NOAA Technical Memorandum NOS OMA 37

National Status and Trends Program for Marine Environmental Quality Specimen Bank Project: Field Manual

Gunnar G. Lauenstein, Stephen A. Wise, Rolf Zeisler,
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Rockville, Maryland
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U.S. DEPARTMENT OF COMMERCE
NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
National Ocean Service

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Gunnar G. Lauenstein[a], Stephen A. Wise[b], Rolf Zeisler[b],
Barbara J. Koster[b], Michele M. Schantz[b], and Sandra L. Golembiewska[a]

[a] Ocean Assessments Division, Office of Oceanography and Marine Assessment, National Oceanic and Atmospheric Administration, Rockville, MD. 20852

[b] National Bureau of Standards, Gaithersburg, MD. 20899

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UNITED STATES DEPARTMENT OF COMMERCE
C. William Verity, Jr.
Secretary

National Oceanic and Atmospheric Administration

National Ocean Service
Paul M. Wolff
Assistant Administrator

Coastal and Estuarine Assessment Branch
Ocean Assessments Division
Office of Oceanography and Marine Assessment
National Ocean Service
National Oceanic and Atmospheric Administration
U.S. Department of Commerce
Rockville, Maryland

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THE NATIONAL STATUS AND TRENDS
Specimen Bank Project
1987

Gunnar G. Lauenstein[a], Stephen A. Wise[b], Rolf Zeisler[b],
Barbara J. Koster[b], Michele M. Schantz[b], and Sandra L. Golembiewska[a]

1. INTRODUCTION

In 1980, a pilot National Environmental Specimen Bank Program was established in the United States at the National Bureau of Standards (NBS), sponsored in part by the U.S. Environmental Protection Agency. Since then, other Federal agencies, including the Food and Drug Administration, Department of Agriculture, National Cancer Institute, and the National Oceanic and Atmospheric Administration (NOAA), represented by the Ocean Assessments Division, have joined in the specimen banking activities at NBS.

In fiscal year 1984, NOAA's Ocean Assessments Division (OAD) initiated a new program, called the National Status and Trends (NS&T) Program, within which activities are being undertaken to quantify the current status and long-term temporal and spatial trends of key contaminant concentrations and biological indicators of contaminant effects in the nation's coastal and estuarine environments.[1] The program's purpose is to provide highly reliable data on concentrations of toxic chemicals in marine fishes, shellfishes, and sediments; to measure biological parameters that accurately reflect anthropogenic stress; to assess marine environmental quality; and to recommend Federal actions needed to maintain or to improve it.

One of the elements of the NS&T Program is the archiving of samples for retrospective analyses. The methods of collection, preparation, and storage of samples for a specimen banking program are critical to the scientific accuracy of the analysis and comparison of these data. The methods used in the specimen banking component of NOAA's National Status and Trends Program are described in this report. Also summarized are the National Status and Trends Program specimen banking operations for its Benthic Surveillance[2] and Mussel Watch component for fiscal years 1985, 1986, and 1987. Protocols used for preparation and storage of samples are also described in detail.

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[a] Ocean Assessments Division, Office of Oceanography and Marine Assessment, National Oceanic and Atmospheric Administration; Rockville, Maryland.

[b] National Bureau of Standards; Gaithersburg, Maryland.

2. CRYOGENIC STORAGE FACILITY

Because of its long-term experience with cryogenic specimen storage and related specimen banking activities, facilities of the National Bureau of Standards were selected for the National Status and Trends Program's Specimen Bank. The facilities available at the National Bureau of Standards for storage of NS&T materials include (1) a class-100 clean-air laboratory designed to minimize sample contamination from the laboratory environment during sample handling and processing, and (2) a specimen storage room. The laboratory has two sections where samples are prepared for organic and inorganic analysis. The specimen storage room, designed for long-term storage of environmental and biological samples, contains two NS&T liquid nitrogen vapor freezers with a volumetric capacity of 500 liters each, which are maintained at temperatures from -110 to -150 $^{\circ}$C.

The National Bureau of Standards facility currently receives samples from two NS&T activities--the Benthic Surveillance Project and the Mussel Watch Project. Each year benthic fish samples (muscle and liver tissue) and sediment samples collected from 6 of the 50 coastal and estuarine sampling sites nationwide in the Benthic Surveillance Project are archived along with bivalve molluscs and sediments from 30 of 150 nationwide sampling sites in the Mussel Watch Project (Figures 1 & 2). The inventory of samples stored in the specimen bank are summarized in Tables 1 and 2.

3. BENTHIC SURVEILLANCE SPECIMEN BANKING PROTOCOLS

Samples of sediment and fish tissue (liver and muscle) are collected as part of the NOAA National Status and Trends (NS&T) Benthic Surveillance Project for storage in the National Biomonitoring Specimen Bank (NBSB) at the National Bureau of Standards. Samples to be stored in the NOAA NS&T Specimen Bank must be collected, processed, and packaged according to the protocols described in this document.

Target fish (e.g., flounder, croaker) are collected by otter trawl and returned to a shipboard laboratory. After selected specimens are weighed and measured, they are moved to a laminar-flow, clean-air work station, where tissue samples are excised according to the protocols described below. Sediment samples are collected with box cores or grab samplers.

The collection protocols for the NOAA NS&T specimens consist of three stages: sample collection and preparation, fish dissection/sediment processing, and sample packaging and shipping. The division of the protocols into stages is used as an aid in organizing and simplifying the collection procedures. All information regarding the sample preparation is recorded on the Sampling Data form (NOAA NS&T Benthic Surveillance Project -- see Appendix A).

Figure 1. Specimen Bank Sampling Sites
Mussel Watch Project
1986 & 1987

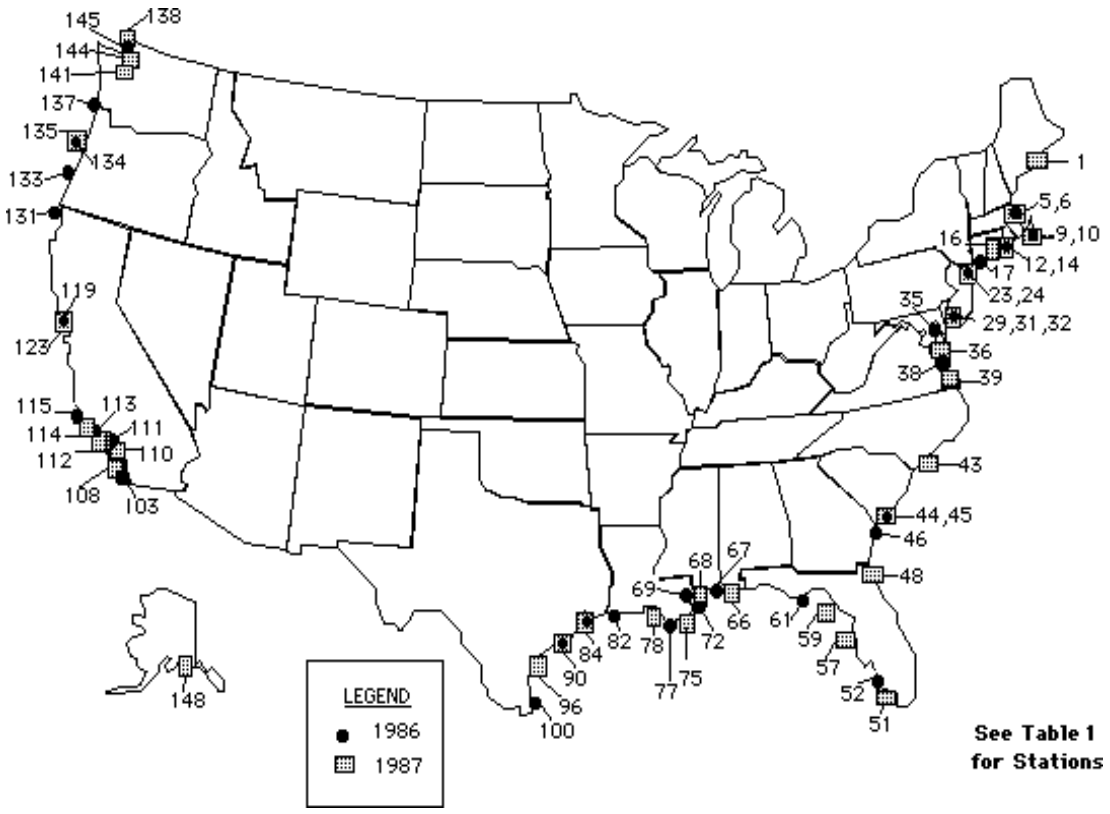


TABLE 1. Specimen Bank Inventory for the Mussel Watch Project (1986-87)

SITE NO.	CODE	TYPE	SITE NAME	SITE AREA	LAT.	LONG.	DATE COLLECTED	SPECIES	NBS SAMP NO.
1	PBSI	Mussel	Sears Island	Penobscot Bay	44 27.1	68 53.4	27Mar87	*M. edulis*	MW2M178
1	PBSI	Sediment	Sears Island	Penobscot Bay	44 27.1	68 53.4	27Mar87		MW2S179
5	BHDB	Mussel	Boston Harbor	Dorchester Bay	42 18.2	71 02.1	13Jan86	*M. edulis*	MW1M048*
5	BHDB	Sediment	Boston Harbor	Dorchester Bay	42 18.2	71 02.2	16Jan86		MW1S049*
6	BHHB	Mussel	Hingham Bay	Boston Harbor	42 16.5	70 53.3	02Mar87	*M. edulis*	MW2M163*
6	BHHB	Sediment	Hingham Bay	Boston Harbor	42 16.5	70 53.3	02Mar87		MW2S164*
9	BBRH	Mussel	Buzzards Bay	Round Hill	41 32.3	70 55.6	23Jan86	*M. edulis*	MW1M046
9	BBRH	Sediment	Buzzards Bay	Round Hill	41 32.4	70 55.0	05Feb86		MW1S047
10	BBAR	Mussel	Anglica Rock	Buzzard's Bay	41 34.6	70 51.8	09Mar87	M. edulis	MW2M165
10	BBAR	Sediment	Anglica Rock	Buzzard's Bay	41 35.2	70 52.7	07Mar87		MW2S166
12	NBCI	Mussel	Narragansett Bay	Conanicut Island	41 29.8	71 23.2	03Feb86	M. edu.lis	MW1M050
12	NBCI	Sediment	Narragansett Bay	Conanicut Island	41 29.8	71 23.1	03Feb86		MW1S051
14	NBDI	Mussel	Dyer Island	Narragansett Bay	41 36.2	71 17.4	12Mar87	M. edulis	MW2M176
14	NBDI	Sediment	Dyer Island	Narragansett Bay	41 36.2	71 17.4	12Mar87		ME2S177
16	LICR	Sediment	Long Island Sound	Connecticut River	41 15.8	72 20.4	11Nov86		MW2S118
17	LIHR	Mussel	Long Island Sound	Housatonic River	41 10.1	73 06.6	12Feb86	*M. edulis*	MW1M053
17	LIHR	Sediment	Long Island Sound	Housatonic River	41 10.5	73 07.0	13Feb86		MW1S054
23	HRUB	Sediment	Raritan Estuary	Lower Hudson Bay	40 41.4	74 02.6	07Dec87		MW2S126*
24	HRLB	Mussel	Hudson Raritan	Lower Bay	40 34.0	74 03.1	15Mar86	*M. edulis*	MW1M075
24	HRLB	Sediment	Hudson Raritan	Lower Bay	40 34.1	74 03.3	13Mar86		MW1S076
29	DBFE	Sediment	Delaware Bay	False Egg Island Pt.	39 12.7	75 11.5	16Dec86		MW2S124
31	DBAP	Mussel	Delaware Bay	Arnolds Point Shoal	39 23.0	75 25.4	25Mar86	*C. virginica*	MW1M077
31	DBAP	Sediment	Delaware Bay	Arnolds Point Shoal	39 23.3	75 25.9	25Mar86		MW1S078
32	DBKI	Oyster	Delaware Bay	Kelly Island	39 12.2	75 21.3	07Jan87	*C. virginica*	MW2Y119
32	DBKI	Sediment	Delaware Bay	Kelly Island	39 12.2	75 21.3	07Jan87		MW2S120
35	CBMP	Oyster	Baltimore Harbor	Mountain Point Bar	39 04.4	76 24.7	12Mar86	*C. virginica*	MW1Y073*
35	CBMP	Sediment	Baltimore Harbor	Mountain Point Bar	39 04.4	76 24.6	12Mar86		MW1S074*
36	CBHP	Oyster	Chesapeake Bay	Hackett Point	38 58.4	76 25.0	21Jan87	*C. virginica*	MW2Y133
36	CBHP	Sediment	Chesapeake Bay	Hackett Point	38 58.4	76 25.0	21Jan87		MW2S134
38	CBIB	Oyster	Ingram Bay	Chesapeake Bay	37 47.6	76 17.1	04Mar86	*C. virginica*	MW1Y162
38	CBIB	Sediment	Ingram Bay	Chesapeake Bay	37 47.5	76 17.2	04Mar86		MW1S063
39	CBCC	Oyster	Cape Charles	Chesapeake Bay	37 17.6	76 00.6	09Jan87	*C. virginica*	MW2Y121
39	CBCC	Sediment	Cape Charles	Chesapeake Bay	37 17.6	76 00.6	09Jan87		MW2S122
43	CFBI	Oyster	Cape Fear	Battery Island	33 54.9	78 00.6	07Feb87	*C. virginica*	MW2Y149
43	CFBI	Sediment	Cape Fear	Battery Island	33 54.9	78 00.6	07Feb87		MW2S150
44	CHFJ	Oyster	Charleston Harbor	Fort Johnson	32 43.9	79 51.7	12Feb86	*C. virginica*	MW1Y044*
44	CHFJ	Sediment	Charleston Harbor	Fort Johnson	32 43.5	79 52.0	11Feb86		MW1S045*
45	CHSF	Oyster	Charleston Harbor	Shutes Folly	32 46.8	79 55.0	13Feb87	*C. virginica*	MW2Y151
45	CHSF	Sediment	Charleston Harbor	Shutes Folly	32 46.8	79 55.0	13Feb87		MW2S152
46	SRTI	Oyster	Savannah River	Tybee Island	31 58.8	80 50.6	04FEB86	*C. virginica*	MW1Y035
46	SRTI	Sediment	Savannah River	Tybee Island	31 58.8	80 50.5	05FEB86		MW1S036
48	SJCB	Oyster	Chicopit Bay	St. John's River	30 23.0	81 26.6	16Feb87	*C. virginica*	MW2M161
48	SJCB	Sediment	Chicopit Bay	St. John's River	30 23.0	81 26.6	17Feb87		MW2S162
51	EVFU	Oyster	Faka Union Bay	Everglades	25 54.3	81 30.6	02Mar87	C. virginica	MW2M171
51	EVFU	Sediment	Faka Union Bay	Everglades	25 54.3	81 30.6	02Mar87		MW2S172
52	RBHC	Oyster	Rookery Bay	Henderson Creek	26 01.8	81 43.8	20Feb86	*C. virginica*	MW1Y066
52	RBHC	Sediment	Rookery Bay	Henderson Creek	26 01.8	81 43.8	20Feb86		MW1S065
57	TBMK	Oyster	Mullet Key Bay	Tampa Bay	27 37.3	82 43.6	01Mar87	*C. virginica*	MW2M169
57	TBMK	Sediment	Mullet Key Bay	Tampa Bay	27 37.3	82 43.6	01Mar87		MW2S170
59	CKBP	Oyster	Black Point	Cedar Key	29 10.3	83 03.0	28Feb87	*C. virginica*	MW2M167
59	CKBP	Sediment	Black Point	Cedar Key	29 10.3	83 03.0	28Feb87		MW2S168
61	APCP	Oyster	Cat Point Bar	Apalachicola Bay	29 43.0	84 52.5	27Feb86	*C. virginica*	MW1Y072
61	APCP	Sediment	Cat Point Bar	Apalachicola Bay	29 43.0	84 52.5	27Feb86		MW1S071
66	MBCP	Oyster	Cedar Point Reef	Mobile Bay	30 19.4	88 07.3	13Jan87	*C. virginica*	MW2Y129*
66	MBCP	Sediment	Cedar Point Reef	Mobile Bay	30 19.4	88 07.3	14Jan87		MW2S130*
67	MSPB	Oyster	Mississippi Sound	Pascagoula Bay	30 21.1	88 37.0	05Feb86	*C. Virginica*	MW1Y068*
67	MSPB	Sediment	Mississippi Sound	Pascagoula Bay	30 21.1	88 37.0	05Feb86		MW1S067*
68	MSBB	Oyster	Biloxi Bay	Mississippi Sound	30 23.4	88 15.4	16Feb87	*C. virginica*	MW2M157
68	MSBB	Sediment	Biloxi Bay	Mississippi Sound	30 23.4	88 15.4	16Feb87		MW2S158
69	MSPC	Oyster	Mississippi Sound	Pass Christian	30 19.8	89 19.6	06Feb86	*C. virginica*	MW1Y070
69	MSPC	Sediment	Mississippi Sound	Pass Christian	30 19.8	89 19.6	06Feb86		MW1S069
72	BSBG	Oyster	Brenton Sound	Bay Garderne	29 35.9	89 38.5	26Mar86	*C. virginica*	MW1Y089
72	BSBG	Sediment	Brenton Sound	Bay Garderne	29 35.9	89 38.5	26Mar86		MW1S090
75	TBLF	Oyster	Lake Felicity	Terrebonne Bay	29 16.0	90 24.5	13Feb87	*C. virginica*	MW2M159
75	TBLF	Sediment	Lake Felicity	Terrebonne Bay	29 16.0	90 24.5	13Feb87		MW2S160
77	CLCL	Oyster	Caillou Lake	Caillou Lake	29 15.3	90 55.5	29Mar86	*C. virginica*	MW1Y091
77	CLCL	Sediment	Caillou Lake	Caillou Lake	29 15.3	90 55.5	29Mar86		MW1S092
78	ABOB	Oyster	Oyster Bayou	Atchafalaya Bay	29 13.0	91 08.0	11Feb87	*C. virginica*	MW2M153
78	ABOB	Sediment	Oyster Bayou	Atchafalaya Bay	29 13.0	91 08.0	11Feb87		MW2S154
82	CLSJ	Oyster	Calcasieu Lake	St. John's Island	29 51.0	93 23.0	18Jan86	*C. virginica*	MW1Y043
82	CLSJ	Sediment	Calcasieu Lake	St. John's Island	29 51.0	93 23.0	18Jan86		MW1S042
84	GBCR	Oyster	Confederate Reef	Galveston Bay	29 15.8	94 50.5	11Jan86	*C. virginica*	MW1Y030*
84	GBCR	Sediment	Confederate Reef	Galveston Bay	29 15.8	94 50.5	11Jan86		MW1S031*
84	GBCR	Sediment	Confederate Reef	Galveston Bay	29 15.8	94 50.5	16Dec86	*C. virginica*	MW2Y127
84	GBCR	Sediment	Confederate Reef	Galveston Bay	29 15.8	94 50.5	16Dec86		MW2S128
90	MBGP	Oyster	Matagorda Bay	Gallinipper Point	28 35.0	90 63.4	31Jan86	*C. virginica*	MW1Y039
90	MBGP	Sediment	Matagorda Bay	Gallinipper Point	28 35.0	96 34.0	31Jan86		MW1S038
90	MBGP	Oyster	Matagorda Bay	Gallinipper Point	28 35.0	90 63.4	13Jan87	*C. virginica*	MW2Y129

TABLE 1 (Cont.). Specimen Bank Inventory for the Mussel Watch Project (1986-87)

SITE NO.	CODE	TYPE	SITE NAME	SITE AREA	LAT.	LONG.	DATE COLLECTED	SPECIES	NBS SAMP NO.
90	MBGP	Sediment	Matagorda Bay	Gallinipper Point	28 35.0	96 34.0	14Jan87		MW2S130*
96	CBCR	Oyster	Capano Reef	Capano Bay	28 08.20	97 07.58	14Jan87	*C. virginica*	MW2Y131
96	CBCR	Sediment	Capano Reef	Capano Bay	28 08.20	97 07.58	14Jan87		MW2S132
100	LMSB	Oyster	L. Laguna Madre	South Bay	26 02.6	97 10.5	24JAN86	*C. virginica*	MW1Y041
100	LMSB	Sediment	L. Laguna Madre	South Bay	26 02.6	97 10.5	24JAN86		MW1S040
103	IBNJ	Mussel	San Diego	Imperial Beach	32 40.5	117 08.1	11JAN86	*M. californianus*	MW1M029*
103	IBNJ	Sediment	San Diego	Imperial Beach	32 36.0	117 10.0	20JAN86		MW1S032*
108	LJLJ	Mussel	LaJolla	Point LaJolla	32 56.5	117 14.6	29Dec86	*M. californianus*	MW2M140
108	LJLJ	Sediment	LaJolla	Point LaJolla	32 48.8	117 19.7	12Mar87		MW2S173
110	NBWJ	Mussel	Newport Bay	Balboa Channel Jetty	33 35.9	117 53.1	07Dec86	*M. californianus*	MW2M137
110	NBWJ	Sediment	Newport Bay	Balboa Channel Jetty	33 35.1	117 53.7	10Dec86		MW2S138
111	ABWJ	Mussel	Anaheim Bay	West	33 44.0	118 07.0	27JAN86	*M. californianus*	MW1M034
111	ABWJ	Sediment	Anaheim Bay	West Jetty	33 44.1	118 07.0	12Feb86		MW1S052
112	SPFP	Mussel	San Pedro Harbor	Fishing Pier	33 42.3	118 16.3	07Dec86	*M. californianus*	MW2M175*
112	SPFP	Sediment	San Pedro Harbor	Fishing Pier	33 42.6	118 16.4	07Dec86		MW2S139*
113	PVRP	Mussel	Palos Verdes	Royal Palms State Pk	33 43.3	118 19.3	27JAN86	*M. californianus*	MW1M033
113	PVRP	Sediment	Palos Verdes	Royal Palms State Pk	33 43.5	118 21.0	18Feb86		MW1S064
114	MDSJ	Mussel	Marina Del Rey	South Jetty	33 57.7	118 27.4	03Dec86	*M. edulis*	MW2M135*
114	MDSJ	Sediment	Marina Del Rey	South Jetty	33 59.5	118 32.0	03Dec86		MW2S136*
115	PDPD	Mussel	Point Dume	Point Dume	34 00.2	118 48.2	06FEB86	*M. californianus*	MW1M037
115	PDPD	Sediment	Point Dume	Point Dume	33 59.9	118 46.9	01Mar86		MW1S061
119	SFDB	Sediment	Dunbarton Bridge	San Francisco Bay	37 31.7	122 08.3	12Mae87		MW2S174
123	SFSM	Mussel	San Mateo Bridge	San Francisco Bay	37 35.8	122 14.5	11Apr87	*M. edulis*	MW1M094
123	SFSM	Sediment	San Mateo Bridge	San Francisco Bay	37 21.4	122 14.5	03Apr87		MW1S093
131	SGSG	Mussel	Point St. George	Point St. George	41 44.5	124 12.3	29Mar87	*M. californianus*	MW1M096
131	SGSG	Sediment	Point St. George	Point St. George	41 43.2	121 13.6	21Apr87		MW1S195
133	CBRP	Mussel	Coos Bay	Russell Point	43 26.1	124 13.0	06Feb86	*M. edulis*	MW1M057
133	CBRP	Sediment	Coos Bay	Russell Point	43 25.8	124 13.0	07Feb86		MW1S059
134	YBOP	Mussel	Yaquina Bay	Oneatta Point	44 35.0	124 00.1	03Dec86	*M. edulis*	MW2M147
134	YBOP	Sediment	Yaquina Bay	Oneatta Point	44 34.8	124 00.8	03Dec86		MW2S148
135	YHYH	Mussel	Sally's Slough	Yaquina Head	44 40.4	124 04.4	04Feb86	*M. californianus*	MW1M056
135	YHSS	Sediment	Sally's Slough	Yaquina Head	44 37.0	124 00.4	08Feb86		MW1S060
137	CRSJ	Mussel	Columbia River	Fort Stevens	46 13.9	124 00.5	04Mar86	*M. edulis*	MW1M081
137	CRYB	Sediment	Columbia River	Fort Stevens	46 10.9	123 51.9	04Mar86		MW1S082
138	BBSM	Mussel	Saqalicum Marina	Bellingham Bay	48 45.3	122 29.1	27Jan87	*M. edulis*	MW2M145
138	BBSM	Sediment	Saqalicum Marina	Bellingham Bay	48 44.8	122 30.7	24Nov87		MW2S146
141	SSBI	Mussel	Budd Inlet	South Puget Sound	47 05.9	122 53.6	12Dec86	*M. edulis*	MW2M141
141	SSBI	Sediment	Budd Inlet	South Puget Sound	47 06.0	122 54.7	17Nov86		MW2S142
144	SIWP	Mussel	Waterman Point	Sinclair Inlet	47 35.1	122 34.2	18Dec86	*M. edulis*	MW2M143
144	SIWP	Sediment	Waterman Point	Sinclair Inlet	47 33.1	122 37.6	14Nov86		MW2S144
145	WIPP	Mussel	Possession Point	Whidbey Island	47 53.2	122 25.3	21Jan86	*M. edulis*	MW1M055
145	WIPP	Sediment	Possession Point	Whidbey Island	47 54.7	122 20.7	29Jan86		MW1S058
148	PVMC	Mussel	Mineral Creek Flats	Port Valdez	61 07.6	146 27.5	25Mar87	*M. edulis*	MW2M180
148	PVMC	Sediment	Mineral Creek Flats	Port Valdez	61 07.6	146 27.5	25Mar87		MW2S181

*These locations have been selected for analysis.

Figure 2. Specimen Bank Sampling Sites
Benthic Surveillance
1985, 1986, & 1987

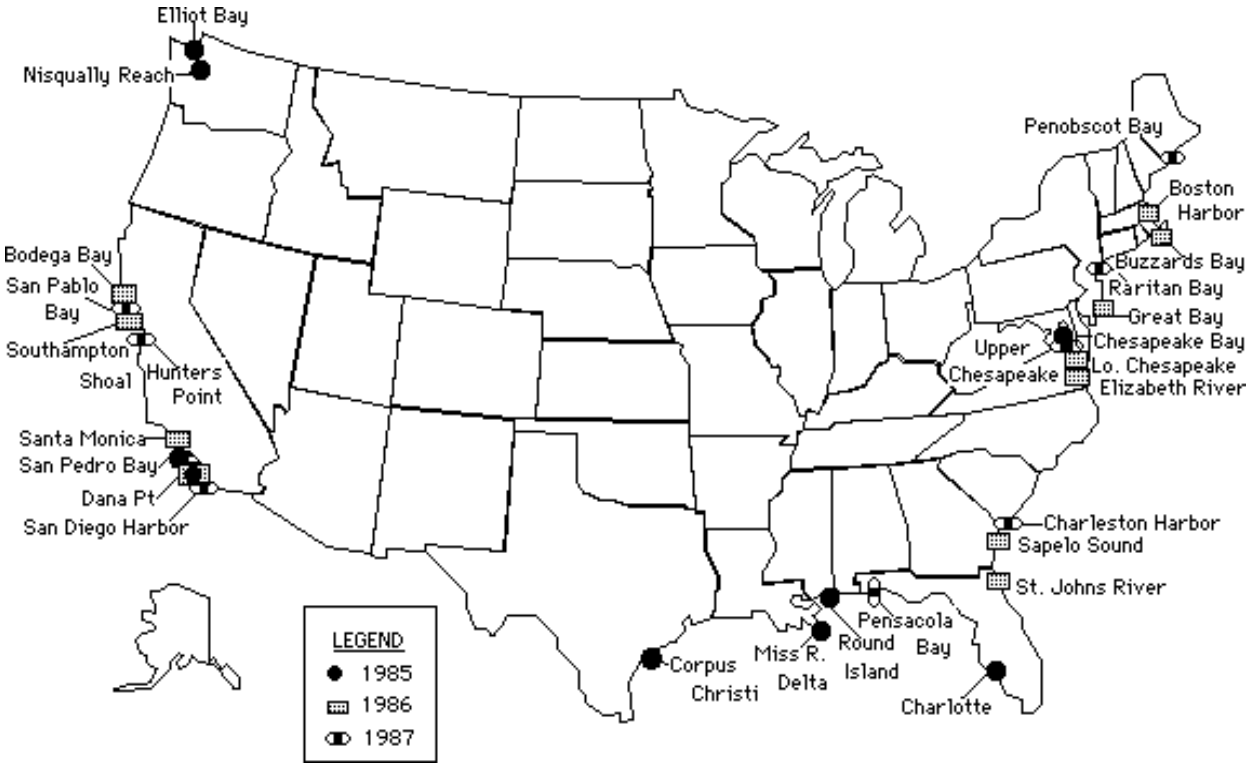


Table 2. Specimen Bank Inventory for the Benthic Surveillance Project (1985-1987)

CODE	TYPE	SITE NAME	SITE AREA	LAT.	LONG.	COLLECTED	SPECIES	SAMP NO.
DAN	Sediment	Dana Point	Southern California	33 26.8	117 41.5	19Jul85		BS1S001*
DAN	Fish Muscle	Dana Point	Southern California	33 26.8	117 41.5	19Jul85	White croaker	BS1F002
DAN	Fish Liver	Dana Point	Southern California	33 26.8	117 41.5	29Jul85	White croaker	BS1L003*
DAN	Fish Muscle	Dana Point	Southern California	33 26.8	117 41.5	18Jul85	White croaker	BS1F005
DAN	Fish Liver	Dana Point	Southern California	33 26.8	117 41.5	18Jul85	White croaker	BS1L006
SPB	Sediment	San Pedro Bay	San Pedro Bay	33 42.0	118 15.7	21Jul85		BS1S007
SPB	Fish Muscle	San Pedro Bay	San Pedro Bay	33 42.0	118 15.7	20Jul85	White croaker	BS1F008
SPB	Fish Liver	San Pedro Bay	San Pedro Bay	33 42.0	118 15.7	20Jul85	White croaker	BS1L009
ELL	Sediment	Elliott Bay	Puget Sound	47 35.5	122 21.0	12Aug85		BS1S010*
ELL	Fish Muscle	Elliott Bay	Puget Sound	47 35.5	122 21.0	12Aug85	English sole	BS1F011
ELL	Fish Liver	Elliott Bay	Puget Sound	47 35.5	122 21.0	12Aug85	English sole	BS1L012*
NIS	Sediment	Nisqually Reach	Puget Sound	47 08.0	122 44.5	14Aug85		BS1S013*
NIS	Fish Muscle	Nisqually Reach	Puget Sound	47 08.0	122 44.5	14Aug85	English sole	BS1F014
NIS	Fish Liver	Nisqually Reach	Puget Sound	47 08.0	122 44.5	14Aug85	English sole	BS1L015*
ROU	Sediment	Round Island, MS	Miss. River Delta	30 19.0	88 36.0	05Sep85		BS1S016
ROU	Fish Muscle	Round Island, MS	Miss. River Delta	30 19.0	88 36.0	04Sep85	Atlantic croaker	BS1F017
ROU	Fish Liver	Round Island, MS	Miss. River Delta	30 19.0	88 36.0	04Sep85	Atlantic croaker	BS1L018
MRD	Sediment	Miss. R. Delta	Miss. River Delta	29 05.0	89 04.0	09Sep85		BS1S019*
MRD	Fish Muscle	Miss. R. Delta	Miss. River Delta	29 05.0	89 04.0	08Sep85	Atlantic croaker	BS1F020
MRD	Fish Liver	Miss. R. Delta	Miss. River Delta	29 05.0	89 04.0	08Sep85	Atlantic croaker	BS1L021*
CCB	Sediment	Corpus Christi	Corpus Christi Bay	27 50.0	97 17.0	23Sep85		BS1S022*
CCB	Fish Muscle	Corpus Christi	Corpus Christi Bay	27 50.0	97 17.0	20Sep85	Atlantic croaker	BS1F023
CCB	Fish Liver	Corpus Christi	Corpus Christi Bay	27 50.0	97 17.0	20Sep85	Atlantic croaker	BS1L024*
CHR	Sediment	Charlotte Harbor	Charlotte Harbor	26 51.0	82 07.0	14Oct85		BS1S025
CHR	Fish Muscle	Charlotte Harbor	Charlotte Harbor	26 51.0	82 07.0	14Oct85	Spot	BS1F026
CHR	Fish Liver	Charlotte Harbor	Charlotte Harbor	26 51.0	82 07.0	14Oct85	Spot	BS1L027*
UCB	Sediment	Chesapeake Bay	Chesapeake Bay	38 55.7	76 25.0	17Jul85	Spot	BS1S028*
GRB	Fish Muscle	Great Bay	Great Bay	39 31.0	74 23.0	27Mar86	Winter flounder	BS1F079
GRB	Sediment	Great Bay	Great Bay	39 31.0	74 23.0	25Mar86		BS1S080
BOS	Fish Liver	Boston Harbor	Boston Harbor	42 20.0	71 00.0	08Apr86	Winter flounder	BS1L083*
BOS	Fish Muscle	Boston Harbor	Boston Harbor	42 20.0	71 00.0	08Apr86	Winter flounder	BS1F084
BOS	Sediment	Boston Harbor	Boston Harbor	42 20.0	71 00.0	08Apr86		BS1S085
BUZ	Fish Liver	Buzzards Bay	Buzzards Bay	41 35.0	70 45.0	07Apr86	Winter flounder	BS1L086*
BUZ	Fish Muscle	Buzzards Bay	Buzzards Bay	41 35.0	70 45.0	07Apr86	Winter flounder	BS1F087
BUZ	Sediment	Buzzards Bay	Buzzards Bay	41 35.0	70 45.0	08Apr86		BS1S088
BOD	Fish Muscle	Bodega Bay	Bodega Bay	38 18.0	123 02.0	29Jun86	Starry flounder	BS1F097
BOD	Fish Liver	Bodega Bay	Bodega Bay	38 18.0	123 02.0	29Jun86	Starry flounder	BS1L098*
BOD	Sediment	Bodega Bay	Bodega Bay	38 18.0	123 02.0	30Jun86		BS1S099
SHS	Fish Muscle	Southampton Shoal	San Francisco Bay	37 54.0	122 25.0	08Jul86	Starry flounder	BS1F100
SHS	Fish Liver	Southampton Shoal	San Francisco Bay	37 54.0	122 25.0	08Jul86	Starry flounder	BS1L101*
SHS	Sediment	Southampton Shoal	San Francisco Bay	37 54.0	122 25.0	08Jul86		BS1S102
SMB	Fish Muscle	Santa Monica Bay	Santa Monica Bay	33 53.0	118 26.0	16Jul86	Hornyhead turbot	BS1F103
SMB	Fish Liver	Santa Monica bay	Santa Monica Bay	33 53.0	118 26.0	16Jul86	Hornyhead turbot	BS1L104*
SMB	Sediment	Santa Monica Bay	Santa Monica Bay	33 53.0	118 26.0	16Jul86		BS1S105
DAN	Fish Muscle	Dana Point	Southern California	33 27.0	117 42.0	19Jul86	Hornyhead turbot	BS1F106
DAN	Fish Liver	Dana Point	Southern California	33 27.0	117 42.0	19Jul86	Hornyhead turbot	BS1L107
DAN	Sediment	Dana Point	Southern California	33 27.0	117 42.0	19Jul86		BS1S108
ELR	Sediment	Elizabeth River	Chesapeake Bay	36 50.0	76 22.0	28Jul86		BS1S109
LCB	Sediment	Lower Chesapeake	Chesapeake Bay	37 10.0	76 10.0	26Jul86		BS1S110
SJR	Sediment	St. Johns River	St. Johns R. Estuary	30 24.0	81 33.0	18Aug86		BS1S111
SJR	Fish Liver	St. Johns River	St. Johns R. Estuary	30 24.0	81 33.0	19Aug86	Atlantic croaker	BS1L112*
SJR	Fish Muscle	St. Johns River	St. Johns R. Estuary	30 24.0	81 33.0	29Aug86	Atlantic croaker	BS1F113
SAP	Sediment	Sapelo Sound	Sapelo Sound	31 33.0	81 14.0	15Aug86		BS1S114
SAP	Fish Liver	Sapelo Sound	Sapelo Sound	31 33.0	81 14.0	13Aug86	Atlantic croaker	BS1L115
SAP	Fish Muscle	Sapelo Sound	Sapelo Sound	31 33.0	81 14.0	14Aug86	Atlantic croaker	BS1F116
RAR	Fish Liver	Raritan Bay	Raritan Bay	40 28.8	74 02.0	10Apr87	Winter flounder	BS2L182
RAR	Fish Muscle	Raritan Bay	Raritan Bay	40 28.8	74 02.0	10Apr87	Winter flounder	BS2F183
RAR	Sediment	Raritan Bay	Raritan Bay	40 28.8	74 02.0	13Apr87		BS2S184
PNB	Fish Liver	Penobscot Bay	Penobscot Bay	44 15.0	68 50.0	24Apr87	Longhorn sculpin	BS2L185
PNB	Fish Muscle	Penobscot Bay	Penobscot Bay	44 15.0	68 50.0	24Apr87	Longhorn sculpin	BS2F186
PNB	Sediment	Penobscot Bay	Penobscot Bay	44 15.0	68 50.0	27Apr87		BS2S187
PAB	Fish Liver	San Pablo Bay	San Pablo Bay	38 03.0	122 17.0	17Apr87	Starry flounder	BS2L189
PAB	Fish Muscle	San Pablo Bay	San Pablo Bay	38 03.0	122 17.0	17Apr87	Starry flounder	BS2F190
PAB	Sediment	San Pablo Bay	San Pablo Bay	38 03.0	122 17.0	19Apr87		BS2S191
HUN	Fish Liver	Hunters Point	San Francisco Bay	37 42.0	122 22.0	26Jun87	White croaker	BS2L192
HUN	Fish Muscle	Hunters Point	San Francisco Bay	37 42.0	122 22.0	26Jun87	White croaker	BS2F193
HUN	Sediment	Hunters Point	San Francisco Bay	37 42.0	122 22.0	29Jun87		BS2S194
UCB	Sediment	Up. Chesapeake Bay	Chesapeake Bay	38 55.7	76 25.0	13Jul87		BS2S195
SDF	Fish Liver	San Diego Harbor	San Diego Bay	32 43.0	117 11.0	22Jul87	Black Croaker	BS2L196
SDF	Fish Muscle	San Diego Harbor	San Diego Bay	32 43.0	117 11.0	22Jul87	Black Croaker	BS2F197
SDF	Sediment	San Diego Harbor	San Diego Bay	32 43.0	117 11.0	22Jul87		BS2S198
PEN	Fish Liver	Pensacola Bay	Pensacola Bay	30 24.1	87 12.4	10Jul87	Atlantic croaker	BS2L199
PEN	Fish Muscle	Pensacola Bay	Pensacola Bay	30 24.1	87 12.4	10Jul87	Atlantic croaker	BS2F200
PEN	Sediment	Pensacola Bay	Pensacola Bay	30 24.1	87 12.4	10Jul87		BS2S201
CHS	Fish Liver	Charleston Harbor	Charleston Harbor	32 46.0	79 55.0	16Oct87	Atlantic croaker	BS2L202
CHS	Fish Muscle	Charleston Harbor	Charleston Harbor	32 46.0	79 55.0	16Oct87	Atlantic croaker	BS2F203
CHS	Sediment	Charleston Harbor	Charleston Harbor	32 46.0	79 55.0	16Oct87		BS2S204

Stage I. Sample Collection and Preparation will occur on board the research vessel under field laboratory conditions for the measuring, sacrificing, and cleaning of the fish, and for the sediment sampling.

Stage II. Fish Dissection/Sediment Processing will be performed in a clean-air work station (laminar-air-flow condition), under laboratory conditions. This stage includes the dissection of fish and the extruding of sediment.

Stage III. Sample Packaging and Shipping will be relatively standard for all samples: packaging will be performed in a clean-air work station with precleaned Teflon bags or jars; samples will be shipped at liquid nitrogen temperatures to the National Bureau of Standards.

3.1 Fish Tissue Specimens

3.1.1 Sample Selection

The amount of each sample to be banked should be approximately 150 g. Since true duplicate samples (aliquots A and B) of each sample will be archived, approximately 300 g (2 x 150 g) of each tissue should be collected at each site. An estimate should be made of the number of individual fish required to obtain the necessary quantity of a sample for the Specimen Bank. This estimate should be based on the number and size of fish from a collection site. The number of individual fish to be pooled will be determined by the size of the fish liver. Ideally, equal amounts of liver and muscle tissue should be collected from each fish. If the amount of obtainable liver is limited due to size and/or number of fish at a specific site, the ratio of liver to muscle can be adjusted to 2:3, to obtain a minimum 2 x 100 g liver sample and a full 2 x 150 g muscle sample. For example, the average liver weight of Atlantic croaker and spot should be in the range of 0.7 to 2.0 g, i.e., 200 to 100 fish, respectively, are required; muscle tissue samples should be approximately 1.4-4 g each. The liver of a white croaker weighs 2-3 g, i.e., a minimum of 100 fish are required; the muscle tissue samples should be approximately 3 g each. The overall schematic of the fish sampling and necropsy protocol is illustrated in Figure 3.

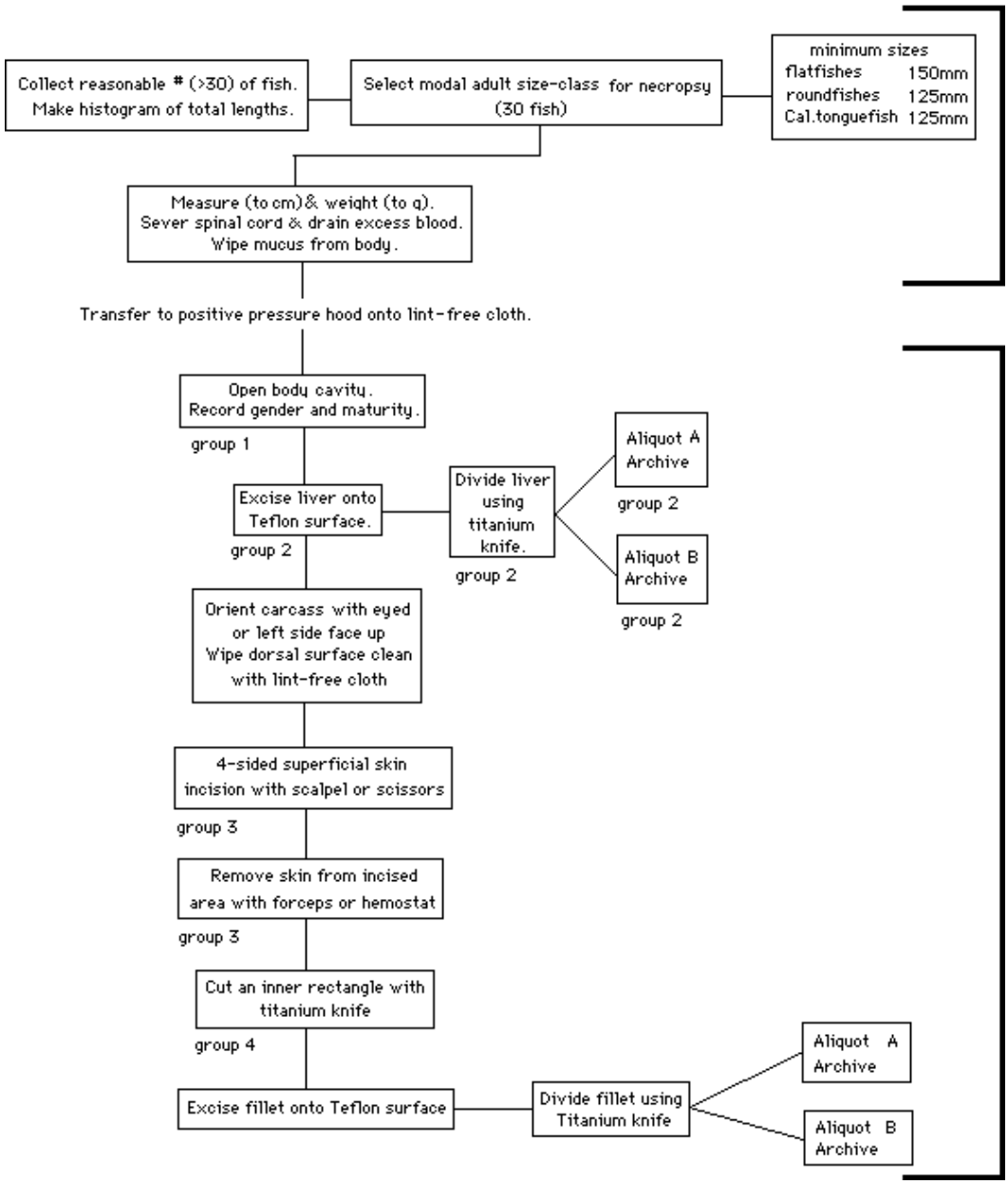
3.1.2 Cleaning of Sampling Instruments

After sharpening the titanium knives and before dissection, the tools are wiped clean, scrubbed in detergent solution, rinsed extensively with tap water, rinsed in distilled/high-purity (HP water) (i.e., milli-Q or HPLC-grade water), transferred to the fume-exhaust hood where they are carefully rinsed with methylene chloride (CH_2Cl_2), and carried on a similarly cleaned cutting board covered with a Teflon sheet to the work station. At the station the knives are transferred to

Figure 3

Necropsy Protocol

Benthic Surveillance Project



clean, lint-free cotton cloths at the rear of the work area. Following specified steps in the fish-dissection process, and between specimens of a dissection group, the tools should be rinsed with HP water. While rinsing, with gloved hands, run fingers over the blade and handle of the knife to help remove any adhering blood or tissue. This process should be performed before any fluid or tissue has a chance to dry on the knife. In the laboratory the knife should be rinsed with HP water again, as described above, and rinsed with ethanol or another suitable solvent. The knife is then placed on a clean surface (do not touch the blade) and allowed to air-dry, preferably in a laminar-flow hood. The knife should then be placed in a Teflon bag for storage and transportation to the next sampling site. The sediment corers should be treated in a similar manner, taking care to clean off any sediment adhering to the walls. After the implements are cleaned, they should not be touched with ungloved hands.

3.1.3 STAGE I. Sample Preparation (Fish Liver and Muscle Tissue)

1. Measure and record the specimen's total length, plot the lengths on the sample record form provided to obtain a histogram, and record the information on the Sampling Data form for the NBSB.

2. Using a filleting knife or a scalpel (not a titanium knife) sever the spinal cord. Wipe the body with a paper cloth to remove as much mucus as is practical.

3. With a clean pair of dust-free, nontalced vinyl gloves, transfer the fish specimen onto a lint-free cotton cloth in the clean-air work station (clean laminar-flow conditions).

3.1.4 STAGE II. Fish Dissection

Four sets of dissection instruments are used in the sample preparation process: Group 1. Scissors, scalpel, and forceps (used to enter sampling field). Group 2. Sharp-pointed scalpel, scissors, forceps or hemostat, and titanium knife (used to excise sample). Group 3. Scalpel or scissors, forceps or hemostat (used to enter sampling field). Group 4. Forceps and titanium knife (used to excise sample).

1. Place the Teflon bags into beakers and then fold the open ends back onto the rim of the beakers.

2. Using the scissors or scalpel and forceps from Group 1, open the body cavity and record the gender. Wipe the instruments with a lint-free cotton cloth, rinse them with HP water, shake dry, and return them to their Group 1 position within the work station.

3. Using the surgical scissors from Group 2, free the liver from the surrounding tissue. Avoid cutting into the liver tissue. Remove the gall bladder. Using the titanium knife, place the liver on a

clean Teflon sheet and divide the liver into two equal parts for A and B samples. Wipe the implements with a lint-free cloth and rinse with HP water after each dissection.

4. Samples A and B of each tissue should be placed in the separate, precleaned Teflon bags supplied by NBS. Care should be taken to avoid getting fluid on the Teflon surface where the bag is to be sealed because it is difficult to get a good seal on wet Teflon surfaces. (Teflon bags and sheets are packaged under clean-room conditions in larger Teflon bags and should be opened only in the clean-air work station; the remaining bags should be resealed in the original Teflon bag.)

5. If a partial tissue sample (i.e., less than the required 150 g) must be stored for a short interval before obtaining the total sample, the Teflon bag should be placed in a covered glass jar and cooled on ice, or for longer periods, refrigerated. After collection of the complete pooled sample, proceed with packaging and shipment procedure (Stage III).

6. Begin the dissection of the muscle tissue sample with the stainless-steel scalpel or scissors from the third group of instruments. Place the specimen with the eyed or left side facing up. A series of four cuts is made into the dorsal section to expose a rectangular subsection of muscle (Figure 4a). The first cut should be 100 to 150 mm long and extend from behind the head towards the tail, just above the lateral line. This cut is parallel and about 5 to 10 mm dorsal to the lateral line. The next cut is above and parallel to the first, just below the fin ridge. Then two perpendicular cuts are made at the ends of the parallel cuts to complete a rectangular incision. The scalpel is wiped and rinsed with HP water between cuts to remove scales and as much mucus as possible.

7. Use the scalpel from Group 3 to lift the edge of the skin along the cut line at the posterior end of the rectangular cut. Then hold the fish tail with one hand, and use the forceps or hemostat in the other hand to tightly grasp the free edge of the skin. Pull the skin back from the rectangular cut to expose the muscle tissue mass (Figure 4b). (Note: A layer of adipose tissue lies along the dorsal fin ridge. This tissue is not to be taken with the muscle tissue subsample.)

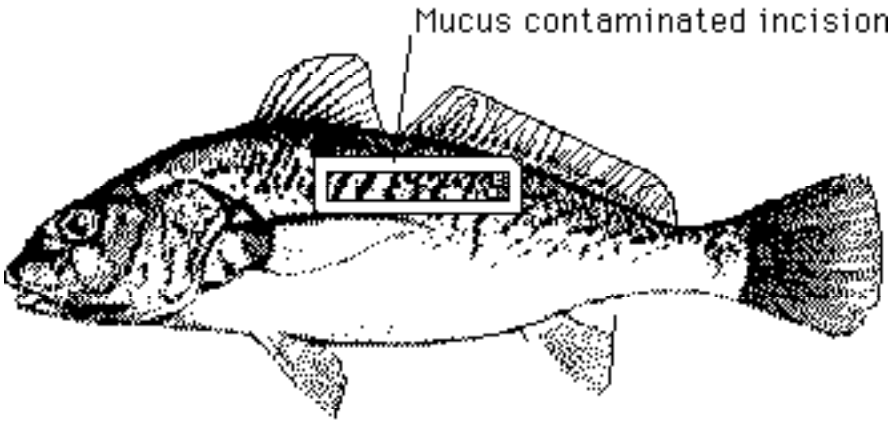
8. Use the titanium knife from Group 4 to obtain a "core" of the muscle tissue mass within the rectangular cut (Figure 4c). Extreme care must be taken to assure that neither the contaminated rectangular cut line (including the area where the skin was lifted) nor the fish exterior is contacted either by the titanium knife or by the cored muscle sample. The titanium knife is then used to transfer this uncontaminated muscle tissue core to a clean Teflon sheet where the muscle tissue is divided with a titanium knife into A and B duplicates.

9. Proceed with the collection of the liver and the muscle tissues until sufficient tissue has been obtained, as described above.

10. Record time of sample preparation on the Sampling Data form. Refer to Stage III for packaging and shipping.

Figure 4a

Three-step Muscle Dissection Sequence

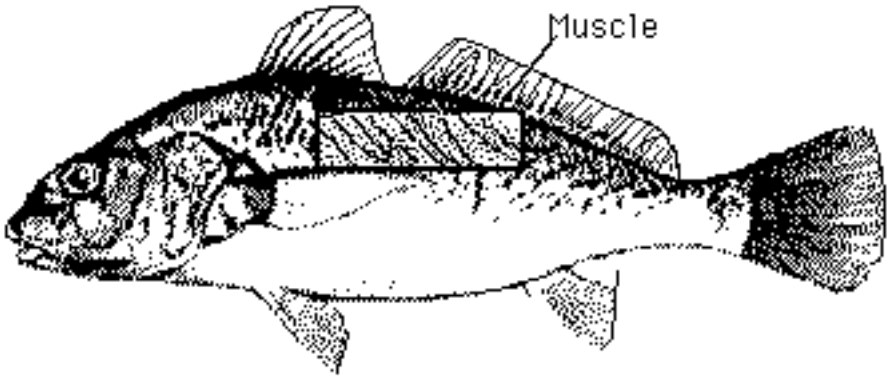


ATLANTIC CROAKER *(Micropogon undulatus)*

Step 1. Muscular incision using stainless steel knife or scissors.

Figure 4b

Three-step Muscle Dissection Sequence

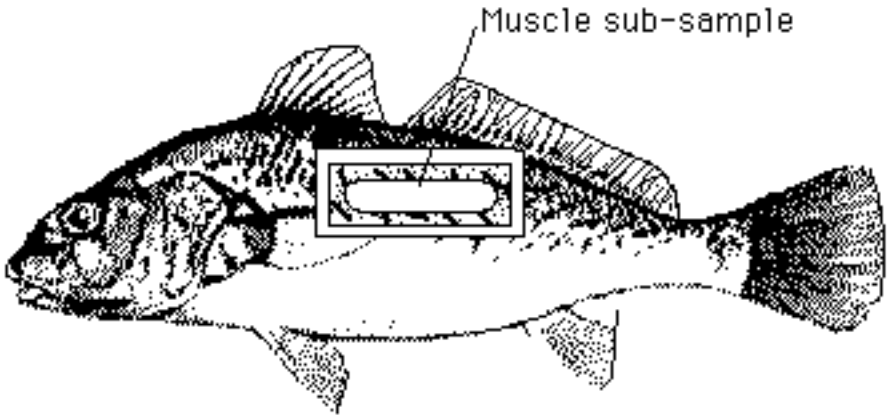


ATLANTIC CROAKER *(Micropogon undulatus)*

Step 2. Epidermis torn away exposing underlying muscle

Figure 4c

Three-step Muscle Dissection Sequence



ATLANTIC CROAKER *(Micropogon undulatus)*

Step 3. Muscular sub-sample taken with titanium knife

3.2 Sediment Specimens

3.2.1 Sediment Collection

A sediment sample is required from selected sites for Specimen Banking. The sample will consist of pooled sediment cores from one grab at each of the three stations at the site. Three box core samples will be taken from each station. From one of the box corers, two equivalent cylindrical core samples of approximately 50 mL will be taken; one will be designated as portion A and the other as portion B. Initially, three cylindrical core samples are taken from which two suitable ones can be selected to form the A and B portions of the respective box core. The A portions from each station will be pooled to provide sample A (150 mL) for the site; the B portions from each station will be pooled to provide sample B. The cylindrical sample cores are taken with the [120 mm x 42 mm (internal diameter)] Teflon tubes supplied by NBS. A core length of 30 mm equals approximately 50 mL.

3.2.2 Stage I. Sediment Collection

1. Insert the Teflon cylinders into the box core (with the water layer remaining in the box core), close the top with the screw cap, and withdraw the cylinder from the box core. The bottom end is also capped for intermediate storage, or the foam plug (see directions below) is inserted for immediate extrusion.

2. The Teflon cylinders containing the sediment cores are transported to the clean work station (laminar flow hood) for processing.

3.2.3 Stage II. Sediment Processing

1. The sediment corers shall be stored on ice or in the freezer until all six samples have been collected.

2. At the clean work station, the A and B portions of the sediment core are extruded into the precleaned Teflon jars (180 mL).

3. The samples are extruded from the tubes by inserting foam plugs into the bottom of the tubes. When a seal is achieved, the top cap is unscrewed. The water on top is removed either with a pipette (Pasteur) or by holding the corer containing the sediment over a beaker and allowing the contained water to overflow into the beaker as the sediment is pushed upwards. The sediment is pushed upwards until its surface reaches the rim of the tube. Use one of the 30 mm long labels to mark the length of the path over which the plug must be pushed upwards. Push the plug to the marked endpoint. A 30 mm sediment core will be above the rim of the tube. Use a titanium knife to cut off this portion and place it into the appropriate Teflon jar.

4. If the sampling procedure described above for the collection of sediment cores from the box cores is not possible, the appropriate portions should be obtained by scooping the sediment. The Teflon tubes should be used to define the approximate sample size.

5. If the sediment core is too fluid to permit an accurate extrusion and collection of the sediment plug, the core tubes may be placed in a freezer until just frozen; sediment tubes are then removed from the freezer and the frozen sediment is easily extruded.

6. The pooled sediment portions are weighed, subtracting 145 g to tare the weight of the jar or use a jar to tare the balance. The weights are recorded on the sampling data form and the sample labels. The labels are completed with the site ID, sample type, date of collection, and cruise number; then the labels are affixed to the jars.

7. The samples are then ready to be frozen for shipment.

8. Proceed with the packing and shipping procedures beginning with 3.2.4 Stage III Step 6.

3.2.4 Stage III. Sample Packaging and Shipment

1. After collection of the complete composite fish tissue samples, the Teflon bags containing the tissues are heat-sealed. Workable settings on the heat-sealer are marked. However, if sealing problems still occur, vary the current/temperature and duration settings.

2. It is important to seal the bag with as little air remaining inside as possible. Air should be squeezed out. Alternatively, seal the bag with as little air remaining as possible; then squeeze the remaining bubble of air to a corner, puncture this corner with the titanium knife, squeeze out the remaining air, and reseal the corner. The seal can be tested by gently squeezing the bag to see if it holds pressure. A second seal is then made slightly away from the first and parallel to it to provide a double seal.

3. The pooled samples are weighed using an empty bag to tare the weight of the Teflon bag. The weights are recorded on the sampling data form and the sample labels. The labels are affixed to the bag.

4. Each labeled bag is placed into a second bag and a double seal is made, as described above in 2. (Note that the label is between the two bags.)

5. The double-bagged tissue samples are placed in the cylindrical cardboard containers provided. The cardboard containers are labeled with sample type, weight in grams, location, date of collection,

and cruise number. The cardboard cylinders are then immersed in liquid nitrogen LN_2 in the provided plastic dewar for 10 minutes to rapidly freeze them.

6. The jars containing the sediment samples are immersed in LN_2 in the provided plastic dewar for 10 minutes for rapid freezing.

7. The LN_2 shipper should be filled with liquid nitrogen for at least 6 hours to prepare it for shipping. This time is necessary to fully saturate the absorbent inside the shipper. Before placing the frozen samples in the shipper, the excess LN_2 must be poured off. (Note: LN_2 should not be stored in sealed containers. Personnel handling LN_2 are cautioned to wear boots, cuffless trousers, nonabsorbent aprons, insulating gloves, and face shields.)

8. The LN_2 shipper should not be used for freezing the samples, but only for shipping. If no separate supply of LN_2 is available, it may be convenient to fill the shippers completely with LN_2 before departure on sampling trips. Excess LN_2 may be used to quick-freeze the samples in the supplied plastic dewar.

9. The frozen tissue and sediment samples are transferred to the LN_2 shipper for storage. The shippers will hold at least 10 sample boxes. Once the shippers are full, they are shipped to the National Bureau of Standards; the samples are not to be stored in intermediate freezers.

10. Make sure all entries on the sampling data forms are complete. Any deviations or modifications of this protocol must be noted on the sampling data form. In addition to the forms provided, include a copy of the applicable site log containing station latitude and longitude, geographic site name, and associated National Status and Trends assigned site code. Place a copy of the completed forms in the shipper, and retain another copy for your project records.

11. The frozen specimens and their corresponding Sampling Data sheets should be shipped by an overnight express carrier. Maximum holding time for the shippers is 10 days. Send the shippers C.O.D. to National Bureau of Standards using the shipping labels provided; i.e., the Federal Express form has a box to check if the recipient is being billed. No account number should be supplied.

12. Notify the NBS Specimen Bank personnel by telephone as soon as possible after the specimens are shipped: Barbara Koster (301) 975-6291, or if she is unavailable leave a message on the answering machine. However, if for some reason you need to talk to someone, telephone: Rolf Zeisler (301) 975-6290, Steve Wise (301) 975-3112, or Michele Schantz (301) 975-3106.

4. MUSSEL WATCH SPECIMEN BANKING PROTOCOLS

The Mussel Watch Project resembles the Benthic Surveillance Project in that both biota and sediments are sampled at predesignated sites, and each site consists of three stations from which sample sediments are composited to provide an integrated site specimen. Unlike the sampling procedures used in the Benthic Surveillance Project, bivalve mollusc tissues are not removed in the field for the Mussel Watch specimens.

In general, the collection protocol for the NOAA NS&T specimens consists of three stages: bivalve/sediment collection and bivalve sorting, bivalve/sediment processing, and sample packaging and shipping. The division of the protocol into stages is used as an aid in organizing and simplifying the collection procedures. All information regarding the sample preparation is recorded on the Sampling Data form (NOAA NS&T Mussel Watch Project) (see Appendix B).

Stage I. Bivalve/Sediment collection and Bivalve sorting -- will occur aboard the vessel under conditions of limited control; samples are counted, sorted, measured, and cleaned.

Stage II. Bivalve/Sediment processing -- will occur upon return to shore, in a controlled environment using precleaned Teflon bags or jars; samples are properly labeled and heat-sealed.

Stage III. Sample packaging and shipping -- performed in a controlled environment, shipped on dry ice in shipping containers provided.

4.1 Bivalve Specimens

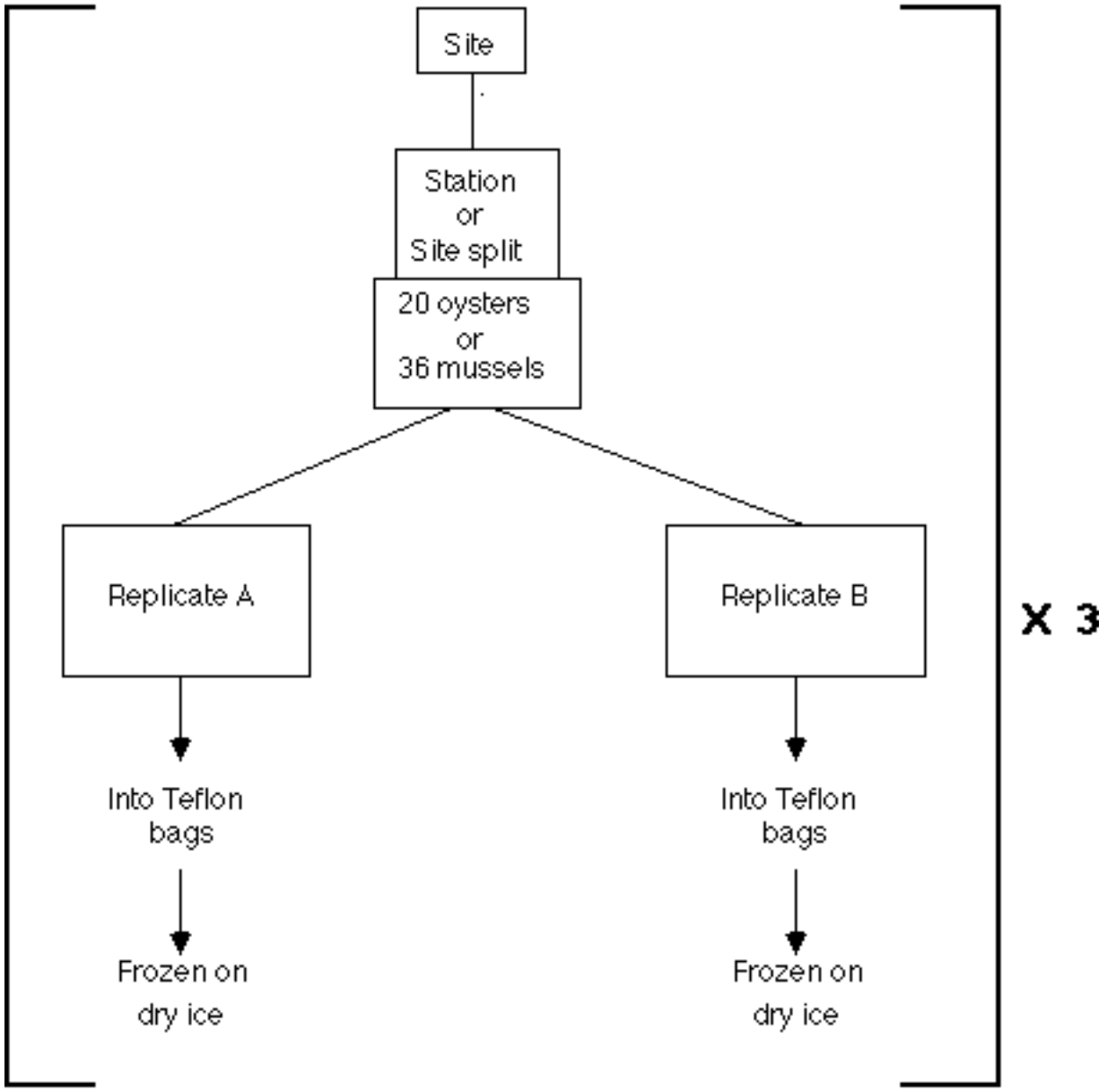
4.1.1 Sample Selection

A composite bivalve sample is obtained from each of the 30 sites nationwide (10 sites per coast) specified for specimen banking. Bivalve and sediment samples are taken from the three stations at the same site.

Each station provides two batches of approximately 16-18 mussels (*Mytilus edulis/M. californianus*) or two batches of 10 oysters (*Crassostrea virginica*), one batch designated as A and the other as B. Since there are three stations per site, the total combined sample for each site is 50 mussels or 30 oysters for each of the A and B duplicates (total of 100 mussels and 60 oysters, see Figure 5).

Figure 5

Bivalve Speciman Bank Sampling Schematic
samples are taken at 10 sites/coast



Pooled "A" replicates from each site = 30 oysters or 50 mussels

Pooled "B" replicates from each site = 30 oysters or 50 mussels

4.1.2 Stage I. Sample Collection and Sorting (Bivalves)

1. Oysters or mussels are collected by: dredge in water depth of 2-3 m or more, tongs in water depth of 2-2.5 m, sampling fork in water depth less than 1 m, or by hand along shoreline.

2. After the collection is complete, the bivalves are separated, if they are clumped, and sorted to ensure acceptable samples have been collected. Mussels less than 5 cm and greater than 8 cm long are discarded. Any bivalve that does not have a tightly closed shell is discarded. Oysters retained are in the size range of 7-10 cm. (Mortality is high for oysters larger than this.) Oysters are examined in the field to ensure that more than an empty shell is being taken.

3. After examination, bivalves are placed on a sorting tray which has holes in the bottom for drainage. The samples are rinsed with seawater and scrubbed with a noncontaminating brush (nylon or natural fiber brush). Then the samples are rinsed a second time with seawater.

4. With clean, nontalced vinyl gloves, fold the open end of a 14 x18 inch precleaned Teflon bag over and keep the bag as free of contamination as possible. When the samples are placed in the Teflon bags, care should be taken to avoid getting fluid on the Teflon surface where the bag is to be sealed because it is difficult to get a good seal on wet Teflon surfaces. If moisture gets on the rim of the Teflon bag use a clean, lint-free cotton cloth to remove it, or put the partially sealed bag into another bag. (Teflon bags and sheets are packaged under clean-room conditions in larger Teflon bags and should be carefully opened and kept as clean as possible; the remaining bags should be resealed in the original Teflon bag.)

5. Place 15-18 mussels or 10 whole oysters per station for each duplicate sample into the Teflon bag. The total A sample for each site will consist of 50 mussels or 30 oysters pooled from the three stations of a site, the resulting full sample for the A and B duplicates is 100 mussels or 60 oysters.

6. Affix the label provided by NBS to the Teflon bag, designating the sample as duplicate A or B, and ensure that all the information (site ID, site number, date, etc.) has been filled in (immediately transfer samples to an ice chest containing dry ice).

7. Complete the NBSB Mussel Watch Project sampling data form. Any deviation or modification of this protocol must be noted on the sampling data form.

4.1.3 Stage II. Bivalve Processing

1. The Teflon bag containing the bivalve samples is heat-sealed using the portable heat-sealer provided by NBS. Workable settings on the heat-sealer are marked. However, if sealing problems still occur, vary the current/temperature and duration settings.

2. It is important to seal the bag with as little air remaining inside as possible. Air should be squeezed out. The seal can be tested by gently squeezing the bag to see if it holds pressure. A second seal is then made slightly away from and parallel to the first to provide a double seal.

3. Each labeled bag is placed into a second bag, and double seals are made, as described above. (Note: the sample label is between the two bags.)

4. The double-bagged samples are placed in an ice chest containing dry ice.

5. Refer to Stage III for packaging and shipping information.

4.2 Sediment Specimens

4.2.1 Sample Selection

Two composite sediment samples are required from each of the 30 nationwide sites (10 per coast) designated for specimen banking. Sediment will be collected at three stations from within the designated bivalve site. A Kynar-coated modified Van Veen-type grab sampler is used to collect the A and B duplicate samples of sediment (Figure 6).

4.2.2 Stage I. Sediment Collection

1. Using a Kynar-coated sample scoop, a 60 mL volume (approximately 2 scoops) of the top 1 cm of sediment is removed from the modified Van Veen-type grab.

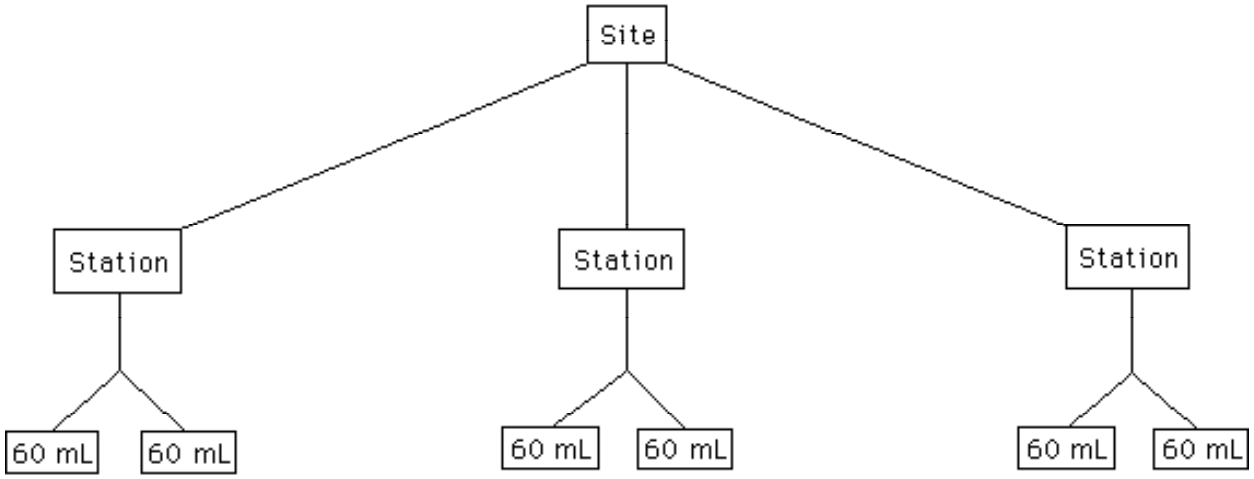
2. Immediately transfer the sample to a precleaned, 180 mL Teflon jar; close the jar between the three samplings.

3. From the same grab cast, collect a second duplicate 60 mL volume (2 scoops) of surface sediment and place the sample into a second Teflon jar. These two samples respectively constitute the A and B duplicates.

Figure 6

Sediment Specimen Bank Sampling Schematic

at 10 selected sites/coast



Pooled "A" Samples = 180 mL-> into Teflon bags ----> frozen on dry ice

Pooled "B" Samples = 180 mL-> into Teflon bags ----> frozen on dry ice

4. The combined sediment samples are stored cool (on ice) until returning to shore. (Eight hours maximum storage on ice.)

5. Care must be taken to wash the grab with seawater between each station and to clean the sampling scoop by: soap and water wash, water rinse, distilled water rinse, methanol rinse, and methylene chloride rinse.

4.2.3 Stage II. Sediment Processing

1. After the three stations have been combined to form the A and B duplicates for the site, the sediment duplicates are weighed subtracting 145 g to tare the weight of the jar. The weights are then recorded on the sampling data form and on the sample labels. Finally, the sample labels are completed with the site ID, sample type, and date of collection, and then affixed to the jars.

2. Complete the NBSB Mussel Watch Project Sampling Data form. Any deviations or modifications of this protocol must be noted on the sampling data form.

3. The samples are now ready to be frozen for shipment. Make sure the lid is tightly secured.

4. Proceed with the packing and shipping procedure beginning with Stage III.

4.2.4 Stage III. Sample Packaging and Shipment

1. Frozen or chilled samples will be repacked with fresh dry ice in insulated dry ice shipping containers and shipped by overnight express to National Bureau of Standards. Since dry ice has a limited capacity to keep samples cold over extended periods of time, samples packed in dry ice are not to be shipped on Friday or before holidays.

2. Notify the NBS Specimen Bank personnel by telephone as soon as possible after the specimens are shipped: Barb Koster (301) 975-6291. If she is unavailable leave a message on the answering machine. However, if for some reason you need to talk to someone, telephone: Rolf Zeisler (301) 975-6290, Steve Wise (301) 975-3112, or Michele Schantz (301) 975-3106.

5. SAMPLE HOMOGENIZATION AND ANALYSES

The environmental samples collected as part of the NS&T program are analyzed to determine aromatic hydrocarbons, chlorinated pesticides, polychlorinated biphenyls (PCBs), and trace elements (see Tables 3 and 4). As part of the specimen banking activity within the NS&T program, NBS has analyzed selected specimens (approximately 20% of the banked specimens) for the determination of these same contaminants. These analyses serve two purposes: (1) to provide

Table 3. Trace Organic Contaminants Determined by the NS&T Programs' Specimen Banking Project (Alternate names as listed in The Merck Index).

	Chemical Abstracts Registry Number	Alternate Name
Aromatic Hydrocarbons		
Acenaphthene	83-32-9	1,2-Dihydroacenaphthylene
Anthracene	120-12-7	Paranaphthalene
Benz[*a*]anthracene	56-55-3	1,2-Benzanthracene
Benzo[*a*]pyrene	50-32-8	3,4-Benzpyrene
Benzo[*e*]pyrene	192-97-2	1,2-Benzpyrene
Biphenyl	92-52-4	Diphenyl; phenylbenzene
Chrysene	218-01-9	1,2-Benzphenanthrene
Dibenzanthracene	53-70-3	Dibenz[*a,h*]anthracene
Fluoranthene	206-44-0	1,2-(1,8-naphthylene)benzene
Fluorene	86-73-7	*o*-Biphenylenemethane
Perylene	198-55-0	Dibenz[*de,kl*]anthracene
Phenanthrene	85-01-8	- - - - - - - - - - -
1-Methylphenanthrene	832-69-9	- - - - - - - - - - -
Pyrene	129-00-0	Benzo[*def*]phenanthrene
Chlorinated Compounds		
Aldrin (HHDN)	309-00-2	1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-endo-exo-1,4:5,8-dimethanonaphthalene
alpha-Chlordane (cis-chlordane)	5103-71-9	1,2,4,5,6,7,8,8-Octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene
2,4'-DDD (*o,p'* -TDE)	53-19-0	1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene
4,4'-DDD (*p,p'* -TDE)	72-54-8	1,1-Dichloro-2,2-bis-(p-chlorophenyl)ethane
2,4'-DDE	3424-82-6	1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethynyl]benzene

Table 3 (cont)

	Chemical Abstracts Registry Number	Alternate Name
4,4'-DDE	72-55-9	1,1'-(dichloroethenylidene)-bis(4-chlorobenzene)
2,4'-DDT	789-02-6	1-chloro-2-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene
4,4'-DDT	50-29-3	1,1'-(2,2,2-trichloroethylidene)-bis[4-chlorobenzene]
Dieldrin (HEOD)	60-57-1	1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro endo-exo-1,4:5,8-dimethanonaphthalene
Endrin	72-20-8	1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octohydro-endo,endo-1,4:5,8-dimethanonaphthalene
Heptachlor	76-44-8	1,4,5,6,7,8,8-Heptachloro-3a,4,7,7a-tetrahydro-4,7-methano-1H-indene
Heptachlor epoxide	1024-57-3	1,4,5,6,7,8,8-Heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan
Hexachlorobenzene (HCB)	118-74-1	Perchlorobenzene
Lindane (γ-BHC)(γ-HCH)	58-89-9	1a,2∝,3ß,4∝,5∝,6ß,hexachloro-cyclohexane
Mirex	2385-85-5	1,1a,2,2,3,3a,4,5,5a,5b,6-Dodecachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*] pentalene
trans-Nonachlor	39765-80-5	1,2,3,4,5,6,7,8,8-nonachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1*H* -indene

Table 3 (cont)

	Chemical Abstracts Registry Number	Alternate Name
PCBs		
Dichlorobiphenyls	- - -	dichloro-1,1'-biphenyl
Trichlorobiphenyls	25323-68-6	- - -
Tetrachlorobiphenyls	26914-33-0	- - -
Pentachlorobiphenyls	25429-29-2	- - -
Hexachlorobiphenyls	26601-64-9	- - -
Heptachlorobiphenyls	28655-71-2	- - -
Octachlorobiphenyls	- - -	- - -
Nonachlorobiphenyls	- - -	- - -

Table 4

Major and Trace Elements Determined for Archived Samples

(in order of atomic number)

		Chemical Abstracts Registry Number
Al	Aluminum	7429-90-5
Si	Silicon	7440-21-3
Cr	Chromium	7440-27-3
Mn	Manganese	7439-96-5
Fe	Iron	7439-89-6
Ni	Nickel	7440-02-0
Cu	Copper	7440-50-8
Zn	Zinc	7440-66-6
As	Arsenic	7440-38-2
Se	Selenium	7782-49-2
Ag	Silver	7440-22-4
Cd	Cadmium	7440-43-9
Sn	Tin	7440-31-5
Sb	Antimony	7440-36-0
Hg	Mercury	7439-97-6
Pb	Lead	7439-92-1

accurate baseline data to evaluate the storage stability of various marine specimens in the specimen bank, and (2) to allow for comparison with data on similar samples collected at the same time from the same sites and analyzed by other NOAA laboratories and contractors using different analytical methods.

Prior to analysis the specimens are homogenized and subsampled. Bivalve and fish liver samples are homogenized frozen using a cryogenic homogenization procedure, whereas the sediments samples are thawed and homogenized by stirring. The cryogenic homogenization procedure was developed at NBS[3] and uses a Teflon disk mill. This homogenization procedure reduces the loss of volatile components and reduces the likelihood of changes in sample composition due to thawing and refreezing of the specimen. Experiments with mussel tissue indicate that after homogenization using this procedure virtually all the frozen material passes through a 0.46 mm sieve[3]. The tissue homogenate is aliquoted into ten 17 mL Teflon jars (approximately 6-8 g tissue homogenate/jar) and from one to five 90 mL jars (30-35 g tissue homogenate/jar) depending on the amount of homogenate available. After homogenization the sediment samples are subsampled in a similar manner for analyses and storage. Aliquots of the sample homogenates (tissue and sediment) that are not used for analyses are stored for future evaluation.

During the first two years of the NS&T Specimen Bank Program, 12 samples of fish liver and six samples of sediment from the Benthic Surveillance project, and six samples of bivalves from the Mussel Watch project were analyzed at NBS. In the third year of the program six sediment and six bivalve samples from the Mussel Watch project and six sediment samples from the Benthic Surveillance project will be analyzed.

Trace elements are determined in the sediment and bivalve samples using a combination of instrumental procedures involving neutron activation analysis and x-ray fluorescence analysis. After freeze-drying and pelletizing the samples, no additional sample preparation is necessary for analysis. For the bivalve samples the combination of the two instrumental techniques provided data for 44 elements[4] including all the elements in Table 4 with the exception of Si. For the sediment samples, several elements (Cd, Cu, Ni, Pb, and Sn) could not be determined by x-ray fluorescence analysis due to matrix interferences.

For the determination of the organic pollutants, the sample homogenate is mixed with sodium sulfate and extracted with methylene chloride. The compounds of interest are isolated from the extract using a two-step liquid chromatographic cleanup procedure[5]. The aromatic hydrocarbons, chlorinated pesticides, and PCBs are isolated into three separate fractions which are then analyzed by GC with either flame ionization (aromatic hydrocarbons) or electron capture detection (chlorinated pesticides and PCBs). For samples with low levels of aromatic hydrocarbons, liquid chromatography with fluorescence detection is used to determine selected aromatic hydrocarbons[5].

6 . CONCLUSION

Sample collection protocols developed and used for the collection of benthic fish, bivalve molluscs, and associated sediments have provided specimens, from over 77 sites nationwide (Tables 1 & 2), which have now been submitted for inclusion in the Specimen Bank housed at the National Bureau of Standards in Gaithersburg, Maryland. Specimens are preserved at liquid-nitrogen temperature with minimal degradation expected for decades. Initial analysis of archived samples provides data that is used for Quality Assurance purposes since the data can be compared to data generated by other laboratories involved in the NS&T projects. In addition, these primary data will allow a quantification of archived sample degradation during storage, if any. Analysis of banked specimens in the future will allow the derivation of baseline values for newly defined environmental contaminants, and the preservation of these samples for retrospective analysis will make historical marine samples available as new and improved analytical procedures are developed.

7. REFERENCES

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3. Zeisler, R., J.K. Langland, and S.H. Harrison. 1983. Cryogenic homogenization of biological tissues. *Analytical Chemistry.* pp. 2431-2434.

4. Stone, S.F., R. Zeisler, and R.W. Sanders. Submitted. Sequential Analysis of 44 Trace Elements in Marine Bivalves, *Analytical Chemistry.*

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APPENDIX A

NATIONAL BIOMONITORING SPECIMEN BANK
Sampling Data - NOAA/NS&T Program
Benthic Surveillance Project

Sample Source _____

Site ID _____ Lat. _____ Long. _____
Geographic Name

Sample Type ☐ Liver ☐ Muscle ☐ Sediment ☐☐

Time of Collection Liver/Muscle ☐☐☐ ☐ Sediment ☐☐☐ ☐
day mo yr hr day mo yr hr

Intermediate Storage (Temp/remarks) _____

_____

_____

Time of Preparation Liver/Muscle ☐☐☐ ☐ Sediment ☐☐☐ ☐
day mo yr hr day mo yr hr

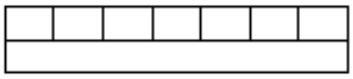
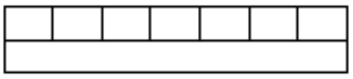
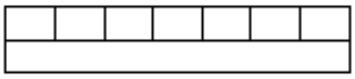
Intermediate Storage (Temp/remarks) _____

_____

_____

Time of LN2 freezing: ☐☐☐ ☐
day mo yr hr

Remarks:

Fish Species _____

Number & Sex in Sample A: _____ B: _____

_____ _____

Sediment Grab (log number) A: _____ B: _____

_____ _____

_____ _____

Sediment Sample Weight A: _____ g B: _____ g

Liver Sample Weight A: _____ g B: _____ g

Muscle Sample Weight A: _____ g B: _____ g

Prepared by: _____
Name (print)

_____
Signature

APPENDIX B

NATIONAL BIOMONITORING SPECIMEN BANK
Sampling Data - NOAA/NS&T Program
Mussel Watch Project

Sample Source _____

Site ID _____ Lat. _____ Long. _____

BIVALVE: Oyster ☐ Mussel ☐

Time of Collection: ☐☐☐ (day mo yr) ☐ (hr) Collected by _____

Intermediate Storage (Temp/remarks) _____

Time of Preparation ☐☐☐ (day mo yr) ☐ (hr) Collected by _____

Time of LN2 Freezing: ☐☐☐ (day mo yr) ☐ (hr) Processor _____

SEDIMENT

Time of Collection: ☐☐☐ (day mo yr) ☐ (hr) Collected by _____

Intermediate Storage (Temp/remarks) _____

Time of Preparation: ☐☐☐ (day mo yr) ☐ (hr) Collection _____

Time of LN2 Freezing: ☐☐☐ (day mo yr) ☐ (hr) Processor _____

Protocol: Standard Modified (Please note modification below)

Remarks:

Site ID _____

Number of Bivalves in sample **A** _____ **B** _____

BIVALVE COLLECTION

Station sample numbers _____

_____

_____

_____ INTERTIDAL _____ SUBTIDAL _____ SAND _____ MUD

_____ SHELL _____ HAND _____ DREDGE _____ FORK

_____ GRAB _____ OTHER

Sediment weight sample (g) **A** _____ **B** _____

SEDIMENT COLLECTION

Station sample numbers _____

_____

_____

Prepared by: _____
Name (print)

_____
Signature