NISTIR 7708

MARINE ENVIRONMENTAL SPECIMEN BANK: Specimen Banking Protocols for the National Status and Trends Mussel Watch Program

Amanda J. Moors[1]
Rebecca S. Pugh[1]
Lauren B. Rust[1]
Barbara J. Porter[2]
Kimani L. Kimbrough[3]
Gunnar G. Lauenstein[3]
Paul R. Becker[1]
Stephen A. Wise[2]

[1] National Institute of Standards and Technology
Chemical Science and Technology Laboratory
Hollings Marine Laboratory
Charleston, South Carolina 29412

[2] National Institute of Standards and Technology
Chemical Science and Technology Laboratory
Gaithersburg. Maryland 20899

[3]National Oceanic and Atmospheric Administration
National Ocean Service
National Centers for Coastal Ocean Science
Center for Coastal Monitoring and Assessment
1305 East West Highway
Silver spring, Maryland 20910

August 2009

National Institute of Standards and Technology
Technology Administration, U.S. Department of Commerce

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

The authors would like to acknowledge the following individuals and organizations for their support of specimen banking for the National Status and Trends (NS&T) Mussel Watch Project: NOAA NS&T Mussel Watch Team, field collectors from the 1989 EXXON Valdez Oil Spill; field collectors from the 1976 and 1978 Environmental Protection Agency (EPA) Mussel Watch Program; TDI-Brooks International; Bob Hillman and Carol Pevin (New England Marine Laboratory, Duxbury, MA); James Campbell (Marine Research Specialists, Ventura, CA); Eric Crecelius (Battelle Northwest Marine Research Laboratory, Sequim Bay, WA); Roger Fay, Dan Wilkinson, Dennis Weisenburg, and Jim Brooks (Geochemical Environmental Research Group, College Station, TX); Andy Lessner (Science Applications International Co., LaJolla, CA); NOAA Northwest Fisheries Center, Seattle, WA; NOAA Southeast Fisheries Center, Beaufort, NC; and NOAA Northeast Fisheries Center, Highlands, NJ.

DISCLAIMER

Certain commercial equipment or instruments are identified in this paper to specify adequately the experimental procedures. Such identification does not imply recommendations or endorsement by the National Institute of Standards and Technology nor does it imply that the equipment or instruments are the best available for the purpose.

SPECIMEN BANKING FOR THE NATIONAL STATUS AND TRENDS PROGRAM

INTRODUCTION

The National Status and Trends (NS&T) Program, was initiated in 1984 by the National Oceanic and Atmospheric Administration (NOAA) to address concerns about the condition of the Nation's coastal and estuarine ecosystems[1,2]. This Program consists of the National Benthic Surveillance (established in 1984) and Mussel Watch, one of the longest (inaugural year, 1986) nation-wide continuous contaminant monitoring programs in U.S. coastal waters[3]. The goal of the NS&T Program is to quantify current status and long-term trends in concentrations of selected contaminants in biological indicator organisms and environmental matrices of coastal and estuarine areas in the U.S.[2] In the initial years of the Program, benthic fish, shellfish, sediment, and bivalve tissue were collected on a regular basis for real-time chemical analysis from sites along the U.S. In addition to real-time analysis and monitoring of these materials, the Program collects samples of mussels and oysters for long-term banking and future retrospective analyses. Due to a decrease in funding, specimen banking for the Program ended in 1992; however specimen banking for the Mussel Watch portion resumed in 2005[4]. Each collection year, approximately 10% of the samples that are collected from Mussel Watch sites are archived at one of two specimen bank facilities operated by the National Institute of Standards and Technology (NIST)[5]: the National Biomonitoring Specimen Bank (NBSB), established in 1987, located on the main NIST campus in Gaithersburg, Maryland, and the Marine Environmental Specimen Bank (Marine ESB), established in 1998, located at the Hollings Marine Laboratory in Charleston, South Carolina. This report describes the program history, and the detailed collection, processing, and long-term storage protocols practiced by NIST's Environmental Specimen Banking System for Mussel Watch.

PROGRAM BACKGROUND

In 1985, NOAA's NS&T Program added a specimen banking component and selected NIST's NBSB as the banking facility for the archived samples[6,7]. This collaboration between NIST and the NS&T Program resulted in the development of rigorous state-of-the-art protocols designed to collect high quality samples in the marine environment with minimal introduction of extraneous contaminants due to collection and handling[8]. The specimen banking component of the program began in 1985 and represents a major effort in the U.S. to expand environmental specimen banking into the area of marine and coastal environmental research and monitoring. Presently, NIST maintains specimens of mussels, oysters, and sediments collected by the NS&T Program from over 280 coastal Mussel Watch sites, and sediment and fish tissues (liver and muscle) collected by the Program from approximately 80 Benthic Surveillance sites located throughout the U.S. (Fig. 1). In addition, mussels, fish tissues, and sediments collected by NOAA in Prince William Sound, Alaska, for environmental damage assessment following the 1989 EXXON VALDEZ oil spill, and samples of mussels collected from 82 sites between 1976 and 1978 as part of the EPA Mussel Watch Program have also been incorporated into the NS&T Program[9]. The National Benthic Surveillance collected benthic fish tissues and sediments at 50 coastal and

estuarine sites in the U.S. from 1984 to 1993. This part of the NS&T Program was a cooperative effort between NOAA's National Ocean Service (NOS) and NOAA's National Marine Fisheries Service (NMFS), and coordinated by the Coastal Monitoring and Bioeffects Assessment Division (CMBAD) of NOS's Office of Ocean Resources Conservation and Assessment (ORCA). The annual field collections and laboratory analysis were performed by NOAA/NMFS. A portion of the samples collected were also stored at the NIST's NBSB for long-term archival. Additional details and information regarding sampling protocols, analytical methods and results for this project have been described elsewhere[1].

The NS&T Mussel Watch was established in response to a legislative mandate under Section 202 of Title II of the Marine Protection, Research, and Sanctuaries Act (MPRSA) (33 USC1442), which tasked the Secretary of Commerce to "initiate a continuous monitoring of contaminate levels in biota, sediment and the water column.[3]" The specific goal for Mussel Watch is to support eco-based management through an integrated nationwide program of environmental monitoring, assessment and research to describe the status and trends of the nation's estuaries and coasts[3]. Mussels and oysters are collected at designated coastal sites throughout the U.S and are analyzed on an annual basis for approximately 140 contaminants. Additional information on the history of the NS&T Mussel Watch, including a summary of contaminant levels over the last 20 years has been described in detail elsewhere[3].

In 1992, however, the specimen banking component of the NS&T Program ended due to a decrease in overall funding. A subsequent outside review of the Program recognized the inherent value of specimen banking for future research and monitoring and, based on recommendations by the reviewers, the banking of mussels and oysters for the Mussel Watch part of the NS&T Program was resumed in 2005[9]. Presently, specimens collected from 1986-1992 are banked at the NIST NBSB and collections from 2005 to present are banked at the Marine ESB. The protocols for collecting, processing, and banking samples presented in this report refer only to the specimen banking component of the NS&T Mussel Watch Program.

SPECIMEN BANKING FOR MUSSEL WATCH PROGRAM

COLLECTION LOCATIONS

NS&T Mussel Watch sites were selected to be representative of large coastal areas, to avoid small-scale patches of contamination, and to create a nationwide contaminant assessment[3]. The following criteria were used to select Mussel Watch collection locations:

1. The collection location is large enough for repeated sampling; abundance is important;

2. The sampling location is a natural location; collections from caged mussels are not permitted; and

3. Industrial effluents and municipal sewage outfalls are avoided, if possible[3].

Sampling sites are distributed along the U.S. coast within estuaries and embayments including the Great Lakes, Puerto Rico, and Hawaii[3] (Fig. 1). In addition, NS&T Mussel Watch collection

sites were selected to coincide with other mussel and oyster collection programs such as the U. S. EPA's Mussel Watch Sites sampled from 1976 to 1978 and the California Mussel Watch Program[3]. Many Mussel Watch sites were also selected near urban populations with the assumption that chemical contamination is higher, more likely to cause biological effects, and more spatially variable than in rural areas[10]. Using Global Positioning Systems (GPS), field collectors are able to precisely record collection location enabling NS&T collections to be repeated in subsequent years within a few meters[11]. A NS&T Mussel Watch site is assigned two names; a location name and a site name. The location name is a general geographic area name (e.g. Galveston Bay), and the site name is the specific area within the general geographic area (e.g. Hanna Reef)[12].

Map courtesy of K. Kimbrough, NOAA

Figure 1. NS&T Collection Locations

SPECIES COLLECTED

Since there is not a common species of mussel or oyster common to all coasts, a variety of species was collected from Mussel Watch sites in order to gain a national perspective. A target species is identified for each site based on abundance and ease of collection. Mussels (*Mytilus* spp.) are collected from the North Atlantic and Pacific coasts; oysters are collected from Delaware Bay southward and along the Gulf Coast (*Crassostrea virginica*), Hawaii (*Ostrea sandvicensis*), and Puerto Rico (*C. rhizophorae*); smooth-edge jewelboxes (*Chama sinuosa*) are collected from the one site in the Florida Keys; *Dreissens polymorpha* and *D. bugensis* (*Dreissena* spp.) are invasive species collected from the Great Lakes (Table 1; Fig. 1)[3,10]. Oysters range in size from 7 to 10 cm, *Mytilus* species from 5 to 8 cm, and Zebra mussels from 2 to 4 cm.

Table 1. Regional Collections of Mussels and Oysters

Region	Number of Sites	Target Species
Northeast, Southwest, Northwest, and Alaska	108	*Mytilus edulis*, *M. californians*, *M. galloprovinvialis*, and *M. trossulus*
Southwest and Gulf of Mexico	105	*Crassostrea virginica*, *Ostrea sandvicensis*, *C. rhizophorae*, *and Chama sinuosa**
Great Lakes	23	*Dreissena polymorpha* and *D. bugensis*

* smooth-edge jewelbox collected from one site in the Florida Keys

(Note: Table reproduced from NOAA Technical Memo 74[3].)

FIELD COLLECTION AND PROCESSING PROTOCOL

The following collection protocol is taken from NOAA Technical Memorandum NOA OMA 37; National Status and Trends Program for Marine Environmental Quality Specimen Bank Project: Field Manual[7].

The collection protocol for the NOAA NS&T Mussel Watch specimens consists of three stages[7]:

Stage 1: Bivalve Collection and Sorting - occurs aboard the vessel under conditions of limited control; samples are counted, sorted, measured, and cleaned (Figs. 2 and 3).

Stage 2: Bivalve Field Processing - occurs upon return to shore, in a controlled environment using pre-cleaned Teflon bags or jars; samples are properly labeled and heat-sealed.

Stage 3: Sample Packaging and Shipping - performed in a controlled environment, shipped on dry ice in shipping containers provided.

Photo courtesy of K. Kimbrough, NOAA

Figure 2. Field Collections

Photo courtesy of K. Kimbrough, NOAA

Figure 3. Great Lakes Field Sampling

Each year, pooled samples of bivalves are collected from specified sites for specimen banking (sites are chosen from each coast: Pacific, Atlantic, and the Great Lakes). Each collection site is divided into three stations, and each station is divided into an A and B subset. Each A and B consists of approximately 16-18 mussels or 10 oysters. To create duplicate samples, the three A subsets are pooled together and the three B batches are pooled together creating A and B duplicate samples of 50 mussels or 30 oysters for each of the A and B[7]. The A and B duplicate samples are sent to the MESB for banking (Fig. 4).

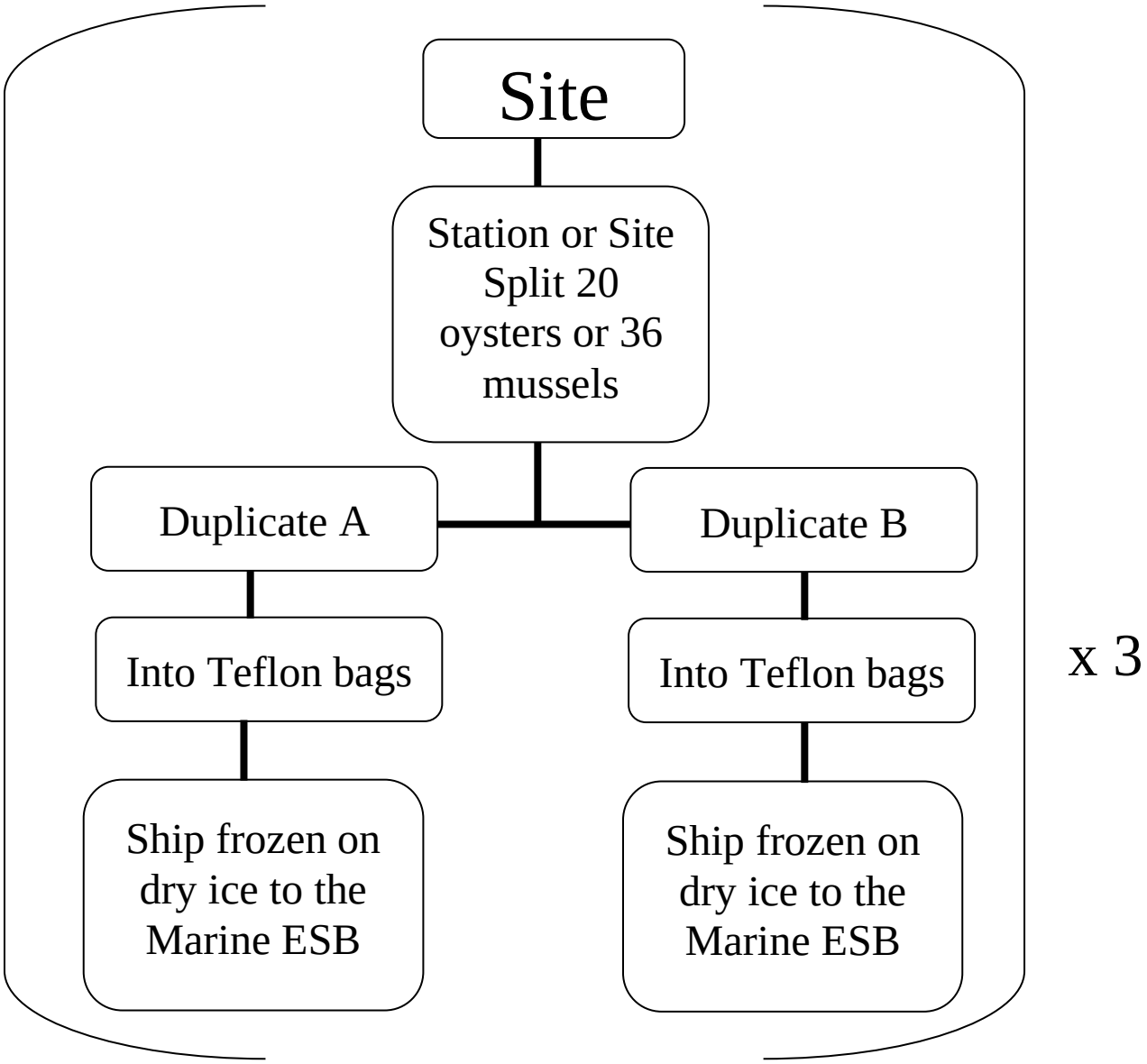

Figure 4. Bivalve Specimen Banking Sampling Schematic at Selected Sites[7]

Stage 1: Bivalve Collection and Sorting

1. Collect the samples by: a) dredging the water depth of 2m - 3m or more; b) placing tongs in water depth of 2m - 2.5m; c) placing a sampling fork in water depth less than 1m; or d) collect by hand along shoreline.

2. Once sampling is completed, separate the bivalves if clumped, and sort to ensure acceptable samples were collected. Guidelines for mussel size are: mussels less than 5cm and greater than 8cm long are discarded; mussels that are not tightly closed are discarded; oysters sized between 7 cm and 10cm are kept; oysters are examined in the field to ensure that more than an empty shell is being collected for storage.

Stage 2: Bivalve Field Processing

3. Inspect the bivalves and place them on a sorting tray, rinse with seawater, and scrub with a noncontaminating brush (nylon or natural fiber brush). The seawater rinse is repeated.

4. While wearing talc-free vinyl gloves, fold open a 35.0 cm x 45.0 cm pre-cleaned Teflon bag keeping the Teflon bag as free of contamination as possible. When the samples are being placed in the Teflon bag, care should be taken not to get fluids on the surface of the Teflon bag where the bag is to be heat sealed. A sufficient seal cannot be achieved if the surface of the Teflon bag is moist. However, if moisture does get on the area to be heat sealed, use a clean, lint-free cloth to remove it, or put the partially sealed bag into another Teflon bag and heat seal the outer Teflon bag.

   Note: The Teflon bags are manufactured and packaged under clean-room conditions and should be kept as clean as possible. The unused Teflon bags should be resealed in the original Teflon bag.

5. Place 15-18 mussels or 10 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 sample of A and B samples from the three (3) stations at one (1) site will therefore be 100 mussels or 60 oysters (see Fig. 4).

6. Attach the label provided by NIST to the outside of the Teflon bag using the tape provided. Designate one Teflon bag as “A” and the other as “B.” Ensure that all of the information for the sample is correct, e.g. site ID, site number, date, etc. Heat-seal the “A” Teflon bag closed. Place the labeled Teflon bag containing the “A” sample in another Teflon bag and heat-seal the outer Teflon bag. The “A” sample should be double bagged now with the label attached to the inside Teflon bag to prevent the label from becoming detached. Repeat this process for the “B” sample.

Stage 3: Sample Packaging and Shipping

7. Immediately transfer both "A" and "B" Teflon bags containing the samples into a cooler containing dry ice.

8. Complete an inventory of collected samples similar to Table 2. Include the 4-letter collection site ID, external ID (if any), site location name, collection date, note indicating if A & B replicates were collected, and the type of bivalves collected. Record any modification or deviation from the sampling protocol and include them with the sample inventory. Ship the coolers containing the samples on dry ice overnight express to the NIST Marine ESB:

   National Institute of Standards and Technology
   Hollings Marine Laboratory
   331 Ft. Johnson Rd.
   Charleston, SC 29412
   Attn: Rebecca Pugh (843) 762-8952 or
   Amanda Moors (843) 762-8953
   NISTESB@noaa.gov

9. Notify either of the Marine ESB staff listed above after samples have been shipped.

CRYOGENIC STORAGE AT THE MARINE ESB

When the specimen banking component resumed in 2005, the samples were shipped to and stored at the Marine ESB facility in Charleston, SC. The sample processing and storage protocols described below are specific to the Marine ESB and pertain to all collections from 2005 to current collections. Samples from previous Mussel Watch specimen bank collections (1986-2002) are currently stored in liquid nitrogen (LN_2) vapor phase freezers (-150 °C) at the Marine ESB facility. The Marine ESB was designed to contain an ISO Class 5 Clean Room for sample processing and ISO Class 7 Freezer Room for cryogenic storage of specimens[13]. These clean air laboratories minimize contamination of the sample when handling, processing and storing. Additional details about the facility have been previously described in Pugh *et al.*, 2007. After samples are received at the Marine ESB they are immediately placed in stainless steel baskets, in LN_2 vapor phase freezers (-150 °C) temporarily until sample processing (Fig. 5).

Figure 5. Banked, Unprocessed NS&T Samples

SAMPLE PROCESSING PROTOCOL

The bivalves are collected and shipped whole to the Marine ESB, therefore, they will need to be further processed in order to remove the meat from the shell. This will allow the samples to be stored in proper storage containers and create additional storage space inside of the LN_2 freezers. The following steps are to be completed as quickly as possible after the samples are received at the Marine ESB:

1. While wearing cleanroom gloves, spray the countertop in the ISO Class 7 cleanroom using Deconahol spray and a wipe with a lint-free Texwipe.

2. Cut a piece of VERSIDRY absorbent material, or benchline paper, and place *absorbent* side up on the clean counter. Place a Teflon sheet approximately the same size of the absorbent sheet on top. This is the clean, shucking surface.

3. Wearing clean gloves, retrieve a 350 mL pre-cleaned Teflon jar and a pre-cleaned titanium shucking knife with a Teflon handle. The Teflon shucking knife should be cleaned according to the titanium cleaning procedure described in NISTIR 7389 to ensure minimal cross contamination[13]. Place the jar on an outside corner of the

Teflon sheet, and place the knife on a Texwipe on the other corner of the shucking surface and cover the shucking knife with a Texwipe (Fig. 6).

4. Using cryogloves, retrieve a bag of mussels or oysters from the LN_2 freezer. Place the sample bag on the clean counter, but without touching the Teflon sheet shucking surface. With clean gloves, cut the Teflon bag using available scissors, without touching any sample inside of the bag.

5. Pour the sample (mussels or oysters) onto the Teflon sheet for partial thawing (≈0 °C). Spread out the samples to allow for faster thawing (Fig. 7). The sample should not thaw out completely, but enough for safe handling. The thaw time will depend on the size of the mussels or oysters.

6. Each NS&T sample bag has a label affixed. Record the NS&T sample four digit code, sampling date, and NIST A or B on the Teflon jar lid label. Place this label in the lid label slot of the Teflon jar lid. Record the same information (NS&T four digit code, sampling date, NIST A or B) in the specimen bank laboratory book. Include the initial mass of the jar, start and stop shucking time, and final jar mass. Affix the label that was included with the sample inside of the specimen bank laboratory book. The sample mass will be calculated after shucking is complete. See Figure 6.

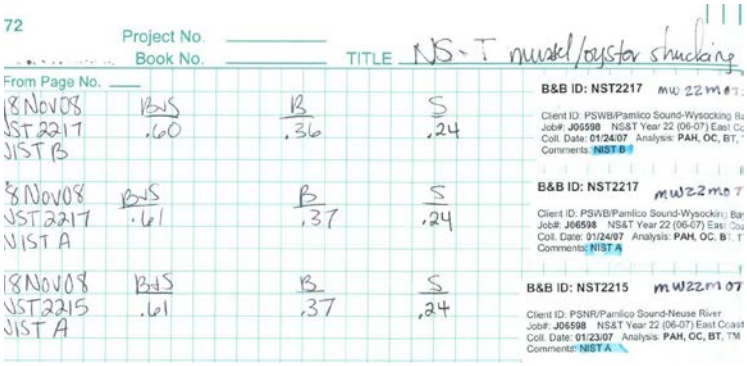

Figure 6. NIST Specimen Bank Laboratory Book

7. After sufficient thaw time, open the NS&T labeled Teflon jar with gloved hands and place the lid to the side on the Teflon sheet, face up. Remove the Texwipe from on top of the shucking knife and begin shucking the mussels or oysters, placing the meat into the 350 mL Teflon jar.

8. All contents inside of the bivalves should be scooped out using the titanium knife and included inside of the Teflon jar, except the "beard" of the mussel. If needed, an additional Teflon jar can be used for the excess bivalve contents. The jars are labeled as "Jar 1" and "Jar 2" of the respective sample. Both jars should be recorded in the lab book as well as the storage data sheet (see Appendix A).

9. After shucking is complete, verify that the labels are correct and place the lid on the Teflon jar (Fig. 8). Weigh the jar with the contents inside and record the mass in the specimen bank laboratory book.

10. Place the labeled Teflon jar in the next available storage position inside of a LN_2 freezer.

11. Discard bivalve shells, absorbent material, and Teflon sheet. Spray Deconahol on the countertop and wipe clean. Clean the titanium knife according to the titanium cleaning procedure in NISTIR 7389[13].

Figure 7. NS&T Sample Processing Set-up

Figure 8. NS&T Oyster samples

Figure 9. Processed NS&T Oyster Sample

SAMPLE ARCHIVAL AND CRYOGENIC HOMOGENIZATION PROTOCOL

The A sub-sample is meant for long-term storage and the B sub-sample is homogenized upon request and is available for analysis. Specimens collected as part of the NS&T Mussel Watch are eventually analyzed to determine aromatic hydrocarbons, chlorinated pesticides, polycyclic biphenyls (PCBs), DDT and its metabolites, organotins, and trace elements[7,11]. The A and B sub-samples are stored in separate freezers in order to minimize sample loss due to equipment failure. After a sample has been processed, a storage data sheet is filled out and a storage ID number is assigned. The storage ID number is assigned as indicated in Fig. 10. The sample data and storage location information is then entered into Freezerworks Unlimited (FUL), a specimen tracking database.

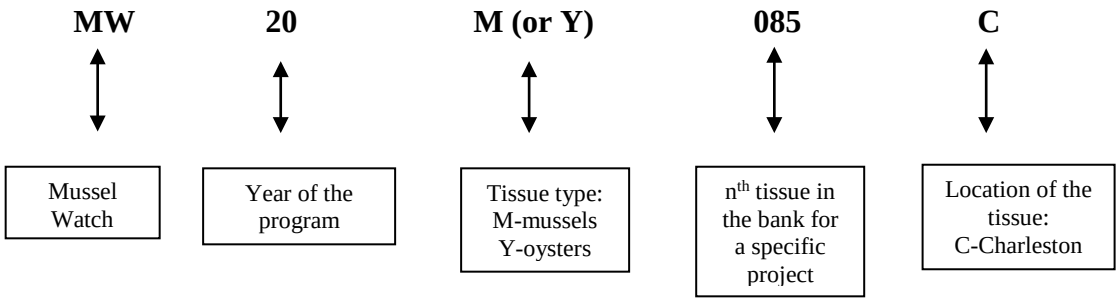

Figure 10. Unique Storage ID for NS&T Mussel Watch Samples

Before analysis on NS&T samples can begin, requested specimens are cryogenically homogenized and sub-samples into smaller Teflon jars. The cryogenic homogenization procedure creates a fresh-frozen powder material from the whole frozen bivalve tissue and creates a homogenous set of samples (Figs. 11 & 12) The cryogenic homogenization procedure is described in detail in NISTIR 7389[13].

Figure 11. Pre-Homogenized Tissue in Teflon Diskmill

Figure 12. Homogenized Tissue and Freezer Set-Up

Figure 13. Adding Rack of Homogenized Samples to LN_2 Vapor Phase Freezer (-150 °C)

SPECIMEN ACCESS POLICY

The NS&T banked specimens are available upon request to the scientific community for research requiring banked samples from the past. All requests for samples from the NS&T Program are considered for approval by the NOAA NS&T Program and NIST Marine ESB. The approval is dependent upon the following criteria:

1. A sufficient amount of a specimen is available beyond the anticipated needs of the NS&T Program;

2. The proposed research can only be achieved using the banked NS&T specimen; and

3. Analysis will be conducted in cooperation between the proposed organization and the NS&T Program

The NS&T Specimen Bank is not meant for readily available samples, but for research requiring banked samples from the past. NOAA makes the final decision to release samples from the NS&T Program. More details about the Specimen Access Policy can be found in Lauenstein, *et al.*, 1996.

SUMMARY AND CONCLUSIONS

NIST provides specimen banking for the NS&T Mussel Watch, one of the longest continuous contaminant monitoring programs in U.S. coastal waters. This specimen collection, extending over 2 decades, represents a valuable resource for investigating newly recognized contaminants of interest and provides an opportunity to apply new analytical techniques to measure analytes with greater degree of accuracy at lower levels of detection than were possible when the collections were first made. This report describes in detail the collection, processing, and long-term storage protocols practiced by NIST's Environmental Specimen Banking System in support of this program.

For further information on NOAA's NS&T Program, the following reports are excellent tools for reviewing current contamination findings from NS&T Mussel Watch collection sites: *NOAA National Status & Trends Mussel Watch Program: An Assessment of Two Decades of Contaminant Monitoring in the Nation's Coastal Zone*[3] and *NOAA National Status & Trends Mussel Watch Program: An Assessment of Polybrominated Diphenyl Ethers (PBDEs) in Sediments and Bivalves of U.S. Coastal Zones*[14].

REFERENCES

1. Lauenstein GG, Cantillo AY, Koster BJ, Schantz MM, Stone SF, Zeisler R, Wise SA. National Status and Trends Program Specimen Bank: Sampling Protocols, Analytical Methods, Results, and Archive Samples. Silver Spring, MD: National Oceanic and Admospheric Administration; 1996. NOAA Technical Memorandum NOS OMA 98.
2. Lauenstein GG, Schantz MM, Wise S, Zeisler R. Specimen Banking in the National Status and Trends Program: Development of Protocols and First Year Results. 1986. 586-589 p.
3. Kimbrough KL, Johnson WE, Lauenstein GG, Christensen JD, Apeti DA. An Assessment of Two Decades of Contaminant Monitoring in the Nation's Coastal Zone. National Oceanic and Atmospheric Administration; 2008. NOAA Technical Memorandum NOS NCCOS 74. 1-105 p.
4. Pugh RS, Becker PR, Porter BJ, Ellisor MB, Moors AJ, Wise SA. Design and Applications of the National Institute of Standards and Technology's (NIST's) Environmental Specimen Banking Programs. Cell Preservation Technology 2008;6:59-72.
5. O'Connor TP. Mussel Watch: Recent Trends in Coastal Environmental Quality. Rockville, MD.: U.S. Dept. of Commerce, National Oceanic and Atmospheric Administration, National Ocean Service; 1992.
6. Wise SA, Koster BJ. Considerations in the design of an environmental specimen bank: experiences of the National Biomonitoring Specimen Bank Program. Environ Health Perspect 1995;103 Suppl 3:61-7.
7. Lauenstein GG, Wise SA, Zeisler R, Koster BJ, Schantz MM, Golembiewska SL. National Status and Trends Program for Marine Environmental Quality Specimen Bank Project: Field Manual. Rockville, MD: National Oceanic and Atmospheric Administration; 1987. NOAA Technical Memorandum NOS OMA 37.
8. Wise SA, Koster BJ, Langland JK, Zeisler R. Current activities within the National Biomonitoring Specimen Bank. Sci Total Environ 1993;139-140:1-12.
9. Becker P, Pugh R, Moors A, Beddia L, Porter BJ, Mackey L, Schantz MM, Zeisler R, Kucklick JR, Vander Pol SS and others. Environmental Specimen Banking at the National Institute of Standards and Technology (USA). 2009.
10. O'Connor TP. National distribution of chemical concentrations in mussels and oysters in the USA. Marine Environmental Research 2002;53(2):117-43.
11. Cantillo AY, Lauenstein GG. Performance-Based Quality Assurance: The NOAA National Status and Trends Program Experience. National Oceanic and Atmospheric Administration; 1998.
12. Shigenaka G, Lauenstein GG. National Status and Trends Program for Marine Environmental Quality: Benthic Surveliance and Mussel Watch Projects Sampling Protocols. Rockville, MD: National Oceanic and Atmospheric Administration; 1988. NOAA Technical Memorandum NOS OMA 40.
13. Pugh RS, Ellisor MB, Moors AJ, Porter BJ, Becker PR. Marine Environmental Specimen Bank: Clean Room and Specimen Bank Protocols. Charleston, SC: National Institute of Standards and Technology; 2007. NISTIR 7389.
14. Kimbrough KL, Johnson WE, Lauenstein GG, Christensen JD, Apeti DA. NOAA National Status & Trends Mussel Watch Program: An Assessment of Polybrominated

Diphenyl Ethers (PBDEs) in Sediments and Bivalves of U.S. Coastal Zones. Silver Spring, MD: National Oceanic and Atmospheric Administration; 2009. NOAA Technical Memorandum NOS NCCOS 94

Table 2. Example of NS&T Collection Information

Collection Site ID	External Sample ID	Site Location	Collection Date	Notes	Comments
LIFI	NST2441	Long Island - Fire Island Inlet	11/14/2007	A & B replicates	mussels
LIJI	NST2442	Long Island - Jones Inlet	11/14/2007	A & B replicates	mussels
LICR	NST2449	Long Island Sound - Connecticut River	11/18/2007	A & B replicates	mussels
DBCH	NST2457	Delaware Bay - Cape Henlopen	11/28/2007	A & B replicates	mussels
CBCI	NST2459	Chincoteague Bay - Chincoteague Inlet	11/28/2007	A & B replicates	mussels
QIUB	NST2460	Quinby Inlet - Upshur Bay	11/30/2007	A & B replicates	mussels
SIWP	NST2467	Sinclair Inlet - Waterman Point	12/7/2007	A & B replicates	mussels
WIP	NST2468	Whidbey Island - Possession Point	12/8/2007	A & B replicates	mussels
PSEH	NST2469	Puget Sound - Everett Harbor	12/8/2007	A & B replicates	mussels
PSMF	NST2470	Puget Sound - Mukilteo Ferry	12/8/2007	A & B replicates	mussels
EBFR	NST2472	Elliott Bay - Four-Mile Rock	12/9/2007	A & B replicates	mussels
ESBD	NST2482	Espiritu Santo - Bill DaysReef	12/16/2007	A & B replicates	oysters
ESSP	NST2483	Espiritu Santo - South Pass Reef	12/16/2007	A & B replicates	oysters
SSBI	NST2492	South Puget Sound - Budd Inlet	1/4/2008	A & B replicates	mussels
CBRP	NST2493	Coos Bay - Russell Point	1/5/2008	A & B replicates	mussels
CBCH	NST2494	Coos Bay - Coos Bay	1/5/2008	A & B replicates	mussels
MSPC	NST2502	Mississippi Soud - Pass Christian	1/13/2008	A & B replicates	oysters
MSBB	NST2503	Mississippi Sound - Biloxi Bay	1/13/2008	A & B replicates	oysters
MSPB	NST2505	Mississippi Sound - Pascagoula Bay	1/13/2008	A & B replicates	oysters
PBPH	NST2504	Pensacola Bay-Public Harbor	01/14/08	A & B replicates	oysters
PBIB	NST2506	Pensacola Bay-Indian Bayou	01/14/08	A & B replicates	oysters
CBSR	NST2507	Choctawhatchee Bay-Off Santa Rosa	01/15/08	NIST A	oysters
CBSR	NST2507	Choctawhatchee Bay-Off Santa Rosa	01/15/08	NIST B1	oysters
CBSR	NST2507	Choctawhatchee Bay-Off Santa Rosa	01/15/08	NIST B2	oysters
CBJB	NST2508	Choctawhatchee Bay-Joe's Bayou	01/15/08	NIST A1	oysters
CBJB	NST2508	Choctawhatchee Bay-Joe's Bayou	01/15/08	NIST A2	oysters
CBJB	NST2508	Choctawhatchee Bay-Joe's Bayou	01/15/08	NIST B 1	oysters
CBPP	NST2509	Choctawhatchee Bay-Postil Pt.	01/15/08	A & B replicates	oysters
BSSI	NST2511	Breton Sound-Sable Island	01/18/08	A & B replicates	oysters
MBDR	NST2512	Mobile Bay-Dog River	01/17/08	A & B replicates	oysters
BSBG	NST2514	Breton Sound-Bay Gardene	01/18/08	A & B replicates	oysters
TBLB	NST2526	Terreborne Bay-Lake Barre	2/10/2008	A & B replicates	oysters
LBMP	NST2527	Lake Borgne-Malheureux Point	2/11/2008	A & B replicates	oysters
LBGO	NST2528	Lake Borgne-Gulf Outlet	2/11/2008	A & B replicates	oysters

APPENDIX A

NIST NS&T Mussel Watch Storage Data Sheet

NIST SPECIMEN BANK

M W 2 3 Y 0 8 9 C

Collection Date - 2/24/2008, Year 23

Sample Storage NST 2552

	Storage ID Fz	Rack	Box	Wt (g)	Date In Day	Mo	Yr	Date Out Day	Mo	Yr	Initials
NIST A	AL	15	91	260	19	08	09				KR

Comments _____

_____

Subsamples	LN2	Other	Storage ID Fz	Rack	Box	Wt (g)	Date In	Date Out	Initials
B001									
B002									
B003									
B004									
B005									
B006									
B007									
B008									
B009									
B010									
B011									
B012									
B013									
B014									
B015									
B016									
B017									
B018									
B019									
B020									