Special Technical Report

An Investigation of Techniques Suitable for Field Extraction of Spionid and Capitellid Polychaetes for Bioaccumulation Testing

Disposal Area Monitoring System DAMOS

Contribution 86
April 1994

US Army Corps of Engineers
New England Division

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	April 1994	Final Report

4. TITLE AND SUBTITLE

An Investigation of Techniques Suitable for Field Extraction of Spionid and Capitellid Polychaetes for Bioaccumulation Testing

5. FUNDING NUMBERS

6. AUTHOR(S)

Isabelle Williams and Donald Rhoads

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Science Applications International Corporation
221 Third Street
Newport, RI 02840

8. PERFORMING ORGANIZATION REPORT NUMBER

SAIC-90/7600 & C94

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Corps of Engineers - New England Division
424 Trapelo Road
Waltham, MA 02254-9149

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

DAMOS Contribution No. 86

11. SUPPLEMENTARY NOTES

Available from DAMOS Program Manager, Regulatory Division
USACOE-NED, 424 Trapelo Road, Waltham, MA 02254-9149

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution.unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

Published techniques for extraction of marine infaunal animals from sediments were reviewed for their potential use in bioaccumulation studies. The goal was to identify the most promising techniques for extraction of small spionid and capitellid polychaetes from fine grained sediments typically found on dredged material mounds. The review identified two categories of techniques: physical disturbance and behavioral reactions. Techniques based on behavioral reactions were selected for testing because the polychaetes live in tubes composed of fine grained sediment and are likely to remain in the tubes despite mechanical separation of the sediments.

Living *Streblospio benedicti* and/or *Polydora cornuta* were subjected to five different extraction techniques: shaking, vibrating, electric current, oxygen depletion with sodium sulfite, and oxygen depletion with gaseous nitrogen. Criteria for selecting the technique of choice included: speed, gentleness, simplicity and efficacy.

Shaking, vibrating and electric current were unsuccessful in driving worms from their tubes. Oxygen depletion with gaseous nitrogen was effective, but slow. Oxygen depletion with sodium sulfite was successful and showed great promise as a technique for rapidly gathering sufficient biomass for chemical analyses.

Based on tests and extrapolation from previous studies, we estimated that 20 g wet weight of tissue could be obtained from four 400 cm^2 grab samples from typical dredged material mounds.

We recommend that the oxygen depletion technique be tested at a suitable disposal site 10-30 days after disposal operations have ended. Tests will require construction of at least 16 test chambers and collection of a minimum of 16 grab samples from a disposal site with recent disposal activity.

14. SUBJECT TERMS

bioaccumulation testing
dredged material
sediments
field techniques
polychaetes

15. NUMBER OF PAGES
26

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified			

AN INVESTIGATION OF TECHNIQUES SUITABLE FOR FIELD EXTRACTION OF SPIONID AND CAPITELLID POLYCHAETES FOR BIOACCUMULATION TESTING

CONTRIBUTION #86

April 1994

Report No.
SAIC- 90/7600&C94

Submitted to:

Regulatory Branch
New England Division
U.S. Army Corps of Engineers
424 Trapelo Road
Waltham, MA 02254-9149

Submitted by:

Isabelle Williams
Donald Rhoads
Science Applications International Corporation
Admiral's Gate
221 Third Street
Newport, RI 02840
(401) 847-4210

US Army Corps
of Engineers
New England Division

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Published techniques for extraction of marine infaunal animals from sediments were reviewed for their potential use in bioaccumulation studies. The goal was to identify the most promising techniques for extraction of small spionid and capitellid polychaetes from fine-grained sediments typically found on dredged material mounds. The review identified two categories of techniques: physical disturbance and behavioral reactions. Techniques based on behavioral reactions were selected for testing because the polychaetes live in tubes composed of fine-grained sediment and are likely to remain in the tubes despite mechanical separation of the sediments.

Living *Streblospio benedicti* and/or *Polydora cornuta* were subjected to five different extraction techniques: shaking, vibrating, electric current, oxygen depletion with sodium sulfite, and oxygen depletion with gaseous nitrogen. Criteria for selecting the technique of choice included: speed, gentleness, simplicity and efficacy.

Shaking, vibrating and electric current were unsuccessful in driving worms from their tubes. Oxygen depletion with gaseous nitrogen was effective, but slow. Oxygen depletion with sodium sulfite was successful and showed great promise as a technique for rapidly gathering sufficient biomass for chemical analyses.

Based on tests and extrapolation from previous studies, we estimated that 20 g wet weight of tissue could be obtained from four 400 cm^2 grab samples from typical dredged material mounds.

We recommend that the oxygen depletion technique be tested at a suitable disposal site 10-30 days after disposal operations have ended. Tests will require construction of at least 16 test chambers and collection of a minimum of 16 grab samples from a disposal site with recent disposal activity.

1.0 INTRODUCTION

Bioaccumulation studies as monitoring tools for contaminated sediments have been performed with relatively large infaunal polychaetes such as *Nephtys incisa* (Peddicord,1988). The advantage of using large taxa is that, because sufficient biomass can be accumulated using fewer organisms, separating the animals from sediment by traditional hand-picking methods is not labor intensive. In addition, laboratory culture of such species is relatively easy. However, these organisms are inappropriate as early indicators of environmental impact in dredged material disposal monitoring programs because they are encountered, more typically, during the final phase of recolonization (Stage III taxa; Rhoads and Germano, 1982). Stage III colonizers may react to contaminated sediments very differently from the small, opportunistic polychaetes that constitute the initial colonizers (Stage I). These Stage I taxa appear to be of first-order importance as food (prey) for predators such as demersal finfish and lobsters; they would be the ideal "early warning" indicators for disposal site bioaccumulation studies.

The potential utility of being able to collect sufficient numbers of these tiny, opportunistic polychaetes for bioaccumulation studies has been recognized by the DAMOS Technical Advisory Committee (TAC) and SAIC senior scientists for the past few years (SAIC, in prep.). Because of the relatively large amount of tissue needed by most chemical laboratories to do body burden analyses (approximately 25-30 gm), the conventional wisdom was that our need to perform bioaccumulation studies on these tiny animals was beyond current technological resources, i.e., we would need to wait until analytical techniques were refined enough to require less tissue. However, the recent work of Oliver (1987) that reported tissue contaminant levels in fresh-water oligochaetes and subsequent discussions during TAC meetings suggested that the exploration of rapid separation techniques for marine Stage I assemblages might be a worthwhile effort.

The objective of this study was to find a practical method of collecting sufficient biomass of small, opportunistic Stage I organisms for field bioaccumulation studies. Such a method would require that very small Stage I infauna (opportunistic polychaetes) be separated from bottom sediments by a rapid extraction technique as opposed to tedious hand-sorting under a microscope. The extraction technique chosen would have to be fast, gentle, and practical for use onboard ship. To obtain accurate data for the less persistent chemical compounds to be analyzed, samples must be sorted and animals frozen within a few hours of collection (Oliver, 1987). Prior to freezing, the animals must remain alive and in good physical condition to avoid post-mortem changes in tissue concentrations of contaminants. The chosen method would have to be simple and take up as little space as

possible so that it could be performed on the small research vessels typically used to collect samples.

2.0 EXTRACTION TECHNIQUES

Techniques developed for the separation of living meio- and macrofauna from bottom sediments fall into two main categories: those that use physical disturbance to sort the animals from the sediment and those that depend on the natural behavior of the animals (Hulings and Gray, 1971).

2.1 Techniques Based Upon Physical Disturbance

Physical disturbances such as sieving, elutriation, or flotation depend on differences in the size or density of animals to be separated from the sediment surrounding them. For sandy sediments, various combinations of decanting and elutriation have been used. The elutriation method, introduced by Boisseau (1957), has been modified by many meiofaunal biologists (Uhlig et al., 1973; Ankar and Elmgren, 1976; Fricke, 1979; Hockin, 1981) and macrofaunal biologists (Eleftheriou and Holme, 1984; Lauff et al., 1961; Pauly, 1973; Worswick and Barbour, 1974). Elutriation depends on passing water upwards through the sample with enough velocity to permit small organisms to float upwards and be caught on an overflow screen. For meiofauna, this technique is most efficient when used in conjunction with an anaesthetic, such as magnesium chloride, so that taxa which normally cling to sand grains are released. The simplest method involves repetitive cycles of mixing a sample with water and decanting the supernatant water through a set of sieves (Pfannkuche and Thiel, 1988).

It is more difficult to separate animals from muddy sediments than from sand, because the samples contain more detritus, and the mud particles have roughly the same elutriation velocity as the organisms. Sieving, using mesh sizes appropriate to the groups of animals to be studied, is a commonly used method to separate animals from fine particles. Sieving is more successful for isolating macrofaunal animals from finer-grained sediment than it is for removing meiofauna. For meiofauna, several flotation methods have been used based on the difference in density between a non-toxic flotation medium and the sediment, including centrifugation of the buoyant fraction. Non-toxic media include "Percoll"-sorbitol (Schwinghamer, 1981) and a saturated sucrose solution (Higgins, 1977).

2.2 Techniques Based Upon Behavioral Reactions

Uhlig (1964) used the behavioral reactions of animals exposed to meltwater of a seawater-ice mixture to separate them from sand. Various modifications of this apparatus have been developed to separate specific meiofaunal taxa (Schmidt, 1968; Hartwig, 1973; Poizat, 1975). It is also possible to use this method on silty sediments that have been sieved (Uhlig et al., 1973).

Oxygen depletion has been used to drive animals upward and out of

muddy sediments. Armonies and Hellwig (1986) covered a layer of muddy sediment with a layer of barely moistened clean sand and left this in the dark for several days. Animals belonging to a variety of taxa, including polychaetes, followed the oxygen gradient upwards into the clean sand and were concentrated by elutriation after the sand layer was removed. No attempt was made by the authors to accelerate this process by initially removing oxygen from the supernatant water. The addition of sodium sulfite or gaseous nitrogen would remove oxygen from seawater (Strickland and Parsons, 1968) and therefore would be a useful modification of this technique.

Trueblood (pers. comm.) has used agitation successfully to drive *Streblospio benedicti* from their tubes and upwards into an overlying water column. The polychaetes then can be concentrated by decanting, elutriation, or by picking them out by hand.

2.3 Extraction Techniques To Be Tested In The Laboratory

Because the polychaetes under consideration live in tubes constructed from grains of the surrounding sediment and because the sediments inhabited by them are muddy, the simplest decanting and elutriation techniques were not expected to separate the majority of the worms. The techniques of choice were those that depend on the natural behavior of organisms to drive them from their tubes and out of the sediment. The technique of Armonies and Hellwig (1986) that slowly removed the oxygen supply to benthic organisms by adding a layer of sand to the sediment surface takes several days and therefore would be unacceptably long (the tissue concentrations of pollutants could change over this period of time). The initial addition of sodium sulfite or gaseous nitrogen may lower the oxygen tension fast enough to permit the polychaetes to be collected before the organisms modify or eliminate from their tissues the chemicals to be tested. Both of these techniques were selected for evaluation and compared for speed. Armonies and Hellwig (1986) collected their organisms in an overlying layer of sand and then concentrated them by decanting. Having a collecting layer of water and subsequent decanting were considered as appropriate techniques for *Streblospio*; another alternative considered was to drive the worms out of the mud onto glass plate collectors.

The agitation technique of Trueblood (pers. comm.) also was evaluated. This consisted of sieving the sample through an appropriate mesh screen to remove fine material. A thin layer of sediment was spread over the surface of a white tray and gently agitated by hand for about 10 minutes. Theoretically the worms would swim into the overlying water where they could be picked out or concentrated by decanting or elutriation.

A common technique for driving earthworms from soil is the application of a low electric current (G. Hampson, pers. comm.). The efficacy

of this technique for marine polychaetes could be tested easily in the laboratory by planting low voltage electrodes into a shallow dish of sediment with only a thin overlying layer of water. Different D.C. voltages were scheduled to be tested to see if the worms could be driven from the sediments quickly and without injury. If successful, worms would be collected as described above.

In summary, we planned to conduct the following tests to extract living *Streblospio benedicti* and/or *Polydora cornuta*:

1) two physical agitation techniques (shaking and vibrating);

2) an electric current technique; and,

3) two oxygen depletion techniques, using sodium sulfite and gaseous nitrogen to lower the ambient oxygen.

Once the animals were driven from their tubes and out of the sediment, they would be concentrated by various combinations of picking, decanting, elutriation, and/or glass plate collectors. All these various techniques were scheduled to be compared for ease and speed.

3.0 METHODS

3.1 Polychaete Collection and Holding Procedures

Large populations of opportunistic spionid polychaetes (e.g., *Streblospio benedicti* and *Polydora cornuta*) inhabit local marshes and shallow, muddy embayments. We were successful in obtaining dense spionid samples at low tide on mud flats located near the University of Massachusetts, Boston campus.

Bulk samples were obtained by coring the mud flats to a depth of about 4 cm with a 1 pound coffee can. Six cores were collected at each of three sites and transferred to a large shallow plastic box. The collected sediment was covered with water for transportation back to the wet laboratory at the Woods Hole Oceanographic Institution, where the boxes were submerged carefully in the running seawater aquarium. The populations were maintained by feeding the worms a slurry of finely ground (using a mortar and pestle) Gerber baby oatmeal. This food periodically was poured over the plastic trays.

3.2 Extraction Techniques Tested in the Laboratory

Tubicolous polychaetes were concentrated prior to all three experiments by wet-sieving sediment through an 0.6 mm mesh screen. This initial processing eliminated 60 to 90 percent of the sediment volume and allowed us to concentrate the worms for each experiment. The retained residue largely consisted of polychaete tubes, plant fragments, and shell detritus. Extraction techniques described in the following sections were all performed using this pre-sieved residue.

3.2.1 Agitation Techniques

3.2.1.1 Shaking

The sieved residue was spread in a thin layer over the bottom of a 100 mm diameter Petri dish. The Petri dish was half filled with seawater and shaken by hand (low frequency) for 5-10 minutes. Periodically, shaking was interrupted and the supernatant water and sediment surface inspected for free-swimming worms. Observations were made both by naked eye and with the aid of a dissecting microscope.

3.2.1.2 Vibration

A 4 cm diameter rubber disc attached to a therapeutic massage device transmitted a high frequency vibration to the Petri dish containing the sieved residues. This vibrator was held against the bottom of the Petri dish and observations on polychaete behavior made with the naked eye and under a dissecting microscope.

3.2.2 Low Voltage Direct Current

A 6 or 12 volt direct current supplied by door-bell batteries was applied to the sediment containing the polychaetes. The positive and negative electrodes were inserted from

underneath the bottom of a shallow plastic box through small holes about 5 cm apart into the 3 cm thick sediment layer within. A 2 cm layer of water covered the sediment.

3.2.3 Oxygen Depletion

Oxygen depletion experiments were performed on sieved residues in either a 50 or 100 ml graduated cylinder or in a specially designed plexiglas sampling box (worm trap; Figure 3-1). Ten to 25 cc of residue were introduced into each test chamber to produce a 1 to 2.5 cm thick layer on the bottom. A thin layer was used so that polychaetes buried within the sediment could escape easily through pore spaces to the overlying water column.

To reduce initial levels of pore-water oxygen, anoxic water was used to introduce the sieved residue into the test chambers. The tall and narrow shape of these chambers helped to prevent re-oxidation of the water through exposure of the surface to the atmosphere. The small surface area to volume ratio of these containers also provided a large wall area for the collection of worms as they moved upward from the anoxic sediment. The worm trap (Figure 3-1) has the advantage of a partition that can be moved to isolate the worms and supernatant water column from the underlying sediment once the worms have moved up onto the sides of the container. Once this partition is in place, the worms can be washed easily from the wall onto a filter, free and clean of sediment.

All experiments were performed in the dark to prevent any possible behavioral artifacts caused by phototropism. This was achieved by wrapping the cylinders and worm trap in aluminum foil.

3.2.3.1 Sodium Sulfite

After the physical agitation and electric current trials were completed, the first of the two oxygen depletion techniques was evaluated. Theoretically, the addition of 100 mg of sodium sulfite (Na_2SO_3) to one liter of seawater should sequester free oxygen and render the water anoxic[1]. This amount of sodium sulfite is theoretically sufficient to scavenge free oxygen from cold seawater; less should be required in warmer water. We

1 The upper limit of dissolved oxygen in the sea is 0.75 mg-atoms oxygen, although this value may be exceeded in areas of low temperature and intense photosynthesis (Sverdrup, et. al., 1942). Because a gram-atom equals the mass in grams numerically equal to the atomic weight (i.e., 1 gm-atom oxygen = 16), we can calculate that the number of grams of oxygen in 1 liter of saturated seawater = 16 x 0.00075 = 0.012 gm. From the balanced reaction equation:

$$2Na_2SO_3 + O_2 = 2Na_2SO_4;$$

therefore,

$$252 \text{ gm } Na_2SO_3/32 \text{ gm } O_2 = x/0.012 \text{ gm},$$

where x = the number of grams of sodium sulfite necessary to render 1 liter of seawater anoxic. Therefore, we calculate that:

$x = 0.0945$ gm Na_2SO_3
$x = 100$ mg Na_2SO_3 (rounded off).

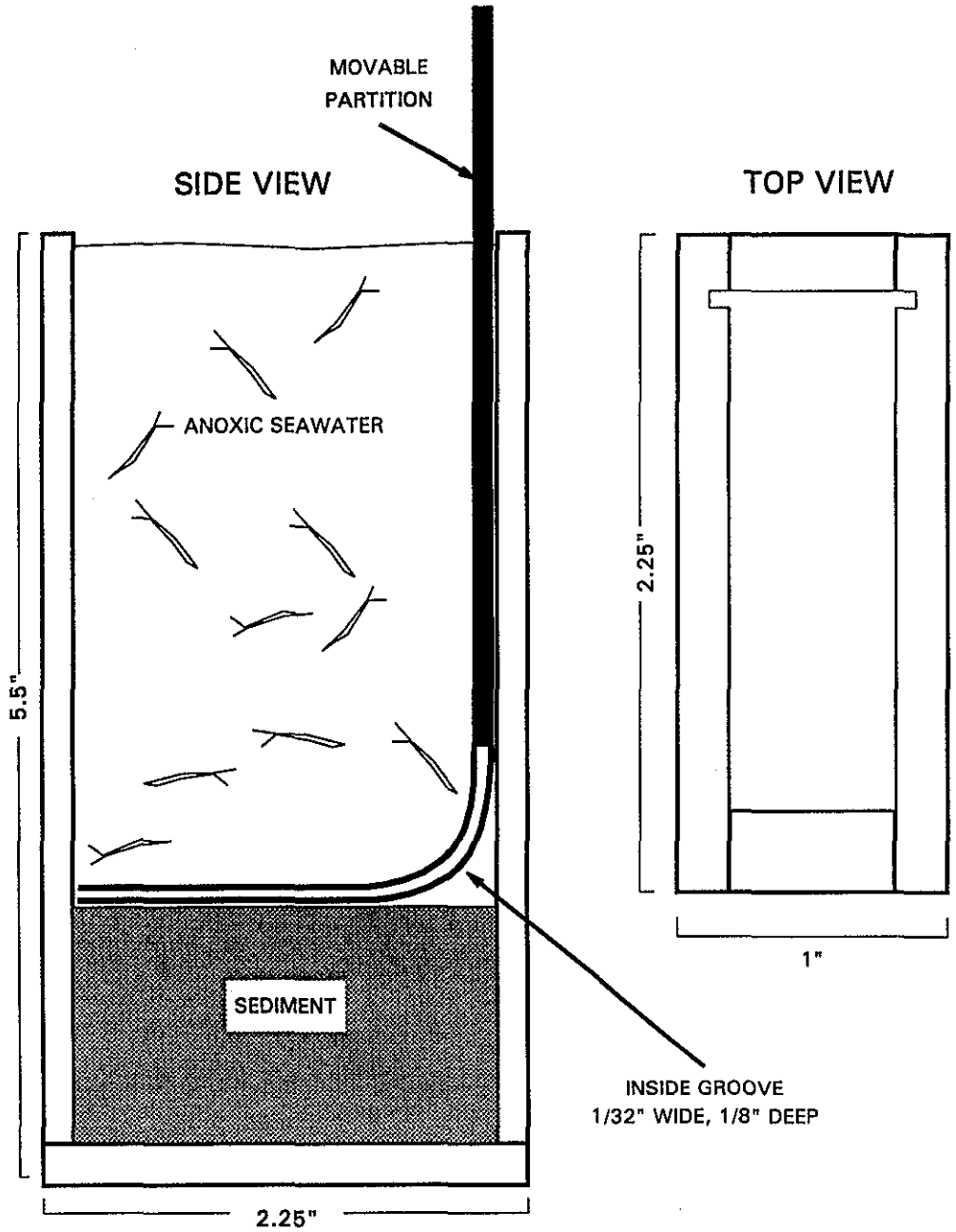

Figure 3-1. Plexiglas sampling box with sliding separator.

suggest, however, that 100 mg would be a minimum addition and that an excess of up to 200-400 mg sodium sulfite would be necessary to insure anoxia in cold water. This excess amount also would scavenge oxygen that might enter the test chamber during the experimental set-up or by diffusion from above during the extraction period.

This addition of sulfite does not increase salinity noticeably. Adding 400 mg Na_2SO_3 of seawater raises the salinity by only 0.6 ppt; in our experiment, salinity increased from 33.5 ppt to 34.1 ppt. This small increase in salinity is well within the tolerance limits of these opportunistic polychaetes.

3.2.3.2 Nitrogen Gas

Bubbling nitrogen gas through water also renders the water anoxic by removing the dissolved oxygen. Strickland and Parsons (1968) state that to remove dissolved oxygen quantitatively from 100-150 ml of water it is necessary to bubble nitrogen gas vigorously through the water for at least 30 minutes; to make 250 ml of water anoxic requires bubbling with nitrogen gas for several hours. This degassing also can be done by first bringing seawater to a simmer and then bubbling it vigorously with nitrogen gas for at least one hour as it cools to room temperature (J. Muramoto, pers. comm.). The water must then be used immediately. The long bubbling time required to scavenge oxygen and the concern about rapid reoxygenation make this method of deoxygenation unattractive for future Stage I bioaccumulation studies, especially because this procedure would have to be done in the field.

However, in the event that less bubbling time might be sufficient, one experiment was performed with water that had nitrogen gas bubbled through it for only 15 minutes. Nitrogen was bubbled from an aquarium stone at the bottom of a one liter jar filled to the brim with seawater, and nitrogen was used to fill the air space as the cap was screwed on.

4.0 RESULTS

4.1 Agitation Techniques

Although Trueblood (pers. comm.) reported successfully driving spionid worms from sediment by shaking, we found that neither manual shaking nor high frequency vibration would drive significant numbers of spionids from their tubes.

4.2 Low Voltage Current Technique

The application of 6 or 12 volt direct current voltages into the sieved residue did not drive spionid polychaetes out of their tubes either. The animals did respond to the current by writhing within their tubes, but they did not attempt to evacuate these tubes and swim into the water column.

4.3 Oxygen Depletion Techniques

The use of anoxic water did drive polychaetes out of their tubes successfully and out of the sediment at a faster rate than found in the controls. To insure anoxia, a concentration of 200 mg of sodium sulfite (Na_2SO_3) in seawater was used in most experiments. This concentration drove spionids out of their tubes and the sediment within 1½ - 2½ hours, whereas worms in oxygenated water remained out of sight within the sediment for more than 5 hours (Table 4-1). From 10 cc of sieved sediment (initial volume approximately 100 cc) placed in anoxic water, in each of three test containers, 2 to 4 worms crawled out of their tubes and up the walls within 2 hours, whereas no animals had appeared on the walls of their tubes in the control. After 5½ hours, more than 20 worms, in each of 2 test containers, had crawled totally out of the sediment when the water was treated with Na_2SO_3 while in the control no animals had crawled up the walls and only 2 had crawled out of their tubes and up onto the sediment surface.

The nitrogen bubbling experiment showed intermediate results; two worms moved out of the sediment within 2 hours but only 8 worms had

Table 4-1

Results from a deoxygenation experiment showing numbers of spionid polychaetes driven above or onto (second number) the sediment surface. Volume of sieved sediment in each container was 10 cc. (N_2 bubbled through seawater for 15 minutes; Na_2SO_3 concentration equal to 200mg/l)

Time	Control	N_2	Na_2SO_3	Na_2SO_3*
2.0 hours	0	2(1)	2(2)	4
5.5 hours	0(2)	8	>20	>20

* Plexiglas sampling box. Other three experiments performed in cylinders.

climbed the walls by the conclusion of the experiment (5½ hours).

All spionid and capitellid polychaetes driven out of the sediments were weighed. A total of 2.3 mg dry-wt biomass was obtained from the 70 polychaetes collected from approximately 25 cc of sieved residue (original volume prior to sieving was 250 cc) placed in the worm trap. The wet weight of these polychaetes was 37.6 mg, that is, 16 times the dry weight. We estimate that dry wt. biomass of an individual worm is about 0.03 mg (wet wt. = 0.5 mg).

5.0 DISCUSSION

The purpose of this study was to identify a practical field protocol for efficiently isolating small polychaete worms from their tubes and associated sediment for bioaccumulation studies. Important criteria for the technique of choice included speed, gentleness, and simplicity. Agitation and low voltage direct current were unsuccessful in driving worms from their tubes and are not worth further investigation. Oxygen depletion techniques were successful, however, and showed great promise in achieving the desired objective of obtaining sufficient biomass for chemical analyses.

Anoxia does drive infauna up and out of sediment. Confining organisms in a chamber with water that is anoxic at the start drives them from their tubes and up out of the sediment at a rate faster than achieved in ambient seawater. The addition of 100-400 mg of sodium sulfite to a liter of seawater is sufficient to render it anoxic. The oxygen depletion technique using anoxic seawater and the plexiglas sampling box made it possible to collect milligram quantities (wet weight) of these tiny polychaetes in our experiments within a few hours of sampling.

To collect gram quantities of wet weight of these tiny polychaetes requires that pooled samples be taken at locations supporting dense populations of Stage I infauna. To obtain 20 gm wet weight of tissue for bioaccumulation analysis, 40,000 worms are needed (1 worm = about 0.5 mg wet weight). The population density of opportunistic polychaetes at dredged disposal sites is extremely high, much higher than in the sediments tested by us. In a recolonization experiment in Long Island Sound, McCall (1977) found *Streblospio benedicti* at a maximum density of 418,315 individuals/m^2 ten days after the start of the experiment; *Capitella capitata* reached peak density (80,305 individuals/m^2) later, 29 to 50 days after the start. Conservatively, even if only half of these densities are found, total density for these two species may be 25 animals/cm^2 (=250,000 animals/m^2). Thus, 10,000 polychaetes may be collected in one Van Veen grab sample (area sampled = 400 cm^2) and 40,000 polychaetes in four grab samples. Removing the top 1 cm of sediment, where the worms congregate, and reducing the sediment volume 90% by sieving, would yield 40 cc/sample suitable for processing by the oxygen depletion technique.

6.0 RECOMMENDATIONS

We recommend that the oxygen depletion technique using Na_2SO_3 to render the water anoxic be tested at a suitable disposal site. Stage I infauna should reach peak population densities within 10 to 30 days after disposal operations have ended for the season. Prior to such a field study, multiple (at least 16) test chambers must be constructed so that several replicate samples can be processed simultaneously at a site. Chambers holding twice as much sieved residue should be built so that double the volume (50 cc) of sieved residue can be processed at a time. Doubling the length of the chamber would permit twice the volume of mud to be processed and still maintain the same surface area/volume ratio. The width (0.75") should remain the same to minimize sloshing; in addition, the same plastic separator (a 12-inch flexible ruler) can be used. Retaining a large height/width ratio should limit reoxygenation by diffusion.

The sieved residue from the top 1-cm of each grab sample (400 cm^2 x 1 cm = 400 cc reduced by 90% = 40 cc) should be contained within one separation chamber (vol. below separation groove = 50 cc) and four chambers should be adequate for the four samples needed to collect 40,000 animals (= 20 gm wet wt. or 1.25 gm dry wt. of tissue). Of course, these numbers would be refined in the field. Numbers of samples could be doubled or tripled, if necessary, to arrive at the required biomass.

7.0 LITERATURE CITED

Armonies, W. and M. Hellwig. 1986. Quantitative extraction of living meiofauna from marine and brackish muddy sediments. Mar. Ecol. Prog. Ser., 29:37-43.

Ankar, S. and R. Elmgren. 1976. The benthic macro- and meiofauna of the Askö-Landsort area (Northern Baltic Proper). Cont. of the Askö Lab. Univ. Stockholm, 11:1-115.

Boisseau, J.-P. 1957. Technique pour l'étude quantitative de la faune interstitielle des sables. Compt. Rend. de Congr. des Soc. Savantes de Paris et des Départ. Sect. Sci., 1957:117-119.

Eleftheriou, A. and N.A. Holme. 1984. Macrofauna techniques. In: Methods for the study of marine benthos, Holme, N.A. and A.D. McIntyre, eds., Blackwell Scientific Publications, Oxford, pp. 140-216.

Fricke, A.H. 1979. Meiofauna extraction efficiency by a modified Oostenbrink apparatus. Helgoländer wiss. Meeresunters., 32:436-443.

Hartwig, E. 1973. Die Ciliaten des Gezeiten-Sandstrandes der Nordseeinsel Sylt II. Ökologie. Mikrofauna des Meeresbodens, 21:1-171.

Higgins, R.P. 1977. Two new species of *Echinoderes* (Kinorhyncha) from South Carolina. Trans. Am. Microsc. Soc., 96:340-354.

Hockin, D.C. 1981. A simple elutriator for extracting meiofauna from sediment matrices. Mar. Ecol. Prog. Ser., 4:241-242.

Hulings, N.C. and J.S. Gray. 1971. A manual for the study of meiofauna. Smithsonian Contr. to Zool., 78, 84 pp.

Lauff, G.M., K.W. Cummins, C.H. Eriksen, and M. Parker. 1961. A method for sorting bottom fauna samples by elutriation. Limnol. Oceanogr., 6:462-466.

McCall, P.L. 1977. Community patterns and adaptive strategies of the infaunal benthos of Long Island Sound. J. Mar. Res., 35:221-266.

Oliver, B.G. 1987. Biouptake of chlorinated hydrocarbons from laboratory-spiked and field sediments by oligochaete worms. Environ. Sci. Technol., 21:785-790.

Pauly, D. 1973. Über ein Gerät zur Vorsortierung von Benthosproben. Ber. der Deut. Wiss. Komm. für Meeresforschung, 2:458-460.

Peddicord, R.K. 1988. Summary of the U.S. Army Corps of Engineers/U.S. Environmental Protection Agency Field Verification Program. Technical Report D-88-6, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Pfannkuche, O. and H. Thiel. 1988. Sample processing. In: Introduction to the study of meiofauna, Higgins, R.P. and H.

Thiel, eds., Smithsonian Inst. Press., Wash. D.C., pp. 126-133.

Poizat, C. 1975. Technique de concentration des gastéropods opistobranches mésopsammiques en vue d'études quantitatives. Cah. de Biol. Mar., 16:475-481.

Rhoads, D. C. and J. D. Germano. 1982. Characterization of organism-sediment relations using sediment profile imaging: An efficient method of Remote Ecological Monitoring of the Seafloor (REMOTS® System). Mar. Ecol. Prog. Ser., 8: 115-128.

SAIC. Unpublished. An Integrated, Tiered Approach to Monitoring and Management of Dredged Material Disposal Sites in the New England Region. SAIC Report No. SAIC-90/7575&234. Submitted to U.S. Army Corps of Engineers, New England Division, Waltham, MA.

Schmidt, P. 1968. Die quantitative Verteilung und Populationsdynamik des Mesopsammon am Gezeiten-Sandstrand der Nordseeinsel Sylt I. Faktorengefüge und biologische Gliederung des Lebensraums. Int. Rev. der gesamten Hydrobiol., 53:723-779.

Schwinghamer, P. 1981. Extraction of living meiofauna from marine sediments by centrifugation in a silica sol-sorbitol mixture. Can. J. Fish. and Aquat. Sci., 38:476-478.

Strickland, J. P. H. and T. R. Parsons. 1968. A practical handbook of seawater analysis. Bull. Fish. Res. Bd. Can. 167: 1-311.

Sverdrup, H. U., W. Johnson and R. H. Fleming. 1942. The Oceans, their Physics, Chemistry, and General Biology. New York, Prentice-Hall, Inc., 1087 p.

Uhlig, G. 1964. Eine einfache Methode zur Extraktion der vagilen mesopsammalen Mikrofauna. Helgoländer wiss. Meeresunters., 11:178-185.

Uhlig, G., H. Thiel, and J.S. Gray. 1973. The quantitative separation of meiofauna. Helgoländer wiss. Meeresunters., 25:173-195.

Worswick, J.M. and M.T. Barbour. 1974. An elutriation apparatus for macro-invertebrates. Limnol. Oceanogr., 19:538-540.