Curation Procedures Applicable to BOEM Invertebrate Collections Transferred to the Smithsonian Institution's National Museum of Natural History

BOEM Voucher Holding Area, NMNH Museum Support Center

Curated BOEM Vouchers in the Collections of the National Museum of Natural History

USNM 182243 **Holotype**

Hesiocaeca methanicola Desbruyères & Toulmond, 1998
Hesionidae Polychaeta Annelida
Id By: Desbruyères, Daniel, Toulmond, A.
No. Spec.: 1

Ethanol - 70%

Label Printed On: 9/30/2009

USNM 182243 Sta.: GC 234
Sample #: MMS-CHEMO/II/GC234-HYD1
North Atlantic Ocean; Gulf of Mexico; ; United States; Louisiana; ; ; Green Canyon site, exposed gas hydrate;
Centroid: 27 44 50 N 91 13 30 W
Date Coll.: Jul 1997 ; Coll.: C. Fisher
Exped.: CHEMO II
Vessel: Edwin Link R/V
Cruise: JSL 97 Gear: Johnson Sea Link II DSR/v
Depth: 538 M Acc.: 421113

Archival Label Prepared for BOEM Ice Worm Voucher

Prepared for BOEM by National Museum of Natural History Department of Invertebrate Zoology Collection Management Staff, September, 2014 **[BOEM Award M13PC00016]**

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Introduction

This document is intended as a general reference for those who are conducting fieldwork or processing specimen samples for a BOEM program that requires the deposition of invertebrate voucher specimens in the National Collections of the Smithsonian Institution's National Museum of Natural History (NMNH). It is not intended as a comprehensive guide to museum collection management practices but it does provide guidelines that will help to make the field processing and eventual transfer and incorporation of BOEM program vouchers into the National Collections as efficient as possible.

If you have collection-related questions that are not answered by the included information please don't hesitate to contact any of the following individuals in the NMNH Department of Invertebrate Zoology:

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CHAPTER 1 – COLLECTION MANAGEMENT CONCEPTS AND TERMINOLOGY

Prepared by Cheryl Bright (brightc@si.edu; 301-238-1756) and Chad Walter

The following terms are commonly used by the NMNH Department of Invertebrate Zoology staff when referring to collections. Some of these terms may be either unfamiliar to those collecting and processing BOEM specimen samples or may have a slightly different meaning in other collections. To help ensure a common understanding of these terms that are used throughout this procedures document, the definitions in use here at the National Museum of Natural History are provided, below.

TYPE(S) – A specimen that has been designated as the standard or exemplar of the characteristics that define a specific taxon. The "*primary type*", typically the **HOLOTYPE**, is a single specimen that has been carefully documented, described and illustrated in a peer-reviewed scientific publication. "*Secondary types*", typically **PARATYPES**, are additional specimens examined by the author of the original species description that were also used to establish the attributes of the taxon. Type specimens must be deposited in an appropriate archival collection where they will be maintained in perpetuity and will be accessible to the international scientific community for comparative examination.

When new taxa are described from BOEM-funded programs the BOEM Program Officer should be consulted regarding the final deposition of the types. Unless otherwise approved by the BOEM Program Officer:

a) The Holotype and at least one paratype must be deposited in the Smithsonian collections along with a voucher series that adequately documents observed intraspecific variation, sexual variation and developmental variations
b) At the discretion of the BOEM Regional Office officials, at least one paratype must be deposited in an appropriate repository located within that region (i.e. a paratype of each new West Coast taxon is deposited in a designated West Coast collection, etc.).
c) At the discretion of the BOEM Regional Office officials, the researcher describing the new taxon may elect to deposit a paratype at up to three other repositories of his/her choice.
d) All remaining paratypes are to be split equally between the Smithsonian and the designated regional repository referred to in "b".

VOUCHER(S) – A specimen that has been identified to the lowest practical level, preferably species, by a taxonomic specialist and used as the identification standard (i.e. an exemplar) for that taxon for the duration of the research program. Often a voucher lot consists of multiple specimens of the same taxon representing the natural variability of that taxon in the collecting area. The variability represented may include sexual dimorphism, developmental stages, color variations, etc. The designated voucher specimens for an ecological or environmental survey are of the same comparative and documentary importance as the type specimens are for a taxonomic study.

LOT (or Specimen **Lot**) – A group of specimens collected from exactly the same place at the same time using the same gear type or methodology. Depending on the sorting and processing status, a **lot** may include multiple taxa or a single taxon. If multiple taxa are present in the **lot**, additional sorting of that single **lot** will likely yield multiple additional **lots**. A single **lot** may be contained in a single jar or in multiple jars. If multiple jars are used to store the contents of a single **lot** the jars should be labeled using sequence numbers, i.e. "1 of 3", 2 of 3" 3 of 3". Each jar must also have a complete and informative label that provides the taxonomic composition of the contents

and the collecting locality data including at least *program name*, *vessel name* and *cruise number*, *station number*, *sample number*, *latitude/longitude*, *water depth*, *date collected*, *gear/collecting method used*. All labels should be prepared on 100% cotton rag paper using solvent stable ink and placed inside the jar with the specimens.

SAMPLE – May refer to the unprocessed contents of a single gear deployment – OR – may refer to a small amount of tissue or part of a specimen that has been set aside for additional analysis.

SAMPLING – The process of selecting one or more entire specimens from a sample or specimen lot for a special use such as molecular analysis – OR – the process of taking a body part or small amount of tissue from a single specimen for a special use such as molecular analysis.

FIELD NUMBER (or SAMPLE NUMBER) - A unique number, usually assigned by the collector, used to track a single specimen or multiple specimens that share the same subset of data. Typically a **field number** or **sample number** is used to link a specimen or specimens to a unique locality record in a field diary, a ship's logbook or an electronic dataset. Specimens identified by the same **field number** or **sample number** typically were collected from the same location, at the same time, by the same individual using the same gear type.

USNM – The acronym for the United States National Museum, the historical name for the Museum that gave rise to the National Museum of Natural History (NMNH). **USNM** is still in use today, especially in literature referencing catalog numbers for collections belonging to the National Museum of Natural History (NMNH).

NMNH – The acronym for the Smithsonian Institution's National Museum of Natural History.

ACCESSION NUMBER - A unique number assigned to a "collection" that has been acquired by the National Museum of Natural History (NMNH) for its permanent collection. The "collection" may include only a single specimen or may include multiple specimens. The **accession number** links the collection and all specimens in the collection to the records that document the circumstances of the acquisition, including the donor name and address, the kind of acquisition (gift, transfer, etc.), donor and NMNH compliance with permit and import laws, and any restrictions that impact the use of the specimen(s). These records are maintained in perpetuity in the NMNH Registrar's Office.

ACCESSIONING – The process of documenting, reviewing, and formally accepting an acquisition for the NMNH's permanent collection.

DONOR – The person or institution who either owned the collection or had the legal right to transfer ownership of the collection to NMNH. An individual donor's right to, and intent to transfer ownership is formally documented through the use of a signed Deed of Gift or Letter of Donation. Institutional transfer of ownership is most often documented through the use of a formal Memorandum of Understanding (MOU), cooperative agreement or ratified contract. The donor of record for all BOEM-funded collections deposited in the NMNH's Department of Invertebrate Zoology is the US Department of the Interior, Bureau of Ocean Energy Management as documented by a ratified contract between the Smithsonian Institution and BOEM.

CATALOG NUMBER – An identification number assigned to a specimen lot at the time the specimen lot is prepared for incorporation into the permanent collection. Within the NMNH, catalog numbers are not unique. However, within a specific collection or sub-collection assigned catalog numbers are unique. In publications, Invertebrate Zoology catalog numbers are preceded by the

prefix "USNM". The catalog number unambiguously identifies a specific specimen or specimen-lot in the collection and is used for inventory control and loan management.

CATALOGING – The process of preparing a specimen or specimen-lot for incorporation into the NMNH's permanent collection. This includes the assignment of a unique catalog number, entry of all available data documenting the identification of the specimen(s), where and how they were collected, who collected them, and linking the catalog record to the corresponding accession record.

DATA (or SPECIMEN DATA) – Information that documents the "what, where and who" about a specimen, a specimen-lot, or a collection. Before an acquisition is accepted and accessioned into the NMNH's permanent collections the available data are reviewed for completeness and accuracy. Collections that do not meet at least our minimum standards for documentation are not accepted and are returned to the donor. Chapter 6 provides extensive information about our data requirements, standards and formats. The minimum standards for collection data include:

TAXONOMIC INFORMATION (IDENTIFICATION) - At least the name of the Family, preferably the full Family/Genus/Species/Author-Date identification, as determined by an expert or based on comparison with a designated voucher specimen. Correct spelling of taxonomic names with the author and year for most marine species and some freshwater species, can be found at http://www.marinespecies.org/. Preferably, the full name of the individual who identified the specimen and the date the identification was made will also be provided.

COLLECTOR – The name of individual or organization that collected the specimens.

LOCALITY INFORMATION (or COLLECTION INFORMATION) – Specific information indicating where, when and how the specimen(s) were obtained. This includes geographic name/locality (i.e. Ocean, Gulf, Country, State, Precise location), latitude/longitude coordinates, water depth in meters, etc.

CHAPTER 2 – COLLECTION MANAGEMENT SUPPLIES: CURATION AND SHIPPING

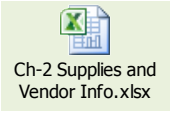
Prepared by Geoff Keel (keelw@si.edu; 301-238-1759)

The NMNH Department of Invertebrate Zoology has identified a standard set of archival curation supplies appropriate for the long-term conservation of its permanent collections. Before a sample is incorporated into the permanent collection an archival sample label is prepared and the specimen is transferred to fresh preservative. If required, the specimen container is replaced with an archival container. The original preservatives and any non-standard containers are discarded.

BOEM contractors are encouraged to use the curation supplies described below as the collections are processed, especially if the specimens will be transferred from the contractor's work site to the Smithsonian in their containers. (Note: Specimens maintained in alcohol, formalin or other fluids must be properly packaged for shipment. Depending on the shipping mode, it may be necessary to remove specimens from their containers. See "Chapter 7 – packing and Shipping".)

The following table lists the most commonly used standard archival curation supplies currently in use in the NMNH Department of Invertebrate Zoology. The column "Product Number" is the manufacturer's product number and the column "X-ref Product" identified either the container a specific closure will fit, or the closure required by a specific container size/style.

Curation/Packing	Category 1	Category 2	Item Name	Description	Product Number	X-ref Product
Curation	Closures	Cotton	Cotton Balls	Medium	07-886	NA
Curation	Closures	Gaskets	Buna-N Rubber - 3/32" (Round)	3-1/8" (OD) x 2" (ID)	NA	FIDO - 200 ml
Curation	Closures	Gaskets	Buna-N Rubber - 3/32" (Round)	3-11/16" (OD) x 2-3/4" (ID)	NA	FIDO - 750 ml & up
Curation	Closures	Screw Capped	OI - Square Flint Cap	33 x 400 Poly	NA	2 oz & 4 oz OI Jars
Curation	Closures	Screw Capped	Qorpak - Square Flint Cap	43 x 400 Cap F217	NA	8 oz
Curation	Closures	Screw Capped	Qorpak - Square Flint Cap	48 x 400 Cap F217	NA	16 oz
Curation	Closures	Screw Capped	Qorpak - Square Flint Cap	58 x 400 Cap F217	NA	32 oz
Curation	Jars	Bail Style	Bormioli Rocca - FIDO	1 L	NA	Gasket, 3-11/16" (OD) x 2-3/4" (ID)
Curation	Jars	Bail Style	Bormioli Rocca - FIDO	2 L	NA	Gasket, 3-11/16" (OD) x 2-3/4" (ID)
Curation	Jars	Bail Style	Bormioli Rocca - FIDO	200 ml	NA	Gasket, 3-1/8" (OD) x 2" (ID)
Curation	Jars	Bail Style	Bormioli Rocca - FIDO	3 L	NA	Gasket, 3-11/16" (OD) x 2-3/4" (ID)
Curation	Jars	Bail Style	Bormioli Rocca - FIDO	750 ml	NA	Gasket, 3-11/16" (OD) x 2-3/4" (ID)
Curation	Jars	Screw Capped	OI - Square Flint Jar	2 oz	NA	33 x 400 Poly Cap
Curation	Jars	Screw Capped	OI - Square Flint Jar	4 oz	NA	34 x 400 Poly Cap
Curation	Jars	Screw Capped	Qorpak - Square Flint Jar	16 oz	NA	48 x 400 Cap F217
Curation	Jars	Screw Capped	Qorpak - Square Flint Jar	32 oz	NA	58 x 400 Cap F217
Curation	Jars	Screw Capped	Qorpak - Square Flint Jar	8 oz	NA	43 x 400 Cap F217
Curation	Supplies	Paper - Resistall	Resistall - 36#	8.5" x 11"	219-368511	NA
Curation	Vials	Shell	Shell Vial	0.25 Dram	03-339-30A	NA
Curation	Vials	Shell	Shell Vial	0.5 Dram	03-339-30B	NA
Curation	Vials	Shell	Shell Vial	1.0 Dram	03-339-30C	NA
Curation	Vials	Shell	Shell Vial	2.0 Dram	03-339-30E	NA
Curation	Vials	Shell	Shell Vial	4.0 Dram	03-339-30G	NA
Curation	Vials	Shell	Shell Vial	6.0 Dram	03-339-30H	NA
Curation	Vials	Shell	Shell Vial	8.0 Dram	03-339-30J	NA
Packing	Containment	Packing Peanuts	Peanuts	Loose Fill Peanuts - 7 Cu Ft	831952	NA
Packing	Containment	PigMat	Absorbent Mat	15" x 20"	MAT203	NA
Packing	Supplies	Shipping Bags & Vials	4 Mil Thick Poly Tubing	10" x 1500' Roll	S-1146	NA
Packing	Supplies	Shipping Bags & Vials	4 Mil Thick Poly Tubing	12" x 1500' Roll	S-1147	NA
Packing	Supplies	Shipping Bags & Vials	4 Mil Thick Poly Tubing	4" x 1500' Roll	S-1143	NA
Packing	Supplies	Shipping Bags & Vials	4 Mil Thick Poly Tubing	6" x 1500' Roll	S-1144	NA
Packing	Supplies	Shipping Bags & Vials	4 Mil Thick Poly Tubing	8" x 1500' Roll	S-1145	NA



Double-Click on the Excel icon, "Ch-2 Supplies and Vendor Info", for website urls and vendor information where these items may be purchased. Please note that these curation

supplies are readily available from a variety of sources. Website and vendor information is provided solely as a starting point. BOEM contractors should consult with their usual suppliers and a variety of other sources to ensure they are receiving the best value.

Oversize specimens, i.e. those specimens too large to fit safely inside of a standard glass jar may be stored in preservative inside of a 2.5 gallon, 5 gallon, or 10 gallon plastic bucket and closed with a threaded, screw-down lid such as a "gamma seal" ™ lid.

Images and descriptions of the various jars and closures listed above are shown:

Piramal jars with Qorpak Green Thermoset F217 and PTFE Lined Caps
From Left to Right
8 oz French Square with 43-400 F217 Cap
16 oz French Square with 48-400 F217 Cap
32 oz French Square with 58-400 F217 Cap

OI Flints (4 oz can also be purchased from Piramal)
From Left to Right
2 oz French Square with 33-400 Polycone Cap
4 oz French Square with 33-400 Polycone Cap

Bormioli Rocco Fido Jars (Sizes available are 5L,4L,3L, 2L,1L, 750ml, 500ml, 200ml, 125ml)
3 Liter jar (square) with White 55-Durometer Buna-N Gasket
750 ml jar (square) with White 55-Durometer Buna-N Gasket
200 ml jar (round) with White 55-Durometer Buna-N Gasket

LeParfait Standard
From Left to Right
3 Liter jar with White 55-Durometer Buna-N Gasket
2 Liter jar with White 55-Durometer Buna-N Gasket
1.5 Liter jar with White 55-Durometer Buna-N Gasket
1 Liter jar with White 55-Durometer Buna-N Gasket

LeParfait Terrine (500 ml not pictured)
From Left to Right
1000 ml jar with White 55-Durometer Buna-N Gasket
750 ml jar with White 55-Durometer Buna-N Gasket
350 ml jar with White 55-Durometer Buna-N Gasket
200 ml jar with White 55-Durometer Buna-N Gasket

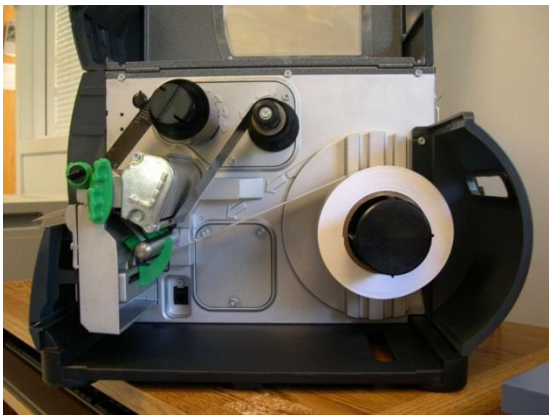
To conserve space and help control the cost of containers and closures, small specimens are curated into shell vials with their labels. The vials are closed using a cotton plug. Multiple vials of the same taxon or from the same station/sample are then "bulk stored" in a larger archival container as shown below.

Temporary labels for fluid preserved specimens are prepared using a #2 pencil or a pen with alcohol-stable ink and "Resistal" ™ 100% cotton rag paper. (Please note that "Resistal" paper is not acid-free and should not be used for dry archival labels.) Laser printed labels are not reliably alcohol-stable and should never be used for temporary labels. Some inkjet inks formulated using pigments rather than dyes are advertised as archival or solvent resistant and may be suitable for temporary labels. Confer with the ink manufacturer before using inkjet printers to prepare specimen labels.

Permanent labels for fluid preserved specimens are prepared using a thermal printer with alcohol-stable plastic paper and a resin ribbon. A variety of thermal printers are commercially available. The types of "paper" and ribbons appropriate for use in alcohol may vary depending upon the brand of thermal printer. Confer with the printer manufacturer to ensure that the printer, "paper' and ribbons are compatible with immersion in alcohols.

A DataMax-O'Neill thermal printer

Thermal printer supplies – a spool of "plastic" paper (also referred to as "tag stock") and a spool of resin ribbon



Paper and ribbon spools loaded on the spindles inside the thermal printer

CHAPTER 3 – CURATION STANDARDS

Prepared by Katie Ahlfeld (ahlfeldk@si.edu; 301-238-1754) and Lisa Burnett

Standards for Curating Wet Specimens

For fluid preservation, collectors may immerse invertebrates in formaldehyde, alcohol, or other solutions. These techniques either kill the specimen or work as fixatives. The type of preservation fluid used depends on the sampling method and the species being collected. (See Chapter 4 – Fixation and Preservation).

1. To be safe, the rate of preservation to specimen/tissue in the vial or jar should be at least 3 parts preservation fluid to 1 part tissue.
2. Specimens should be housed in containers appropriate for their size. **Jars should not be overfilled with specimens because it can damage/squash soft tissues.**
    a. For most small specimens, 4- or 6-dram glass shell vials with straight sides (no shoulders) and filled with preservation fluid are suitable. The vial(s) should be plugged with clean cotton in such a manner that no air bubbles are trapped inside and placed inverted into a 4 oz or 8 oz container, or an appropriately sized glass jar with a proper lid and gasket.
    b. Extremely small specimens may be double vialed, first in a ½ or 1-dram vial and then placed into a 4-dram vial, in order to ensure the specimen is not damaged.
        The purpose of placing the specimens into vials is to protect them from potential damage that could be caused by contact with labels placed into the jar or during removal from the secondary container.
    c. Medium-sized specimens can go directly into appropriately sized glass jars with proper lids and gaskets. The jars should be topped off with preservation fluid to prevent air bubbles being trapped inside.
    d. Oversized invertebrate specimens can be stored in polycarbonate pails with screw top lids. These pails should also be topped off with preservation fluid.
3. All specimens must be identified and sorted by taxon as narrowly as possible, preferably to the species level.
4. Jars should be kept in the dark and not exposed to artificial or sunlight because the specimens will fade.
5. Jars should be checked on a regular schedule to ensure that the specimens remain below the level of the alcohol.

Specimens should never be jammed or forced into the vials or jars.

Required Specimen Label Data – Wet Specimens

1. Labels for Wet Specimens should be made of cotton/rag bond or printed on thermal "plastic" paper.
2. Labels should be completed using a #2 pencil or a pen having indelible ink, or using thermal printer.

3. The following information should be placed on each label:
    a. Name (phylum, class, etc.)
    b. Specific collection event number
    c. Preservation type/solution
    d. County, city/town, and other clearly worded description of collection locality so as to enable another scientist to find the collection locality.
    e. Latitude and longitude in decimal degrees (UTM is not acceptable).
    f. Environmental data regarding habitat (temperature, depth, etc.)
    g. Full names of collector(s) and identifier
    h. Dates of collection and (if available) identification.
        - Dates should clearly indicate day, month and year (e.g. 13 May 1982).
4. Labels should be no larger than 3 x 5 inches and no smaller than 2 x 3 inches.
5. The data may be hand printed with technical pen using #2 pencil or alcohol-proof ink.
    - Laser printed or photocopied labels are not acceptable, as they can fade and bleed. Ballpoint pens and fugitive pencils (such as red pencils) will fade and therefore are also not acceptable for writing on labels.
6. Jars should contain only one species and only specimens from one locality in one collection.
7. For small specimens, labels must be placed in the specimen bottle that contains the specimen vial(s), not directly inside the vials and not attached to the outside of the bottle.
8. For medium or large specimens, labels may be placed directly with specimen, and not attached to the outside of the bottle.

All labels must be legible. If labels are not legible then the specimens will not be accepted or cataloged into the IZ collection.

Standards for Curating Dry Specimens

Some collectors choose to dry their specimens. Dried calcareous materials (shells, corals, echinoderm spines) that have been immersed in formalin and improperly rinsed may be prone to "Byne's Disease." This is a mineral efflorescence that results from the reaction of organic acids, such as formic and acetic acids, with a calcium-based substrate. Byne's Disease usually gives a white powdery appearance to the surface of specimens.

1. Specimens should be housed in containers appropriate for their size
    a. Small specimens may be placed within an appropriately sized glass vial with strait sides (no shoulders) and plugged with cotton or a plastic snap top.
    b. Larger specimens may be housed within appropriate archival-worthy bags or boxes.
    c. Specimens must be sorted and labeled properly, as noted above in the fluid-preserved specimen section. However, labels should be placed into the vial, bag, or box in this case, as there is often no larger secondary container.
        - If the vial is too small to contain the whole specimen label, then a smaller USNM label can be put with the specimen, while the larger specimen label can be put in the box containing the vial.

Required Specimen Label Data – Dry Specimens

a. Name (phylum, class, etc.)
b. Specific collection event number
c. Preservation type
d. County, city/town, and other clearly worded description of collection locality so as to enable another scientist to find the collection locality.
e. Latitude and longitude in decimal degrees (UTM is not acceptable).
f. Environmental data regarding habitat (temperature, depth, etc.)
g. Full names of collector(s) and identifier
h. Dates of collection and (if available) identification.
    - Dates should clearly indicate day, month and year (e.g. 13 May 1982).

Standards for Recording Data in the Field

Researchers collecting in the field must make sure that all specimens preserved are accompanied by the proper location information, collection information and any corresponding notes. Specimens lacking this information will be of less value to other researchers and to the collection as a whole.

Labels for Dry Specimens

1. Label for Dry Specimen should be cotton/rag bond.
2. Label should be completed using a pencil or a pen having indelible ink.
3. The following information will be placed on each tag (also see example below):
    a. Name (phylum, class, etc.)
    b. Collector's name
    c. Locality information
    d. Date of Collection (use the following format 3 November 1946) - do not use a numerical format (3-12-46) because of the possibility of confusing the month and day as well as specimens having been collected in 1846 and 1946)

Labels for Fluid Preserved Specimen

1. The specimens should be preserved in the field using the appropriate preservation fluid.
    a. The type of preservation fluid used depends on the sampling methods and species being collected.
2. Labels for Wet Specimen should be cotton/rag bond.
3. Label should be completed using a pencil or a pen having indelible ink.
4. The following information will be placed on each tag (also see example below):
    a. Name (phylum, class, etc.)
    b. Collector's name
    c. Locality information
    d. Date of Collection (use the following format 3 November 1946) - do not use a numerical format (3-12-46) because of the possibility of confusing the month and day as well as specimens having been collected in 1846 and 1946)
    e. Preservative type

CHAPTER 4 – FIXATION and PRESERVATION of MORPHOLOGICAL VOUCHERS

Prepared by Bill Moser (moserw@si.edu; 301-238-1761) and Courtney Wickel

Introduction

The information in this chapter describes the preservation standards of the NMNH Invertebrate Zoology collection and the appropriate fixation and preservation techniques to be applied to BOEM Invertebrate Zoology voucher specimens prior to accession into the NMNH IZ collection.

Specimens of some taxa can be identified irrespective of the method of preservation used. However, some taxa are unidentifiable unless specific methods are used. Specific fixation and preservation protocols must be used with specimens collected for DNA analysis (see Chapter 6: DNA Vouchers and Sampling).

The organisms targeted in and goals of a specific study ultimately determine which fixation and preservation method or methods are appropriate. This chapter deals exclusively with marine invertebrate specimens that will be deposited at NMNH. A comprehensive guide to fixatives and preservatives for invertebrate samples can be found in Lincoln & Sheals (1979).

I. Fixation

Most fluid-preserved specimens are first "fixed" in a fixative solution, most commonly formaldehyde. The fixation process kills invertebrate animals after they have been collected. The fixative solution coagulates and stabilizes proteins in specimen tissues so they do not deteriorate or become distorted during preservation, study, and storage. The most common fixative solution used is formalin.

1a. Formalin is a diluted solution of formaldehyde. **Formaldehyde** is most commonly sold commercially as a 37.5% aqueous mixture of formaldehyde gas in water. Full strength commercial **formalin** = 100% aqueous formaldehyde (i.e. full strength formalin is a 37.5% aqueous mixture of formaldehyde gas in water). Formalin must be properly diluted before use as a fixative. One part of 100% formalin solution is mixed with 9 parts of water to make a fixative or preservative solution called **10% formalin**. Formalin can be further diluted to **5% formalin** (1.875% formaldehyde); this may be an adequate preservative solution for some small specimens.

1b. The 4% or 5% formalin misunderstanding: It is not uncommon to hear people say "use 5% formalin" either as a fixative or as a long term storage solution. While the use of 4% or 5% formalin may, in certain instances, be appropriate, keep in mind that 5% formalin is a 1.875% formaldehyde solution. Be certain you use the intended concentration of formalin when you are fixing and storing invertebrates.

1c. Buffering: Formaldehyde and formalin are acidic solutions and become more acidic over time. For most applications the final formalin solution should be buffered to help protect the specimens from damage. A number of buffering agents are available. For marine collections, sea water, a natural buffer, is often used in the field to prepare the working dilution of formalin. This is an acceptable buffer for short-term fixation of many invertebrates. For long-term storage in formalin a phosphate buffer is preferred. A suitable phosphate buffer (Sörensen's Buffer) is prepared using 4 grams of monobasic sodium phosphate monohydrate ($NaH_2PO_4 .H_2O$) and 6.5 grams of dibasic sodium phosphate anhydrate (Na_2HPO_4) per liter of a solution of 10% Formalin [100 ml of Commercial Formaldehyde (37 - 40%) and 900 ml of distilled water].

The fixative should be applied as soon as possible after collection and narcotization, if appropriate.

2. Narcotizing: While fixatives will kill and preserve specimens, some soft-bodied specimens should be narcotized ("relaxed") so that they can be fixed without distortion. Narcotization effectively anaesthetizes specimens so muscles do not contract during the fixation process. Narcotization agents also aid in the retention of egg sacs and gut contents during fixation. Narcotization is mandatory for many soft-bodied groups, such as ascidians, anemones, nemerteans, and annelids, in addition to smaller planktonic groups with detailed appendages, like copepods. The following are a few common narcotization agents:

- magnesium sulphate or magnesium chloride added slowly over a period of an hour or so as a 25% solution; small specimens usually can be dropped directly into a solution that is isotonic with seawater (approximately 7.5% for $MgCl_2$ but this needs to be verified with a salinity refractometer)
- chloral hydrate in a 2% solution
- propylene phenoxytol as a 1% solution (this is an emulsion and should be prepared well ahead, with lots of shaking, to fully disperse the oil droplets)
- menthol crystals added to seawater

For more information on narcotization and anesthetization solutions and protocols, please see Lincoln & Sheals (p. 123-126).

II. Preservation

The preservative solution is the long-term storage fluid that the specimen will be housed indefinitely. The purpose of the preservative fluid is to create an environment where the specimen can be stored indefinitely without distortion. A good preservative will arrest autolysis (the self-digestion of cells by enzymes present within them) and kill bacteria and mold. In some cases the preservative is a fresh solution of the fixative solution but, in most cases, the preservative solution used for invertebrate samples is an alcohol.

The majority of the Invertebrate Zoology collection at NMNH is permanently preserved in 70-75% ethyl alcohol (ethanol, grain alcohol). Some taxonomic collections are stored in 10% formalin or 50% isopropyl alcohol (isopropanol, rubbing alcohol).Taxonomic exceptions to this general case are summarized in Table 1. Specimens should not be preserved in methyl alcohol (wood alcohol).

Taxonomic Group	Preservative
Cephalopod	50% Isopropyl Alcohol
Jelly Fish	10% formalin
Porifera	85% ethyl alcohol

Table 1: Taxonomic groups not permanently stored in 70% ethyl alcohol

All BOEM voucher specimens should be housed in their permanent preservative solution prior to transfer to NMNH's Department of Invertebrate Zoology. ***The label inside the container with the specimen must state the preservative used. This is required both for the safety of the individuals who will handle the specimens following transfer to NMNH and to ensure that the specimens are not erroneously transferred to an incorrect preservative during subsequent curation.***

IZ will also accept specimens that have been dried or prepared as slides. Please see the chapter on curation standards (chapter 4) for more information on how these techniques should be applied.

II. Transfer between fluids (from fixative to preservative)

Transferring a specimen from a fixative solution into a permanent preservative requires soaking the specimen in water and then transferring the specimen through a series of graded concentrations of the preservative solution. The specimen should not be placed directly into the full-strength concentration of the preservative; this could cause osmotic imbalances and result in distortion the specimen. For example, if you are moving a specimen from a 10% formalin solution into 70% ethanol, it would be best to place it in staged solutions of water, 20%, 40%, and 60% ethanol for 24 hours each before placing it in the permanent preservative concentration of 70% ethanol.

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CHAPTER 5 – TISSUE SAMPLING and the PREPARATION of DNA and MOLECULAR SAMPLES

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Introduction

The quality of DNA extracted from specimens in the field is dependent on three primary factors: the amount of time between specimen collection and tissue preservation, preservation medium, and the type of tissue sampled.

The Importance of Time

The moment an organism dies, its genetic material begins to degrade. Compounded with high temperatures, moisture, and enzymatic activity, even a brief period between specimen death and tissue sampling can prove highly detrimental to DNA/RNA integrity (Dawson et. al., 1998). Measures must therefore be taken to delay death and to minimize the time between collection and tissue sampling. Temporary storage in seawater, for example, can keep the specimen alive and mitigate the effects of heat exposure, resulting in a higher quality genetic yield.

The Importance of Tissue Type

In order to fit inside of the standard vials, tissue samples should be no more than $1cm^3$ in diameter, or between the volume of a lentil and a pea (Chris Huddleston). Due in part to the small size of the sample, it should be harvested from a genetically dense tissue that doesn't contain degrading enzymes or taxonomically significant features (Predini et. al., 2002).

Generally, muscle tissues are the preferred sample type for invertebrates, while gastric, oral, and exterior tissues are to be avoided. Gastric and oral tissues may contain enzymes that damage DNA, and may also be contaminated with organic matter from food or parasites (Dawson et. al., 1998; Predini et. al., 2002). Exterior tissues may contain parasitic or symbiotic organisms whose own DNA will contaminate the sample, while the pigments and lipids often found in external tissues may inhibit DNA amplification (Borchiellini et. al., 2001, Predini et. al., 2002). In order to remove organic detritus, it is advisable to rinse every tissue sample with seawater.

Forelimb, hind limb, head, tail, mouthpart, and reproductive structures should be avoided unless otherwise specified, as these areas tend to be useful for taxonomic identification. Lastly, exoskeleton and shell portions should not constitute the entirety of the sample, as these tend to contain detrimental pigments and lipids, and bear little DNA (Predini et. al., 2002). The small size of a great portion of invertebrates will not allow for tissue extraction and it is best to preserve the whole specimen. Therefore two representatives of the organism should be taken; one for the specimen voucher and one for the genetic voucher. When practical, photographs of the living animal, with close-ups of the head and other taxonomical features, should be taken.

The Importance of Preservative

Storage in an appropriate preservation medium is of utmost significance. At least a 95% non-denatured ethanol solution must be used to ensure genetic integrity (Predini et. al., 2002). Lower concentration solutions may not preserve the tissues adequately for extraction. Ethanol preserves tissue by drawing out water, effectively desiccating it. In the process, the ethanol itself becomes diluted. It is best to do an initial fixation of non-nitrogen-cooled samples in 10:1 ethanol:tissue ratio and then transfer to a 3:1 ethanol: tissue ratio, to ensure that the ethanol is not diluted to the point of being non-preservative.

- Immediately following labeling and preserving, all prepared tissue samples should be deposited in a liquid nitrogen-cooled tank (Dawson et. al., 1998). While the use of ethanol as a preservative isn't required for liquid nitrogen-cooled samples, it is advised that ethanol be included with those tissue samples to act as a buffer if a problem should arise with the nitrogen tank. The flash freeze will suspend any necrotic activity from further degrading the tissue.
- Use caution when working with liquid nitrogen. Never let any part of your body come in contact with it, ensure you are using appropriate protective equipment, and only use a liquid nitrogen-cooled tank in a well-ventilated area.
- If a liquid nitrogen-cooled tank is not available then the samples should be placed in a freezer on board the collection vessel.
- Combined tissue sample and ethanol volume must not exceed the cryovial's 1.8 mL fill line. Doing so may cause the vial lid to burst when exposed to cryogenic temperatures.

Relaxants

Many organisms will need to be exposed to a relaxant before tissue samples are taken, both to ensure a successful tissue extraction and to avoid inducing the animal to contract, as this can make morphological identification impossible (Templado et. al., 2010).

It is crucial that such organisms be exposed to the relaxant before a tissue sample is taken, as tissue extraction will likely agitate an active specimen and could induce contraction. Attempting to cut a piece of tissue from an active snail, for example, may cause it to contract into its shell, after which it may prove impossible to remove (Templado et. al., 2010).

One can test to ensure a specimen is properly anesthetized by poking it with a probe. Caution should be taken, however, that this test is not performed prematurely, as it may induce irreversible contraction (Templado et. al., 2010).

See the taxa-specific sampling guide and guide to particular relaxants below.

Vials and Labels

The standard vials will be 2.0 ml Simport T310-2A cryovials with CP100 cap inserts and hand-placed Brady FreezerBondz labels ($0.23 per tube; $0.04 per cap insert; $0.08 per label).

Ethanol and tissue added to the vial also must not exceed the 1.8 ml fill line, as overfilling vials may cause lid blowout when frozen.

There will be 3 labels per specimen, each containing its unique ID number. One will be placed on the voucher specimen, another on the tissue sample vial, and a third within the vial.

Labels need to be placed by hand on the 2.0 Simport cryovials while still warm, before placing any tissue samples therein. The label contained within the vial should be handwritten with a graphite pencil on cotton rag paper. Exterior vial labels may fall off during freezing or transport so the internal label provides a failsafe. It is also advisable to write the provided Biorepository Number on the exterior of the tube.

In order to streamline processing and prevent errors and contamination, vials can be prepped before collecting begins. Labels can be placed on the exterior and interior of the vials, and vials can be filled

with ethanol. Doing so will reduce the amount of multitasking necessary during collection, and thereby reduce the likelihood of accidental error or contamination.
Care must be taken to ensure that the specimen label matches its associated tissue sample label. Mix-ups will result in the tissue being attributed to the wrong specimen; and the tissue and associated DNA – as well as all the time and money put into obtaining and processing it – will be worthless.

Prevention of Cross-Contamination

The ability to amplify miniscule amounts of DNA has increased dramatically within recent years, and with it, the potential to accidentally magnify contaminant genetic material. In order to avoid cross-contamination, it is imperative that meticulous attention be paid to cleaning and sterilizing tissue sampling equipment.

Care should be taken not to touch the target tissue or working ends of sampling equipment. Tissue sampling tools such as scissors, scalpels, and forceps need to be sterilized before taking each new sample. This can be performed by rinsing the tool thoroughly with at least 70% ethanol solution, and then igniting with a lighter; or by washing thoroughly with at least 10% sodium hypochloride (bleach) solution, and then rinsing thoroughly in water to remove any residual bleach (Mayden and Dillman, 2008; Prince and Andrus, 1992). If the former option is employed, extreme caution must be used not to accidentally expose ethanol or other flammable chemicals to any open flame. If these sterilization methods prove infeasible, equipment may simply be thoroughly rinsed in between samples (Templado et. al., 2010).

When adding ethanol solution to the vials, care must also be taken to ensure that pipettes and other equipment are adequately sterilized. The best approach is to employ disposable plastic pipettes or 2 ml pipettors with disposable tips that are thrown away after use with each individual sample. If this is not cost effective, it is possible to sterilize pipettes using 10% sodium hypochloride solution and then thoroughly rinsing in water, but this will damage plastic equipment over time, and residual sodium hypochloride may compromise DNA fidelity if pipettes are not adequately rinsed between bleach washes. The best option would be to prepare vials with approximately 1-1.3 ml of ethanol prior to collecting, and then following these procedures to top off the vial after tissue collection.

Procedural Summary

In summation, the tissue sampling procedure should proceed as follows:

1.) Biorepository ID numbers are recorded in the appropriate ledgers prior to collection event.
2.) Prepare all vials prior to the collection event. Appropriate labels are applied by hand to vial exterior. Corresponding labels are placed inside the vial, and the vials filled with 95% non-denatured ethanol solution.
3.) The general sample is taken.
4.) The target specimen is promptly isolated from the general sample, and either processed immediately or briefly stored in cool seawater.
5.) Fresh gloves are put on by anyone working with the specimen and sample.
6.) Tissue sampling equipment is sterilized using either 70% ethanol and fire, or 10% sodium hypochloride solution and water rinse, or a thorough water bath.
7.) If necessary, the specimen is exposed to a relaxant.
8.) A tissue sample between the size of a lentil and a pea is promptly extracted in accordance with the taxa-specific tissue sampling guide below.
    a. If a specimen is too small to extract a tissue sample, the entire specimen is placed in the cryovial, and an identical specimen is kept as the morphological voucher.
9.) The tissue sample is submerged in a water bath to remove organic detritus.

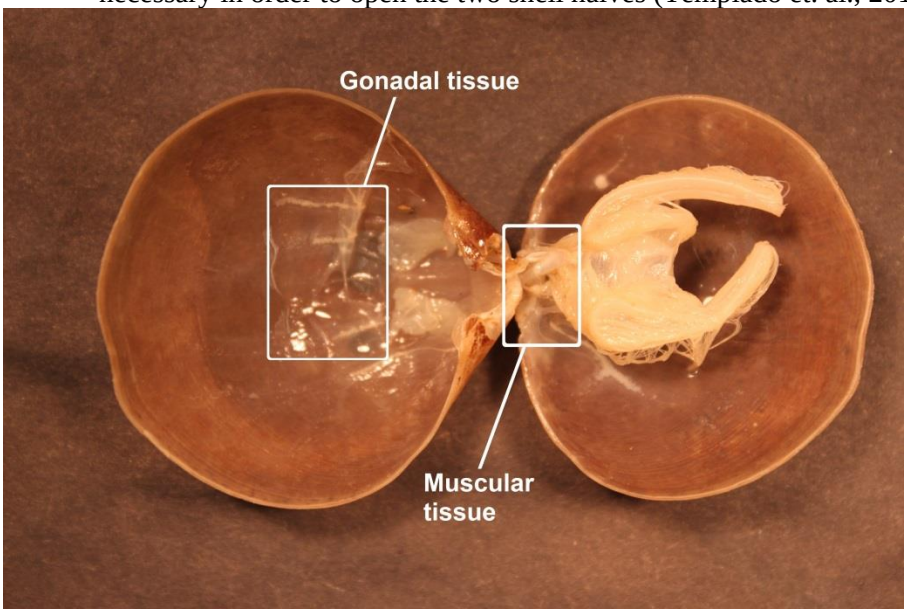
10.) The tissue sample is promptly placed within a new labeled vial, and the vial is topped off with 95% ethanol solution up to the 1.8 ml fill line.
11.) The voucher specimen is placed in a jar featuring the complementary ID label and topped off with the appropriate preservative.
12.) The tissue sample and voucher specimen are promptly moved to liquid nitrogen-cooled storage or a freezer.
13.) Station data and other necessary information are recorded in the appropriate ledgers alongside their corresponding biorepository numbers.

Taxa-Specific Tissue Sampling Guide

Due to their widely varying morphologies, different invertebrate taxa will need to have tissue samples taken from different portions of their anatomy. The following is a general guide to tissue sampling various marine invertebrate groups.

Brachiopoda:

Brachiopods will need to be opened, and the muscular tissues holding the shell halves together sampled. The gonads may also be used if necessary (Stechmann and Schlegel, 1999). A relaxant agent such as magnesium chloride or propylene phenoxitol may be necessary in order to open the two shell halves (Templado et. al., 2010).



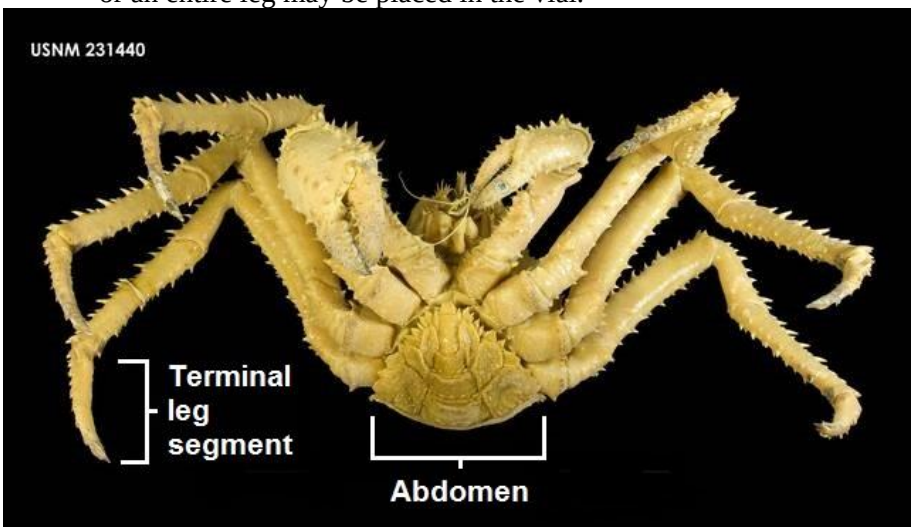
Crustacea:

The legs of a crustacean present the best option for a tissue sample. The sample should be derived from a middle leg, as the fore and hind limbs may be useful for species identification (Radulovici et. al., 2009). Several millimeters of abdominal tissue represent another good sample source, and may be used for any sized specimen where leg samples alone do not provide an adequate sample size, or are difficult or impossible to obtain (Karen Reed). Gonadal tissues may also be used (Geller et. al., 1997).

In large specimens whose terminal leg segments exceed the size of the vial, the terminal leg segment should be clipped, the shell removed, and a portion of the leg tissue stored in the vial.

In specimens whose terminal leg segments fit adequately within the vial, the terminal leg segment may be placed in a vial whole if removal of the shell is difficult or impossible.

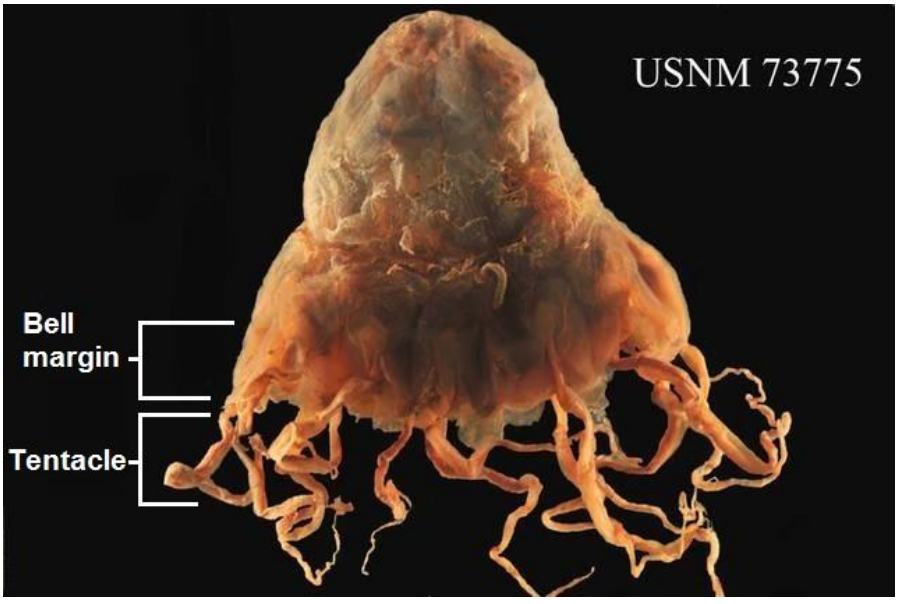
In specimens whose terminal leg segments are relatively miniscule, several leg segments or an entire leg may be placed in the vial.



For the smallest specimens, the entire organism may need to be placed in a vial. Barnacles present a unique challenge, as they do not possess obvious legs from which to sample. The muscular peduncle at the base of the barnacle contains the best tissues to sample; however, this may require some dissection, and not be feasible in the field. A general sample from the barnacle's soft inner tissues may be used instead (Van Syoc, 1994).

Cnidaria:

Typically, a tentacle will be the ideal tissue to sample in Cnidarians. Do not handle tentacles with your hands because some nematocysts remain active. You will get stung! Simply clip a whole tentacle or portion of a tentacle and place it in the vial. In very small specimens, or specimens lacking tentacles, up to half of the bell or main body may be used (Cheryl Ames). Bell margin, oral arm, and gonad tissues may also be used ("Jellyfish Sampling…").



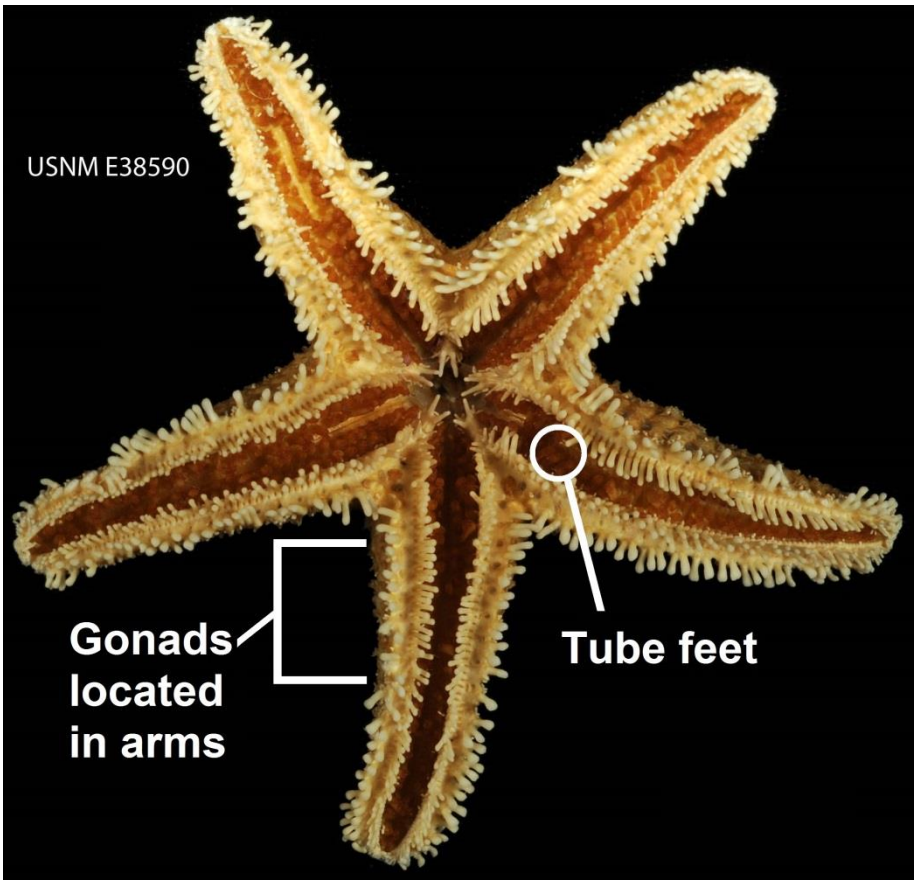
Care should be taken to avoid sampling tissues from or near to the stomach, as these may contain a high concentration of DNA-degrading enzymes.
Anenomes should be relaxed thoroughly with menthol, as they will contract their tentacles if agitated while active (Templado et. al., 2010).
For Anthozoa and other colonial Cnidaria - The polyps should be harvested. If the polyps are not large enough to sample individually then a cluster should be taken.

Ctenophora:

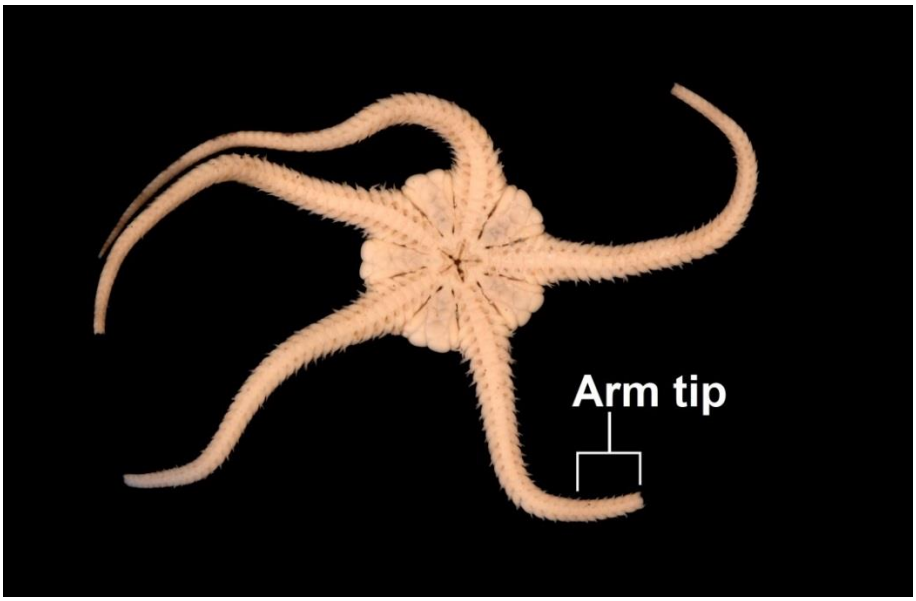
In comb jellies, comb-row tissues provide the best DNA yield. Body tissue may be sampled instead, but care should be taken to avoid sampling tissues from or near to the stomach, as these may contain a high concentration of DNA-degrading enzymes (Dumont et. al., 2004).

Echinodermata:

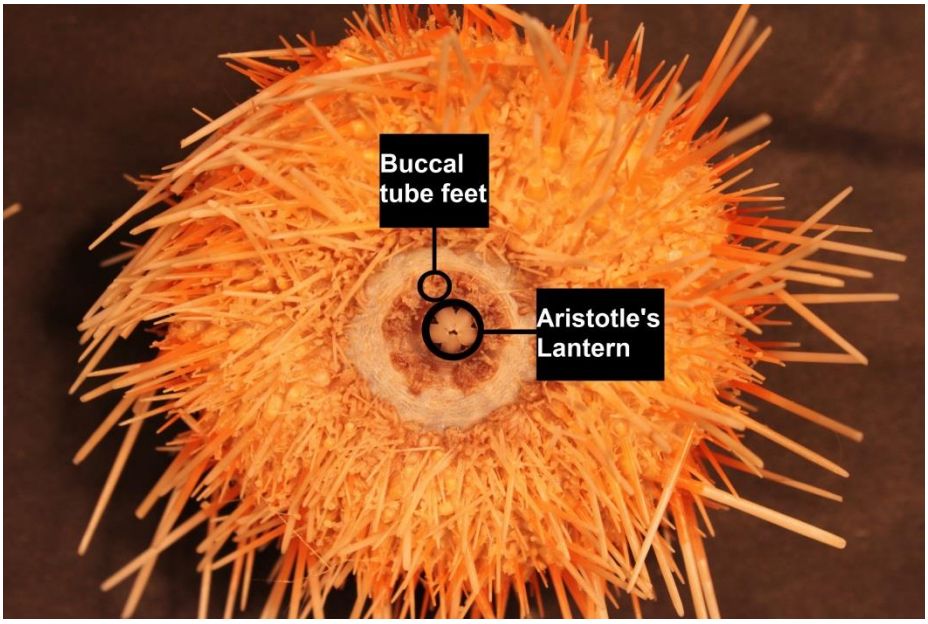
Sea stars should have samples taken from the tube feet, arms, or gonads; these tissues may be sampled by inserting forceps through a cut in the peristomial membrane (Ward et. al., 2008; Templado et. al., 2010).



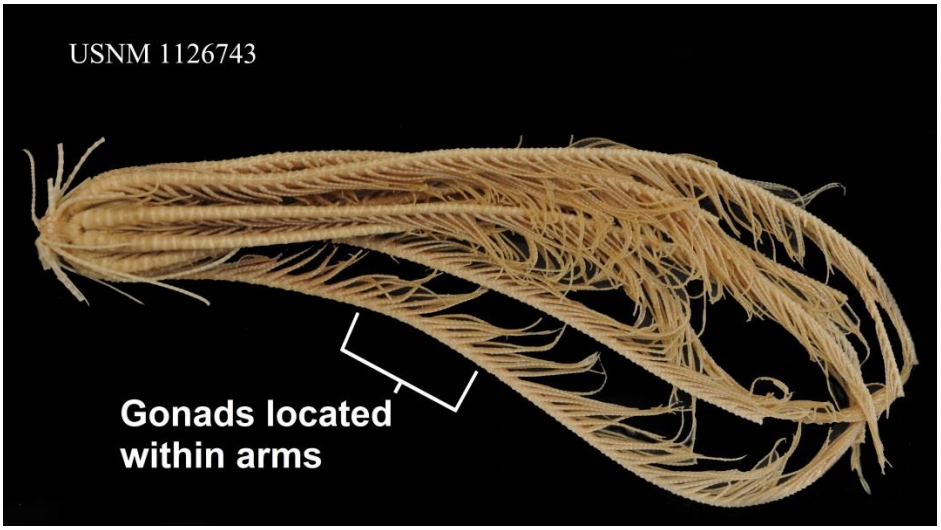
Brittle stars should have samples taken from arm tips (Sponer and Roy, 2002).



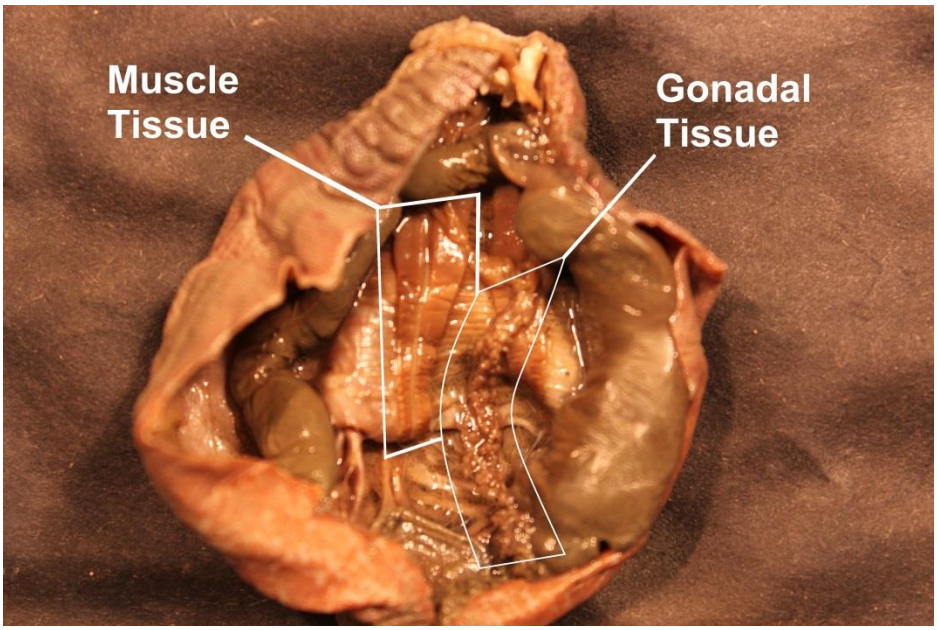
Sea urchins and sand dollars should have samples taken from the tube feet, gonads, or the muscular tissue surrounding Aristotle's lantern (Edmands et. al., 1996).



Crinoids should have samples taken from the gonads. If possible, these samples should be rinsed in ethanol to remove both organic detritus and excess mucus.



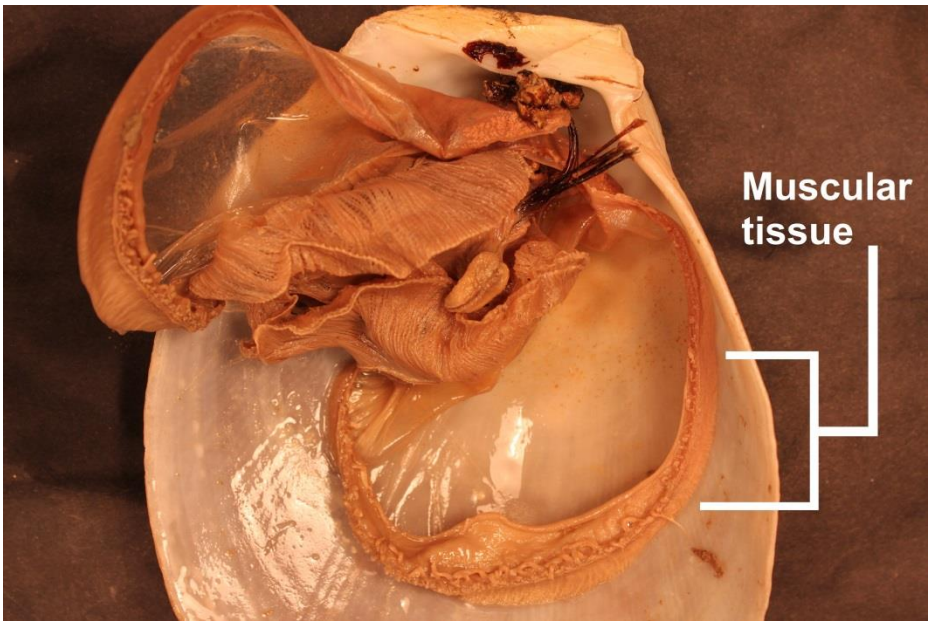
Holothurian gonads, gut, or inner body wall muscle tissues are the best for DNA sampling (Templado et. al., 2010). Alternatively, they may be slit open and the hemolymph "bled" out for a DNA-rich sample (Xu & Doolittle, 1990). Tentacle samples should be avoided if possible, as they may be useful in identification.



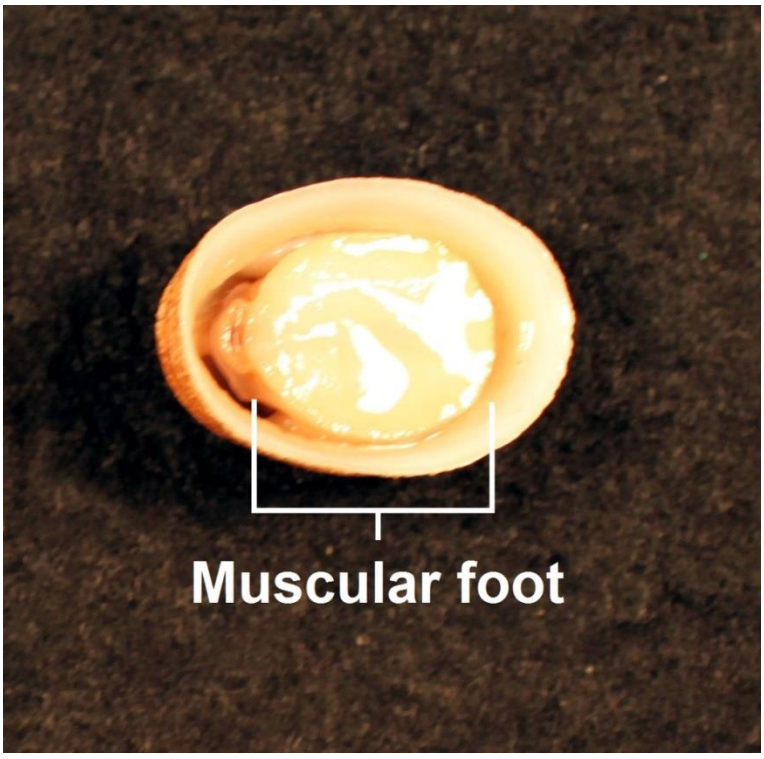
Ophiuroids, asteroids, and echinoids may need to be relaxed in magnesium chloride solution. Holothurians may need to be relaxed in magnesium chloride or chloretone solution.

Mollusca:

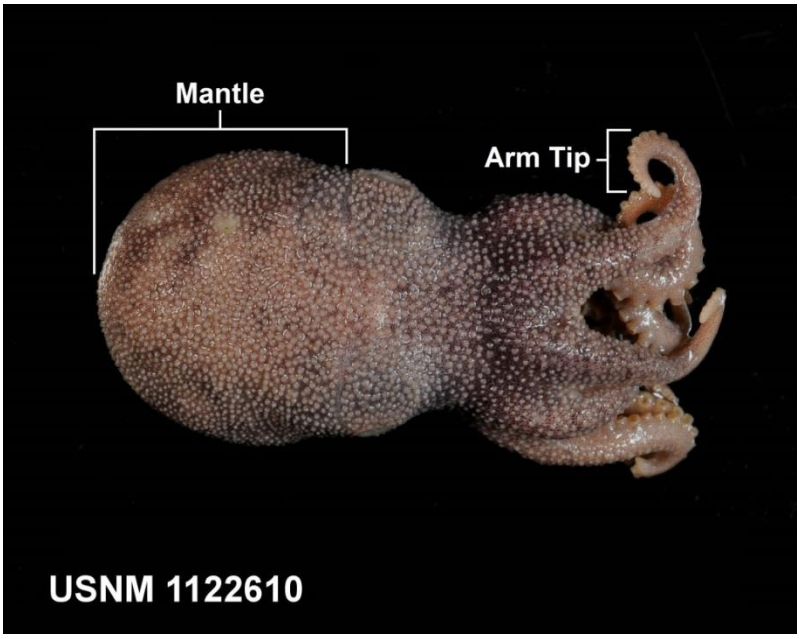
Bivalve mollusks will need to be opened and tissue samples taken from the left and/or right mantle (Fisher and Skibinski, 1990). A relaxant agent such as magnesium chloride or propylene phenoxitol may be necessary in order to open the two shell halves (Templado et. al., 2010).



Gastropods should have samples taken from the muscular foot tissue (Sokolov et. al., 2002). This may prove difficult if the specimen has retracted into its shell, necessitating the use of other methods. One such method is to allow the specimen to crawl along a sterile glass surface, i.e. a petri dish lid, and cutting a tissue sample from the trailing foot with a scalpel. If the shell is unimportant, it can also be drilled with a Dremel tool or other device to extract a tissue sample (Chris Huddleston).



Cephalopods should have samples taken from the arm tips (**not** the tentacle tips – arms are the shorter appendages, tentacles are the two longer ones), or mantle if necessary (Soller et. al., 2000). The tissue sample should minimize the presence of colored skin tissue, as it may contain pigments that inhibit PCR (Predini et. al., 2002).

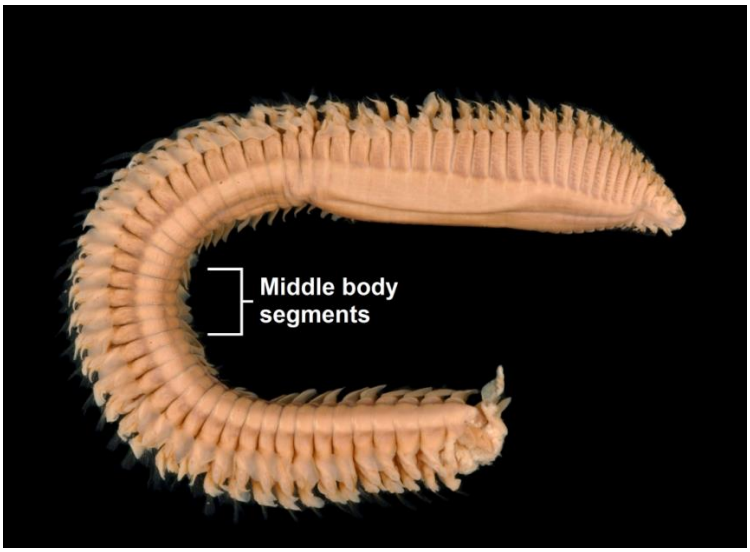


Platyhelminthes:

A small piece of the tail end may be snipped off for tissue sampling (Templado et. al., 2010).

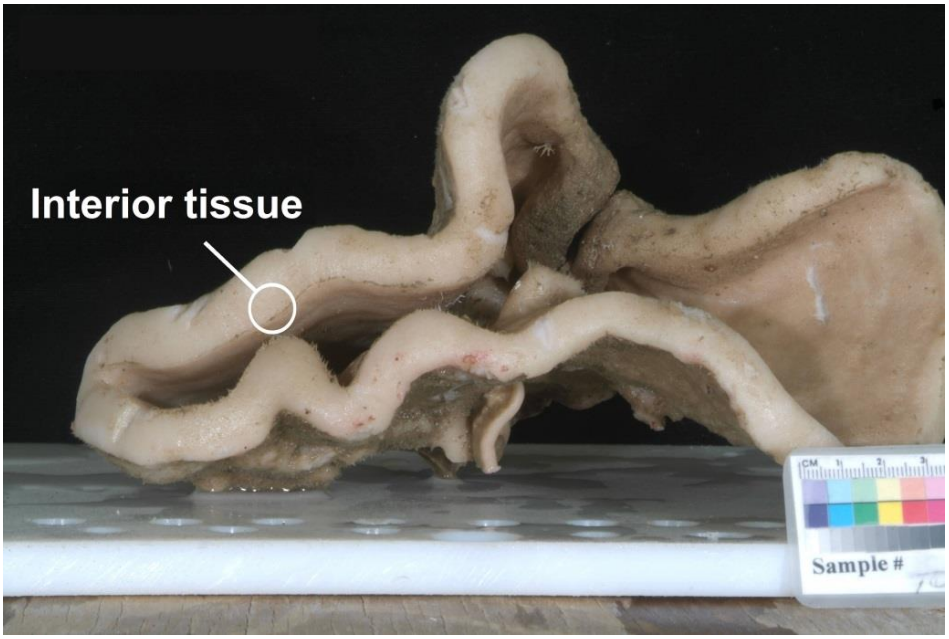
Polychaeta:

Polychaete samples should be taken from one of the middle body segments. The anterior and posterior ends of the polychaete should be avoided, as these may contain taxonomically-significant features (Patti and Gambi, 2001). Specimens may need to be relaxed in magnesium chloride solution prior to tissue sampling (Templado et. al., 2010).



Porifera:

Tissue should be collected from the interior of a sponge to avoid contamination by algae, bacteria, and other commensal organisms living within its surface (Borchiellini et. al., 2001).



Relaxants

<u>This information is taken entirely and nearly verbatim from Templado et. al., 2010.</u>

Magnesium Chloride: a 7.5% by weight solution of $MgCl_2$ and freshwater, mixed in a 1:1 ratio with seawater, will create a good general relaxant. $MgCl_2$ in solution competes with calcium in the muscles and nerves, preventing a specimen from contracting. Exact amounts are unnecessary.
Menthol: works especially well on cnidarians and ascidians. Add crushed crystals directly to a dish containing the specimen, or add drops of concentrated menthol solution prepared in ethanol.
Chloretone/Chlorobutanol: prepared in a concentrated solution with ethanol. A few drops to a dish, or a pipette full to a bucket, works well for echinoderms, especially holothurians.
Clove Oil: prepare a saturated solution in seawater and add to a dish containing the specimens. Works well for crustaceans.
Propylene phenoxitol: a couple of drops added to a dish will rapidly relax bivalves and other invertebrate specimens. If animals come in contact with the oil, which will float on the surface, they may retract.

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Mayden, Richard; Dillman, Casey (2008). "Tissue and Fish Sample Collection Protocols." *Cypriniformes Commons*. Retrieved from: <http://cypriniformes.org/tissue_fish_%20sample_col_protocols.html>.

Ward, R.D.; Holmes, B.H.; and O'Hara, T.D. (2008). "DNA barcoding discriminates echinoderm species." *Molecular Ecology Resources*, vol. 8, iss. 6 (pp. 1202-1211). Retrieved from: <http://onlinelibrary.wiley.com/doi/10.1111/j.1755-0998.2008.02332.x/full>.

Van Syoc, Robert Jay (1994). "Molecular Phylogenetics and Population Structure Derived from Mitochondrial DNA Sequence Variation in the Edible Goose Barnacle Genus *Pollicipes* (Cirripedia, Crustacea)." California Sea Grant College Program, *Research Theses and Dissertations* (pp. 15).

Xu, Xun; and Doolittle, Russel F. (1990). "Presence of a vertebrate fibrinogen-like sequence in an echinoderm." *Proceedings of the National Academy of Sciences USA*, vol. 87 (pp. 2097-2101). Retrieved from: <http://www.pnas.org/content/87/6/2097.full.pdf?origin=publicationDetail>.

CHAPTER 6 – STATION DATA

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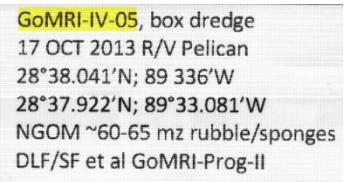
Introduction

Station data refers to all of the parameters that describe a collecting event: the WHERE, WHEN, WHO and HOW of a specimen. WHERE was it collected - essentially the x, y, z coordinates of where the object was located at the time of collection along with a description of the locality. This may include values like latitude, longitude, ocean, sea, country, biogeographic region, microhabitat, depth and elevation. WHEN it was collected is the date and time when the collection was made. WHO collected it may be an individual's name, an organization, or both. HOW it was collected includes the vessel and piece of gear used. Along with the typical station data various ecological parameters may also be gathered as part of the collecting event. These could include measurements of temperature, salinity, light penetration, dissolved oxygen and grain classes and size. The more information we are given about the collection of the object the better. Information regarding WHAT was collected (identification of the specimen(s), who identified it, number of specimens, sex, preservative used, etc.) are specimen data and not considered part of the station data.

A station data record may apply to a single lot, or specimen, or it may represent multiple lots. For example, a deep sea submersible may pick up a single shrimp with one of its suction devices so the station data representing the specific location, point in time and gear applies just to that shrimp. If during the dive a grab device picks up a starfish and places it in a collecting bucket that starfish would have a separate station data record. Many of the elements would be the same in both but because the time and gear are different they would not have the same station data record. On the other hand the station data for a single dredge haul would apply to every shrimp, crab, worm and fish pulled from that haul. Sampling that is done at the same geographic location over several seasons or years are separate collecting events and would have separate station data records.

Relational databases make it possible to create a single station data record for each collecting event and share that record with all of the specimens and their resulting specimen records that came from the event. In order to do this efficiently one needs an identifier that can tie the specimen data with the station data. Ideally, this identifier should be unique or at the very least it should be unique within an expedition or project. For cataloging in Invertebrate Zoology we have made use of the Field or Sample number to provide this link. How the sample number is constructed has varied. Sometimes they are assigned in the field and other times they have been constructed after the fact as a way to identify station data records via the data provided on specimen labels.

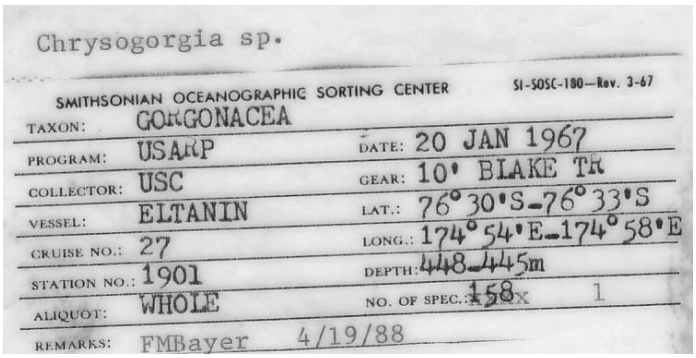
Below is an example of a label with the sample number highlighted. This sample number was assigned by the collector and links 7 specimen records with a single station data (collection event) record. It can save time in the field if you are keeping a master station data file while collecting because you can just write a label that says "GoMRI-IV-05" to drop in the vial with the specimens, you don't need to repeat the date, latitude/longitude etc. on each label.



In the next example there was no field assigned sample number that represented a single collecting event. Upon examining all of the station data it turned out that it took the following to make a unique sample number:

Program/First 2 letters of the Vessel/Cruise #/Station #/Collector

So "USARP/EL/27/1901/USC" is the only piece of data we need to put in the specimen record to link it to the single collection event/station data record. That saves a lot of key strokes and it means all of the attached specimen records, 75 in this example, have the same station data. If an error is found it only needs to be corrected once to have it reflected in all of the specimen records.


Chrysogorgia sp.

SMITHSONIAN OCEANOGRAPHIC SORTING CENTER SI-SOSC-180—Rev. 3-67

TAXON: GORGONACEA
PROGRAM: USARP DATE: 20 JAN 1967
COLLECTOR: USC GEAR: 10' BLAKE TR
VESSEL: ELTANIN LAT.: 76°30'S-76°33'S
CRUISE NO.: 27 LONG.: 174°54'E-174°58'E
STATION NO.: 1901 DEPTH: 448-445m
ALIQUOT: WHOLE NO. OF SPEC.: 158x 1
REMARKS: FMBayer 4/19/88

We have other collections where we have created sample numbers after we have received the collections in order to facilitate cataloging but in each case we have ended up using a different combination of data from the labels to do it. It is much simpler if a sample number similar to the GoMRI example above is created in the field and used on all of the specimen labels.

Consider adding leading zero's to the sample numbers in order to facilitate sorting on sample number. For example, 1, 2, 3, 4, 20 and 30 sort as follows:

Without leading zero's	With leading zero's
1	01
2	02
20	03
3	04
4	20
40	40

Below is the list of the fields we want to have for specimens in our collection. The actual data will vary depending on where you are collecting. We don't expect ocean for specimens collected from the great lakes and nor do we expect elevation for specimens collected in the middle of the Atlantic Ocean.

Field or Sample Number (unique identifier for each collection event)
Station (optional)
Ocean (Sea/Gulf, Bay/Sound)
Country (State, County, City/Town)
Precise Location (text description of locality, nearest named place)
Latitude/Longitude (UTM or Township/Range/Section if its land based collecting)
Date Collected
Time Collected
Collector (Person and/or Organization)

Expedition Name (optional)
Vessel Name (where appropriate)
Cruise or Dive number
Collection Method (Gear)
Depth Collected
Depth (Bottom depth where different from depth of collection)
Elevation (where appropriate)
River Basin (where appropriate)
River Drainage (where appropriate)

Data Standards for Station Data Fields

1. **Field or Sample Number**

Definition: A unique number that is assigned to a collecting event. Ideally, it is assigned in the field. A number unique within a given collection that identifies a specific set of collecting parameters including, but not limited to, location, time, date, collector, and method. When searching for data, EMu, our cataloging software, treats most punctuation as if it's a space allowing one to search for pieces of sample numbers without having to utilize wildcards. By using "MMS" at the front of all of our MMS collections we can easily retrieve all of them and "MMS-Program name" lets us hone in on the records from a single program. The underscore is an exception to this behavior and should be avoided. If "MMS-ASLAR/MA1:01-BC1" were formatted as "MMS_ASLAR_MA1_01_BC1" the record would not be found if you searched for "mms" without using the wildcard "mms*".

Valid Examples:
06B-B3/2
HE/721/1A
772
RH-26
IS/876/115/SOSC
GoMRI-IV-05
MMS-ASLAR/MA1:01-BC1
USGS-GM-LOPH I-2005-04-JSL-4873; Bucket 9

Notes: For programmatic collections and expeditions, the value entered in this field serves as the link to attach the corresponding collecting event and site records to a catalog record

2. **Station**

Definition: The number assigned by the collector to a specific collecting site or locality.

Valid Examples: 50-A, 102, 3-1-N

Notes: The station number is recorded exactly as on the label except in those specific instances where there is an official station number recorded in the field notes or a cruise log.
Include all punctuation such as spaces, dashes, commas, periods and slashes.

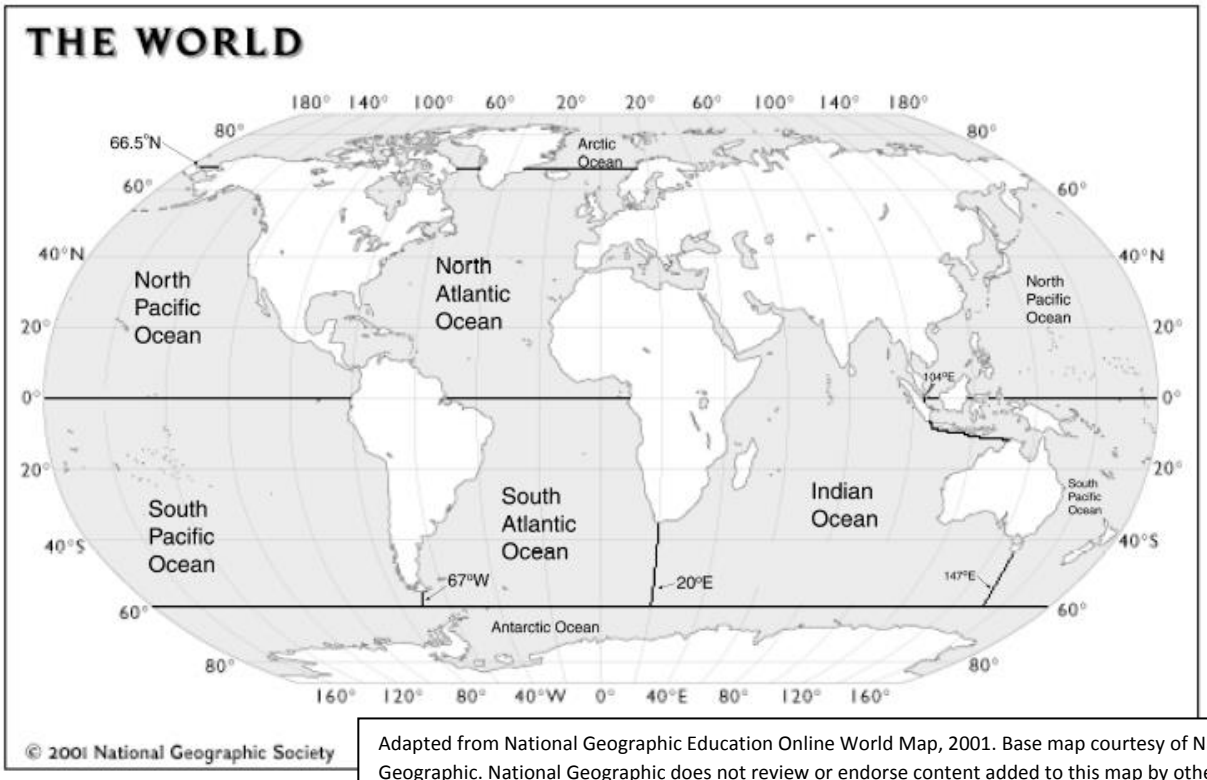
3. **Ocean [mandatory for marine collections]**

Definition: The ocean from which a specimen was collected.

Valid Examples:
Antarctic Ocean
Arctic Ocean
Atlantic Ocean (when you don't know if it's North or South)
North Atlantic Ocean

North Pacific Ocean
Pacific Ocean (when you don't know if it's North or South)
South Atlantic Ocean
South Pacific Ocean
Indian Ocean

Notes: This field must be completed for all marine or estuarine samples. See the Oceans Map below for the boundaries of the oceans.



Adapted from National Geographic Education Online World Map, 2001. Base map courtesy of National Geographic. National Geographic does not review or endorse content added to this map by others.

4. **Sea/Gulf/Bight**

Definition: The sea, gulf, or bight from which a specimen was collected. Separate values with commas.

Valid Examples:
Mediterranean Sea
Mediterranean Sea, Adriatic Sea, Gulf of Trieste
Gulf of Mexico
North Atlantic Bight
Caribbean Sea, Gulf of Honduras

Invalid Examples: Mississippi River Estuary [not a sea, gulf or bight]

Notes: The word "sea", "gulf", or "bight" MUST be considered part of the officially recognized name of the body of water.

5. **Bay/Sound**

Definition: The bay or sound from which a specimen was collected.

Valid Examples:

Achong Bay
Bahia Concepcion, Bahia Coyote
Barkley Sound, Scott Bay
Chesapeake Bay
Kaneohe Bay
Long Island Sound

Invalid Examples: “off Kaneohe Bay” (put that in Precise Locality)

Notes: The word "Bay" or “Sound” MUST be considered part of the officially recognized name of the body of water. We have left many of the locations with the Spanish “Bahia” instead of anglicizing them.

6. **Country [mandatory for terrestrial and freshwater collections]**
Definition: Named country, including independent island nations and sovereignties, from which the specimen was collected.

Valid Examples:
Bahamas
United States
Italy
Marshall Islands

Notes: Guam and Puerto Rico are treated here as if they are a country

7. **State [mandatory for terrestrial collections, desirable for near shore marine collections]**
Definition: The named Division of country, including Province, State, Territory, Department, Region, or Prefecture from which the specimen was collected.

Valid Examples:
Western Australia
Queensland
New York
Mississippi
Aquitaine
Alsace
British Columbia
Quebec

Invalid Examples:
Oregon State,
Washington, DC use District of Columbia in this field and put Washington in the City field

Notes: Include the term "state", "province", "prefecture" in the name only if the term is considered part of the proper name.

8. **County**
Definition: The named District, County, Shire, or Parish from which the specimen was collected.

Valid Examples:
Monroe County
Allen County and Hancock County
Allen County or Hancock County
Hamilton Parish

Invalid Examples: District of Columbia [The District of Columbia is entered in "City"]

Notes: Include the term "district", "county", "shire" in the name only if the term is considered part of the proper name.

Islands are included when they are the equivalent of a state, prefecture, or a county. Example: Vancouver Island, where country is British Columbia and city is Victoria. When in doubt, put the data in Precise Locality.

9. **City/Town**

Definition: The name of the city or town from which the specimen was collected.

Valid Examples:
Chicago
Harrison Township
Paris
Quezon City
Sydney
Victoria

Invalid Examples: Los Angeles County

Notes: Include the term "city", "town" in the name only if the term is considered part of the proper name.

10. **Precise Location**

Definition: The proper names of geopolitical entities that do not appropriately belong in any other field. Any textual locality information that does not belong in one of the other location fields, that is, it is not an Ocean, Country, State, etc. Detailed directional information that clarifies or enhances the other available locality information.

Valid Examples:
3 miles North of Kaneohe Bay
Suzhou, On Rocks in Canal, Near Shanghai,
Vinales Power Canal of Rio San Vicente at Km 35.3
2 miles North West of lighthouse
5 miles east of Highway 64
10 miles east of Oahu

Invalid Examples:
Shanghai (put in City/Town)
Chesapeake Bay (put in Bay/Sound)

Notes:

- Enter more general locality entity to smaller locality entity.
- Every other geographic location on the label goes in this field including the names of lakes, rivers, creeks, peninsula, highways, ocean currents, straits, channels reefs, Islands, Archipelagos.
- In the example, 3 miles North of Kaneohe Bay, you may also enter Kaneohe Bay in the Field: Bay/Sound.
- Directional information must be entered clearly and concisely.
- Spell out standard compass directions (north, south, east, northeast) and distance measures (miles, meters, feet) unless only one interpretation of the abbreviation is possible (consider that the data may be viewed by someone not familiar with casual English abbreviations)

- A string of directional descriptors may be entered separated by commas.

11. **Latitude [Mandatory for BOEM collections]**
The latitude from which the specimen was collected. Enter the value as presented do not add zero's which could affect precision. Enter either a Decimal Latitude or a DMS latitude, do not enter both.

Decimal latitude – latitude presented as decimal degrees from 0 to 90 (positive numbers for Northern latitudes) or -0 to -90 (negative numbers for southern latitudes)

Valid Examples:
33.291
-62.7

DMS latitude –latitude presented as degrees minutes seconds, or degrees minutes decimal seconds. 0 to 90N or 0 to 90S

Valid examples:
50 13 12N
50 15N depending on accuracy of measurements you may also have 50 15 00N but don't add zero's unless it's part of the measurement.
50 23.4S

12. **Longitude [Mandatory for BOEM collections]**
The Longitude from which the specimen was collected. Enter the value as presented do not add zero's which could affect precision.

Decimal Longitude – Longitude presented as decimal degrees from 0 to 180 (positive numbers for Eastern Longitudes) or -0 to -180 (negative numbers for Western Longitudes)

Valid Examples:
134.87
-62.7
-50
180

DMS Longitude –Longitude presented as degrees minutes seconds, or degrees minutes decimal seconds. 0 to 180E or 0 to 180W

Valid examples:
50 13 12E
50 15W depending on accuracy of measurements you may also have 50 15 00W [Don't add zero's unless it's part of the measurement]
50 23.4E

13. **Date Collected [Mandatory]**
Definition: The date(s) when the sample was taken. Usually, this will be a single value placed in the "Starting Date Collected" Field but sometimes a collecting event may start on one date and end on another. For example, a plankton tow starts at 10 pm on June 1 and runs for 3 hours will have different starting and ending dates [01/JUN/2012 TO 02/JUN/2012].

Starting Date Collected: formatted as dd/MMM/yyyy

Valid Examples : 01/APR/2011, 30/SEP/1999

Invalid examples: 06/07/1989 (no idea if the month is June or July); APR/2/2000 (need to have day before month)

Ending Date Collected – add a value here if it's different from the starting date and be sure to then fill in a value for the Date Conjunction field. The date should be formatted the same way as the Starting date.

Date Conjunction – The word describing the link between the starting and ending dates collected.
Valid Examples: To, And, Between, Or

Verbatim Date/Time – With expedition and program collecting you usually have a very specific date recorded as each collecting event is performed but with museum collections one might run into imprecise dates or collecting times. Use this field when you have seasons, dates or times with comments.
Valid Examples: Spr 1995, about June 3 1997, circa 1800, around midday

14. **Time Collected**
    Definition: The time a sample was taken. A 24 hour clock is used so time between 1 minute after midnight and noon go from 00:01 to 12:00. After noon add 12:00 to all values so 1 pm is 13:00, 2:05 pm is 14:05 etc.
    Valid Examples:
    13:05
    1:05
    Enter imprecise times (around noon, morning, etc. in Verbatim Date/Time
    In the case of some collecting methods (tows, dredges) there may be the time the device was deployed and the time it was retrieved in which case enter values in the starting and ending time fields.
    Starting Time: time a device (tow, dredge, etc.) is deployed
    Ending Time: time device is retrieved

15. **Collector**
    Definition: The person(s) or organization that made the collection.
    Person:
    Format for single individual: Last, First Name (or initial) Middle Name or initial
    Walter, T. Chad
    Cleveland, Grover
    Pei, I.M.
    Edwards (if all you have is the last name that's better than nothing)

    Format for multiple individuals: Last, First Name (or initial) Middle Name or initial **and** Last, First Name (or initial) Middle Name or initial
    Edwards, B. and L. Coffin
    Pawson, David E. and Cindy Ahearn

    Organization: Use this if you only have an organization name and no individuals. Spell out the organization name
    Valid Examples: Battelle for MMS
    National Oceanic and Atmospheric Administration
    United States Coast Guard

16. **Expedition Name or Program Name**
    Definition: The name of the formal expedition or program during which the collecting event took place.

Valid Examples: Albatross Philippines Expedition
Antarktis XIV/2
Bathus 4
USGS CHEMO III CRUISE CENTRAL GULF OF MEXICO
ASLAR
CGPS

Notes: Acronyms are acceptable if used consistently. So in the last two above we have used ASLAR instead of the spelled out versions: "Atlantic Slope and Rise" or "Central Gulf Platform Study" which appear elsewhere in the catalog record. The abbreviations fit better on the specimen labels that we print after cataloging.

17. Vessel Name
Definition: The name of the vessel used during a specific collecting event.
Valid Examples:
Albatross IV R/V
Dimitry Mendeleev R/V
Fish Trawler
Fishing Boat

If you wish to include both a submersible and the "mother" ship. Put the submersible first followed by the "mother" ship with a semi-colon and space between.
Alvin DSR/V; Atlantis R/V
Johnson Sea Link II DSR/V; Seward-Johnson R/V
Jason II ROV; Ron Brown R/V

Notes: Record ship designation (i.e. R/V, M/V, ROV etc.) AFTER the vessel name so it will sort by the vessel name rather than the designation.

18. Cruise or Dive number
Definition: The number assigned to a specific portion of an expedition or the specific dive number assigned to a submersible dive.
Valid Examples:
II-27
2B
14
JSLII-09-GOM
SJ-02

Notes: The cruise number should be recorded exactly as it is reported.

19. **Collection Method (Gear)**
Definition: The specific method, gear, or equipment used to collect the specimen. In order to have the pick list we use for gear sort similar items together we have put the "kind" of gear first followed by the details. See the trawl and net examples below.
Notes: Acceptable values include
Rotenone
Rotenone with Palmolive
Trawl - Otter
Trawl - Blake, 5 ft

Trawl - Shrimp
Trawl - 45 ft
Trawl - Tri-net
ROV
Net – Dip
Net – Plankton
Net - Open Plankton, 4 ft

Depth – metric value [desirable for marine collections] – If you are measuring depth using a unit other than meters create new columns and indicate if they are feet or fathoms. In our catalog values added in one of the meters, feet or fathom fields will automatically be converted for the other fields (original value is in black; conversion is in red).

Starting depth– The depth where a gear such as a trawl or net first begins collecting specimens. If you only have a single depth value put it in "Starting depth"

Ending depth- The depth where a gear like a trawl or net is closed or concludes collecting. The ending depth is usually but not always a greater value than the starting depth.

Verbatim depth– use this for values like "intertidal", "near shore", "less than 1 meter".

20. **Bottom depth** – Lowest depth of the body of water where you are collecting. This is generally only captured when the collecting is being done in the water column rather than from the bottom surface.

CHAPTER 7 – PACKING AND SHIPPING COLLECTIONS

Prepared by Kristen Mercer (mercerk@si.edu; 301-238-1772), Paul Greenhall, Bill Moser, Courtney Wickel and Lisa Burnett

Introduction

The purpose of this document is to provide guidance regarding the packaging and shipment of BOEM ESP voucher specimens to the National Museum of Natural History (NMNH). This guide will focus on the packaging and shipment of specimens in regulated fluids (*e.g.* ethanol, formalin, etc.) in accordance with the US Department of Transportation (DOT) and International Air Transport Association (IATA) regulations. Specifically, this guide discusses packaging and shipment of fluid preserved specimens in accordance with IATA Special Provision A180 (and IATA A189) which allows scientific agencies to ship these parcels unrestricted if packed according to the IATA special provision.

Before you start the process of preparing a BOEM ESP voucher collection for shipment to NMNH, please contact Invertebrate Zoology (IZCollections@si.edu) with a detailed summary of the collection including number of lots, types of specimens, and the number and type of packages you are expecting to ship. If possible, send a photograph of the collection to the IZ Collections email so we can estimate the physical size of the collection and prepare to process it and add it to the collections.

*****Please Note: As of 2012, in accordance with the DOT Dangerous Goods Regulations, 49CFR-173.4, full HAZMAT certification training is no longer required to ship scientific research specimens in excepted and limited quantities, (nonetheless, anyone processing specimens according to IATA A180 and A189 Special Provision should be certified to do, and this training may be done by someone already certified). A person processing specimens preserved in a regulated fluid is expected to be informed of the Dangerous Goods regulations. The packer is responsible for selecting the appropriate shipment method.*****

Shipment Methods

It is illegal to Air Mail scientific research specimens in fluid via the US Postal Service (USPS). These shipments must be shipped utilizing a parcel delivery service, such as FedEx, UPS, or DHL. Dry preserved specimens may be mailed via USPS postal accountable, or another accountable delivery service.

There are a variety of different shipment methods available for the shipment of scientific research specimens. The method you choose depends mostly on two factors:

1. Whether you are shipping your specimen in regulated or non-regulated fluid. Most BOEM ESP Voucher collections are preserved in ethanol, formalin, isopropanol or another preservative fluid that is classified as a flammable and combustible liquid. Therefore, we are only going to concentrate on the shipment of Regulated fluids. Non-Regulated fluids (such as water, 24% ethanol) must also be identified prior to shipment.
2. The quantities (volume and weight) of fluid of each inner package (the package which encloses each individual lot) and outer container (total fluid in the total package, either cardboard box or shipping drum). A summary of shipment methods is given in the table on the following page.

Regulated Shipment Types for fluid preserved Scientific Research Specimens

Inner Package	Outer Package	Shipment Type
1 mL	100 mL	De Minimis
30 ml	1 L	IATA A180 or IATA 189
30 mL	29 kg/ 64 lbs	Small Quantity (ground: highway or rail)
30 mL	500 mL	Excepted Quantity (air)
500 mL	1 L	Limited
> 500 mL	30kg / 66 lbs	Full HAZMAT

IATA Special Provision A180

Scientific Research Specimens can be shipped unrestricted according to IATA Special Provision A180 (& A189). This is the preferred shipment method for the Invertebrate Zoology Department at NMNH. We recommend that all loans involving alcohol or other liquid preservatives be shipped according to IATA A180 via a parcel delivery carrier, such as FedEx, DHL, or UPS). IATA A180 (& A189) Shipments must meet the following requirements:

- Specimens are packaged appropriately using a heat sealer (see Section IV. Packing Instructions: IATA Special Provision A180).
- Inner packaging must not exceed 30 mL of free flowing fluid; Outer packaging must not exceed 1 L.
- Waybill is labeled “Scientific Research Specimens, Not Restricted, IATA A180 Applies.
- Also, the ”Package is marked “Scientific Research Specimens, not restricted, IATA A180 applies” See Figure below for an example of package.

Figure 1. Top of package shipped according to IATA A180. Please note the label marked “Scientific Research Specimens, not restricted, Special Provision A180” is affixed under the waybill. Inside the waybill is the shipping invoice.

Packing Instructions: IATA Special Provision A180 (heat sealer required)

Step 1 - Ensure proper, best practice containment of specimens to be shipped.

It is important to note that if a damaged or leaking parcel is intercepted while in-transit, heavy fines and levies will result. If this occurs, the parcel will be sequestered and both sender and receiver will be fined.

1. Specimens must be placed in a plastic bag that is heat-sealed or placed in a container and then in a plastic bag that is heat-sealed. Bag thickness should be no less than 4 mm. See Figure 2 for an example of packing a lot of multiple specimens. See Figure 3 for an example of packing a single specimen (Porifera) that must be shipped in a small container. See Figure 4 for an example of packing multiple small vials. See Figure 5 for an example of a heat sealing.
2. The free liquid within the bag may not exceed 30mL, though it should be as minimal as possible. When practical, it is recommended to drain as much free fluid as possible (note: this may not be possible with certain fragile or small specimens).
   According to IATA A189, less than 10% concentrations of formaldehyde (3.7% formaldehyde = 10% formalin) are acceptable and labeled as "Not Restricted".

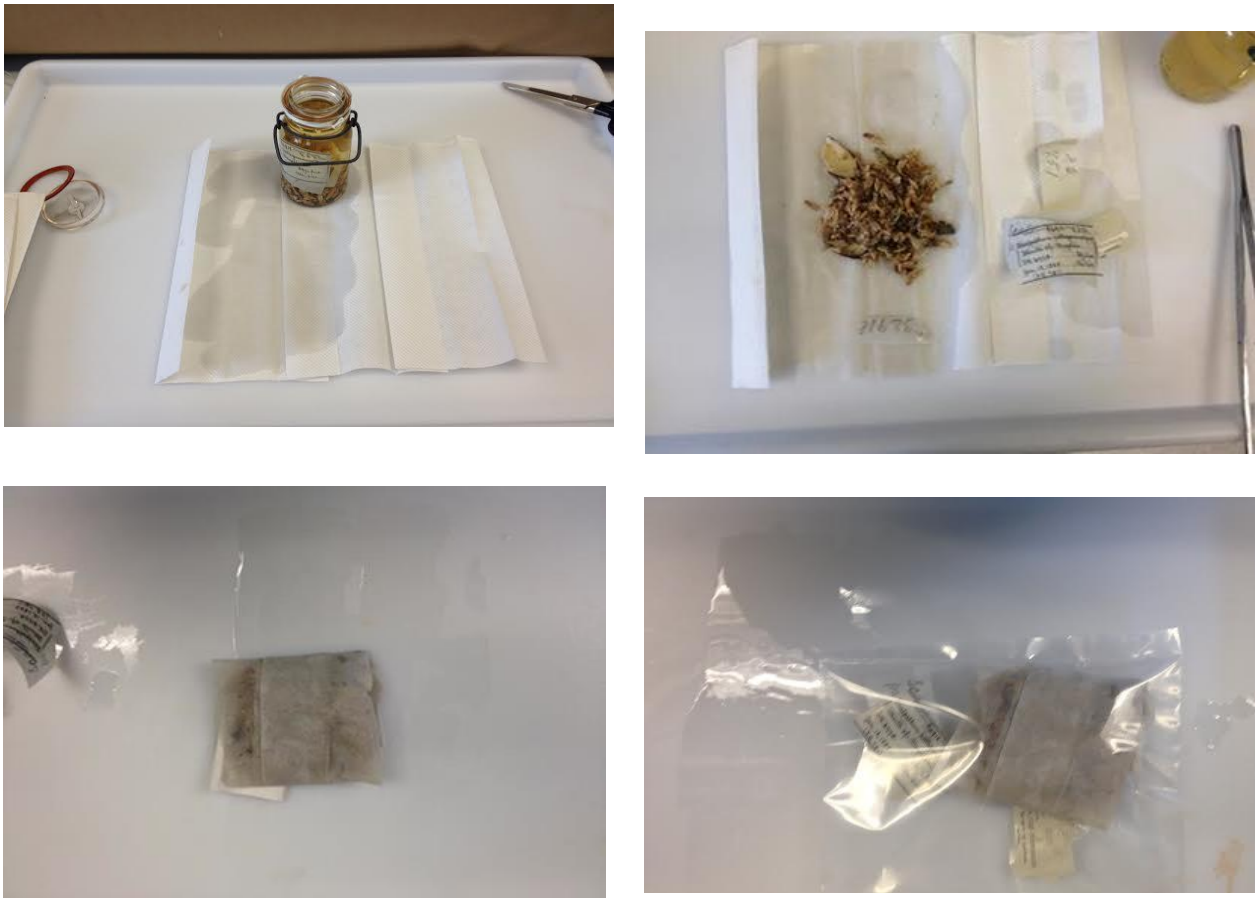


Figure 2. Packing a lot consisting of multiple specimens into innermost, heat sealed bag. Note that these may be placed on a paper towel, hydrated

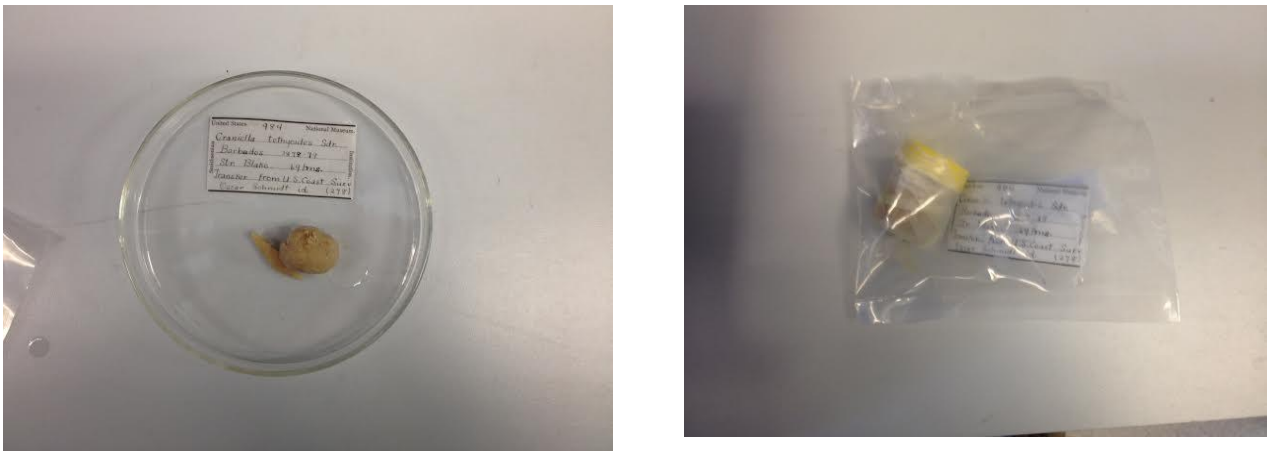


Figure 3. Packing a fragile lot (Porifera) that must be shipped in a small container, inside of a heat sealed bag.

Figure 4. Packing multiple small vials into innermost, heat sealed bag. Note the use of parafilm to secure the top of each vial

Figure 5. Packing using a heat sealer

Step 2 - Double bag and heat-seal specimens

The prepared specimen is then placed in another plastic bag and then heat-sealed.

Step 3 - Triple bag and heat-seal specimens

Bagged specimens must then be placed in another plastic bag and heat-sealed, for atotal containment of 3 heat-sealed plastic bags.

Parcel must contain sufficient absorbent material, such as Bounty-like paper towels, PIG Universal Mat, or vermiculite to absorb the fluid in the event of parcel leakage or damage. See Figure 6 for examples of triple bagged specimens with absorbent material.

Figure 6. Triple bagged and heat sealed specimens with PIG Universal Mat as an absorbent material in the third bag.

Key Things to Remember:

- When practical, it is recommended to drain as much free fluid as possible (note: this may not be possible with certain fragile or small specimens).
- Place specimen in plastic bag and heat-seal or place specimens in a container and then in a plastic bag and heat-seal. Place bagged specimen in a second bag and heat-seal. Finally, place in a third bag with absorbent material and heat-seal for a total containment of 3 heat-sealed plastic bags.
- If a damaged or leaking parcel is intercepted while in-transit, heavy fines and levies will result. If this occurs, the parcel will be sequestered and both sender and receiver will be fined.

Step 4 - Final outer packaging

The finished heat-sealed bags must then be placed in a container, such as a cardboard box, or other suitable shipment worthy container, and include bubble wrap or Styrofoam peanuts that will provide suitable cushioning for specimens.

It is crucial to note that specimens, regardless of whether they are stored in ethanol, isopropanol, or formalin, must be placed in three layers of heat-sealed bags and contain as little free-fluid as possible within the internal bag and solid container (no more than 30mL). The total quantity of liquid contained within the outer package cannot exceed 1L.

Step: 5: Outer package and corresponding paperwork must be clearly marked

All corresponding paperwork should be placed in the waybill pouch. The completed package and must be marked as "Scientific Research Specimens, Not Restricted, Special Provision A-180". This label must be affixed to the top of the parcel below the waybill pouch.

The waybill should contain the statement, "Scientific Research Specimens, Not Restricted, IATA A180". For example, this can be indicated on the waybill at "Your Reference" and "More Options" field online or manually. See Figure 1. for an example.

Key Items to Remember:

- The total quantity of liquid contained within the outer package cannot exceed 1L.
- Label parcel "Scientific Research Specimens, Not Restricted, IATA A180".
- Label the waybill "Scientific Research Specimens, Not Restricted, IATA A180".

Documentation

Any parcel containing Scientific Research Specimens being shipped according to IATA A180 must be accompanied with the following documentation placed inside the shipping waybill pouch:

- Shipping Invoice: this form should list the specimens, counts, preservative, and country of origin. It should be printed on agency letterhead, dated and signed.
- IATA A180 Label: this label should be affixed near the waybill pouch. See Figure 1 for an example.

Please Note: Packages being shipped according to other Dangerous Goods shipping methods, such as Expected Quantities, or Small Quantities for ground, may require specific labeling (such as the red bordered Dangerous Goods Label) and are NOT mailed or shipped unrestricted because they are considered Dangerous Goods. These packages may NOT have an IATA A180 label.