during deepening operations appears to be quite good. The

sublethal effects of sediments composited to a depth of 3m did not produce significant sublethal effects, even for stations which produced low but significant respiration effects in previous studies of surface sediments (Stations E and H; Alden et al., 1981). Thus, the results of the sublethal experiments confirmed the findings of the associated lethal bioassays (Alden et al., 1984) in suggesting that the sediment composites produced by deepening operations would likely dilute whatever contamination is present in the surface sediments with the relatively pristine subsurface materials.

The toxic agent(s) producing respiratory depression and deminishment of osmoregulation capacity is unknown, but previous investigations indicate that this type of sublethal response is not uncommon and may be produced by different types of pollutants. For instance, a decline in the oxygen consumption of decapod crustaceans has been reported to be associated with exposure to heavy metals (Thurberg et al., 1973), petrohydrocarbons (Anderson, 1979) or chlorinated hydrocarbons (Rao et al., 1979). Likewise, osmoregulation capacity of decapods has been observed to be affected by heavy metals (Thurberg et al., 1973), petrohydrocarbons (Anderson et al., 1984; Anderson, 1977, 1979) and chlorinated hydrocarbons (Caldwell, 1974; Nimmo and Bahner, 1974; Roeijadi et al., 1976). Of course, all of these and other potential toxins may be associated with the dredged materials, so pinpointing causative agents for sublethal effects is

problematical. However, the stations producing the greatest effects corresponds to the most highly industrialized region of the Port. This area has been shown to contain the highest levels of heavy metals (EPA, 1976; and Alden et al., 1981, 1982) and polynuclear aromatic hydrocarbons (Alden and Hall, 1984). Although cause and effect relationships cannot be inferred between these sediment contaminants and the effects, the collocation of the biological and chemical "hot spots" does suggest that the materials dredged from the lower reach of the Southern Branch of the Elizabeth River should not be considered for ocean disposal.

SUMMARY AND CONCLUSION

A series of sublethal toxicity tests were conducted on sediments from stations throughout the Port of Hampton Roads, Virginia. The respiration and hyporegulatory capacity of the grass shrimp proved to be sensitive condition indices of sediment quality. On the other hand, hyperregulatory capacity was not affected by any of the experimental conditions.

The suspended solid phase of sediments from Hampton Roads Harbor and most of the main stem of the Elizabeth River were shown to produce no significant sublethal effects on grass shrimp and sheepshead minnows. However, the sediment elutriates from the most industrialized region of the Southern Branch of the Elizabeth River were shown to produce significant sublethal effects.

The present study clearly defined the region of significant contamination identified in previous studies. Sediments from Stations K and M were previously shown to be highly toxic. The present study indicated that toxicity of sediments was restricted to a region 8-10km up the Southern Branch. The sublethal effects were maximum for sediments from Stations N and O, despite the fact that lethal effects during the experiments were apparently diminished by recent dredging operations in the region. It is, therefore, suggested that the region of significant effects between Station J/K and O not be considered for ocean disposal. This is based on respiration and osmoregulation effects which may be indicative of the potential for long-term chronic impacts on the

biological communities living in the vicinity of the disposal site.

The sediments from the Gilmerton region (Stations O/P, P) resulted in nontoxic sublethal tests. However, the experiments which extended the study area further upstream to Stations Q and R did reveal moderate sublethal effects. Since these latter stations were shown to produce moderately low mortalities in the lethal bioassays, the ecological significance of the sublethal effects is somewhat problematical. If sediments from this region would ever be considered for open ocean disposal from a logistical and/or economic point of view, perhaps more intensive experiments (e.g. microcosms) should be conducted to more closely define potential ecological impacts.

The experiments evaluating the sediments composited to a depth of 3m confirmed the findings of the lethal bioassay: the subsurface materials appear to be less contaminated from the surface sediments. No sublethal effects were observed for any of the elutriates of composited sediments, even those taken from stations shown in previous studies to produce significant effects during tests of the surface sediment layers. These findings indicate that the deepening operations should produce dredged materials less contaminated than those routinely taken by maintenance dredging in the same areas.

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