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CURATORIAL STANDARDS FOR MARINE ORGANISMS

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(Cummings; A/EA-1)

CURATORIAL STANDARDS FOR MARINE ORGANISMS

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CURATORIAL STANDARDS

I. Introduction

Heretofore, in the majority of biological sampling and monitoring programs, one of the most neglected areas has been the maintenance of biological collections on which the program reports are based. This most basic of all data, the organisms themselves, has often been discarded or at best, relegated to some storage facility with minimal or no monitoring as to the physical state of the collection. Such material has often been in such a condition that taxonomic validation is difficult if not impossible. It has become obvious that the overall taxonomic validity of many marine ecological surveys cannot be accepted on the printed reports alone. This report provides minimal guidelines for curatorial standards for preparation and maintenance of collections of biological specimens that serve as documentation of survey reports.

II. Materials

A. Fixing and Preserving Fluids

Specimens should be fixed and preserved as soon as possible after death to prevent tissue damages. It is imperative that the volume of fixative equal or exceed the volume of material to be fixed. After fixing, a final preservative must be utilized sufficient to maintain specimens for long-term periods.

1. Formalin

This is a good general fixative for plants and animals. Commercial formalin solution is concentrated and must be diluted before use. It is a saturated solution of gaseous formaldehyde, HCOH, in water. Formalin usually is 39% formaldehyde by volume but may vary from 37 to 40%.

Commercial formalin may decompose in storage, resulting in the production of a free acid that will damage specimens. When decomposition begins the liquid becomes yellow and may turn red-brown with further decomposition. For this reason formalin tends to be acidic and the acid has to be neutralized by buffering. Calcereous structures will dissolve in unbuffered formalin and for this reason formalin must always be buffered.

Either fresh or salt water may be used to dilute formalin. For marine specimens, salt water is preferred. The recommended dilution is 10% (1 part HCOH, 9 parts H_2O). The buffering agent should be added to the diluted formalin solution at least 12 hours prior to its use as a fixative.

Formalin has several practical advantages. Concentrate formalin can be carried in small quantities and diluted with either fresh or salt water to the required concentration as needed. It is non-inflammable and non-volatile. Further, it is not subject to legal restrictions of sale. Formalin has a serious disadvantage. It is highly toxic and prolonged contact may cause skin or respiratory inflammations or other allergenic reactions. For protection one should dilute and use formalin in a well-ventilated area. Rubber gloves and protective eye glasses should be considered when working with formalin of all strengths. All plastic and glass containers used for formalin should be clearly marked since formalin leaves a residue sufficient to kill delicate live specimens.

2. Buffers

Compounds commonly used as buffers include hexamine (hexamethyl-tetramine), ammonia, borax and chalk. Hexamine is recommended due to its ability to maintain a constant pH of approximately 7.8. For each liter of 10% formalin, 30 grams of hexamine should be used. Borax or

chalk are used at the rate of 5 grams per liter. Ammonia is not recommended.

3. Alcohols

Ethyl alcohol (ETOH) is the best general preservative and will maintain specimens in excellent condition for over 100 years. Further, it preserves tissues best for cytological and histological analyses. To denature ETOH, a variety of chemicals are added to ETOH to render it impotable (i.e. acetone, methyl alcohol, etc.). These additives are toxic and prolonged exposure to them without proper ventilation can cause harmful effects. Isopropyl alcohol (ISOH) has a number of serious disadvantages. It has poor tissue penetration properties and as a result may lead to tissue distortion. Further, prolonged exposure to ISOH fumes will cause severe headaches and possible liver damage. As a result, denatured ETOH or ISOH are the only readily available, relatively safe alcohols that can be used. We recommend that non-denatured ETOH be used rather than either denatured ETOH or ISOH.

Alcohol must be diluted prior to its use for preservation. Non-denatured ETOH is not absolute. It contains a small amount of water (5%) and is called "raw alcohol". Raw ETOH should be diluted to strengths not greater than 75 nor less than 70%. ISOH should be used at a 50% dilution. It is recommended that distilled water be used for diluting alcohol rather than tap water. The large amounts of impurities present in tap water will precipitate onto specimens placed in both ETOH and ISOH.

4. Other Fixing and Preserving Fluids

Some systematic studies may require that specimens be fixed and preserved in specialized mixtures of fluids for histological, cytological or other work. For other than routine procedures a specialist should be consulted in advance of the fixing and preserving of specimens.

5. Karo Syrup Mounting Medium for Algae

Some delicate algae and cross-sections for taxonomic identifications are best preserved as mounts on microscope slides. The medium is prepared from commercial grade, clear "Light Karo Syrup," distilled water, and full-strength (100% undiluted) formalin. The formula for 50% Karo syrup is 100 milliliters of syrup, 100 milliliters of water, and 2 drops of formalin. For other concentrations modify the ratio of water and syrup.

B. Containers and Closures

Glass jars or vials are recommended for most specimens of marine organisms. Larger organisms are usually stored in oncorrosive drums, stainless steel tanks, evaporation-proof plastic containers, etc. Closures should be evaporation proof to decrease the maintenance effort in the curation of collections. Lids may be either glass, plastic or metal and should have a plastic liner or rubber gasket to prevent evaporation. An archival institution may be consulted to determine sources of containers and closures in order to ensure compatability of types and sizes if transferal is anticipated.

C. Herbarium Paper

Herbarium paper in a standard size (11½ by 16½ inches) is available from some paper companies (e.g. Carpenter-Offutt in San Francisco) or Biological Supply Comapnies (e.g. Carolina Biological Supply). This paper must be 100% rag composition. Nonpermanent mounting papers of lower rag or cotton content, often labeled "for marine algae," are <u>not</u> satisfactory.

D. Label Papers

In order to insure the permanence of labels placed in solution with wet collections the finest quality label paper is an absolute necessity. Compromise may jeopardize an entire program and may result in a monetary loss many times greater than the relative expense of quality paper stock.

One hundred percent linen paper, treated for water and chemical resistance, or its equivalent, must be used. For many years such a paper could be obtained from L.L. Brown of Massachusetts (i.e. 40 lb. and 110 lb. weight L.L. Brown Resistal Linen Ledger). Though, the original qualities of this product have not been retained, it is still a suitable paper. This paper is presently manufactured by the Weston Paper Company and is distributed on the west coast by Crown Zellerbach. Labels which will not be subjected to heavy use can be printed on 40 lb. stock, otherwise they should be printed on the heavier 110 lb. stock.

In the past few years a number of plastic substitutes have become available. One of these, Polypaper, manufactured by the Nalgene division of the Sybron Corporation of New York, presently meets all requirements for label stock and surpasses all paper products in its resistance to normal fixative and preservative agents. Labels accompanying specimens which are stored dry, such as some invertebrates and algae mounted on herbarium sheets, can be of any high quality, 100% cotton, 24 lb. weight bond paper.

Large organisms should be tagged individually with Dennison waterproof eye tags (1 x 3 inch). These can be tied directly around specimens.

E. Cost Estimates

Every survey budget must contain a line item to cover the cost of curation and maintainence of those collections deemed important for archiving. In preparation of an initial budget the agency conducting the survey should decide whether the specimens are to be retained by the survey agency or deposited in an archival institution. If the collections eventually will be deposited at an institution other than the survey agency, this institution should be consulted in order to standardize materials and develop cost estimates for labor as well as materials.

At present an estimate of $2.00 is suggested for material costs per lot (all specimens of one species collected at each station at a given time). This is a conservative estimation and only includes consumable supplies (i.e., containers, closures, fixing and preserving fluids, labels and their printing costs). Estimation of labor costs for deposition in an archival institution will vary depending on the length of time to be curated (permanent/non-permanent), available staff and space and the processing necessary for final archival status (e.g. standard glassware, permanent labels, etc.).

III. Techniques for Preparation

1. Wet Preservation

Unless the material is to be pressed within a few hours after collection, it should be preserved in a 5% formalin/seawater solution buffered with a teaspoonful of borax per gallon. In order to retain color, specimens should be stored in the dark. A quick, simple method in the field is to place the jar of wet, preserved algae in a paper bag or sealed box.

Specimens wet-preserved in formalin and stored in the dark will last many years without deterioration, although gradual fading does occur. For smaller algae and difficult groups, wet-preserved specimens are far easier to identify than pressed ones. The drying process often obscures many characters and makes interpretation difficult for persons without considerable experience working with dried plants. Once identified, minute forms should be transfered to alcohol or prepared as microscope slide mounts. Larger forms should be pressed on standard herbarium sheets (see materials).

For long-term storage of small algae and sections of larger algae in which examination of anatomical details is necessary, 70% ETOH is preferred.

Identifications normally are made prior to the transfer from formalin to alcohol since alcohol quickly bleaches specimens and makes them brittle. To transfer to alcohol for long-term storage, formalin-preserved specimens should be rinsed briefly in freshwater and placed directly in the alcohol.

2. Dry Preservation (Pressing)

To press algae involves the alteration of specimens spread on herbarium sheets with blotters and cardboards in the following manner. To begin a pile, place a cardboard or two on your table, cover with a blotter (called felt by some), then a herbarium sheet with specimen (see below). Cover the specimen with muslin or cheesecloth (old sheets work well too) to prevent sticking to the blotter above. Waxed paper leaves a film on the specimen and is not satisfactory. Then add a "sandwich" of blotter, cardboard, and blotter. Now, another herbarium sheet with specimen may be added, cover with muslin, another "sandwich" and so on. When the pile becomes high enough, or you have pressed everything, add a couple of cardboards or a piece of plywood to the stack and put a weight on top. Cinder blocks work well.

In spreading the algal specimen on the herbarium sheet, care must be taken to display as much of the plant as possible, including the holdfast, branching pattern, reproductive structures, etc. Larger specimens may be laid directly on the paper and spread out using dissecting needles or forceps to comb the branches.

Smaller specimens must be floated onto the paper underwater to present their features. This seems more like art than science, but a well-spread specimen is scientifically the most valuable. Place the

herbarium paper in a tray large enough to accommodate it (cafeteria trays work well). Add sufficient water to cover the specimen. Sea water is necessary for freshly collected material to prevent plasmolysis. Preserved specimens may be rinsed then arranged in freshwater. Place the specimen on the sheet and arrange it to best display the characters; combing with a dissecting needle works well. Now slowly slide the paper with specimen from the tray. A final arrangement may be made at the air/water interface. Drain and place on a blotter.

The blotters, cardboards, and muslin must be exchanged for dry blotters, etc. daily until the specimens are dry. For kelps and larger specimens, more frequent changing will help prevent mold attack. Specialists of higher plants usually dry specimens in a drying oven with forced warm air. This very rapid method of drying will crack most algae badly.

Many algae will stick to the herbarium paper by their own mucilage. Those that do not must be glued to the sheets with white glue (e.g. Elmer's, Glu-Bird, etc.) after drying. Many herbaria additionally use thin strips of 100% cotton tape to secure the specimen; no other tape (especially "scotch" tape) should be used due to their lack of permanence. One exception to the drying rule is the articulated (jointed) coralline algae. These should be removed from the press at the first change and entirely covered with a thin layer of white glue. This will prevent fragmentation.

Prior to pressing, data sufficient to identify the alga, location, collector, and date must be written on the herbarium sheet with a number 2 pencil. After the specimen is dry, a label with complete data should be attached to the lower right corner of the sheet. White glue again should be used for this; rubber cement, mucilage, and the like are not satisfactory due to the lack of long-term holding power.

Bulky specimens such as crustose coralline algae or crustose forms on rocks may be air dried and placed in boxes. These boxes are stored in trays in separate cabinets and cross-referenced to herbarium sheets.

3. Microscope Slide Mounts

Quite often algal specimens are very small or microscopic. The usual procedure is to mount them on microscope slides and store them in slide boxes. Again they are cross-referenced by herbarium sheets.

The simplest preparation of slides of marine algae is the Karo-Syrup mount. Other methods such as glycerin-jelly preparations, are recommended as superior by some, but are more time consuming. Prior to mounting in Karo, the specimen must be fixed in 5% formalin-seawater for at least ½ hour.

Place the specimen on a clean slide with a couple of drops of a 50%-80% Karo syrup solution, add coverslip slowly to avoid bubbles. Very delicate specimens or sections may plasmolyze in 80% solution and should be placed in 50% solution.

These slides may take several months to completely evaporate to 100% Karo and be permanent, so caution must be used in their handling during that time. Store them horizontally and separately. As they dry it may be necessary to add additional 80% Karo syrup solution at the margins of the coverslip.

Most phycologists stain the specimens prior to mounting to bring out taxonomic details. Information on this may be found by consulting references cited in standard texts. For voucher specimens this is unnecessary.

If a specific repository for marine algal collections has been selected, it is wise to consult with the staff prior to specimen preparation. Advice on procedures and/or information obtained at this stage may save considerable time and expense later.

B. Marine Invertebrates

1. Relaxation and Narcotization:

No standard methods are available to relax or narcotize a broad spectrum of invertebrates. Many of the most effective methods require elaborate techniques and the use of chemicals, which are not readily available. Successful results often may be obtained by placing organisms in sea water and slowly chilling to the freezing point. (Care must be taken not to freeze them solid as this will result in serious tissue damage). Alternative methods, which also provide good results involve: the slow addition of non-denatured ETOH (95 to 75%); isotonic solution of 7.5% magnesium chloride ($MgCl_2$); or floatation of methol crystals on sea water in a closed container. Special methods of relaxation necessary for display of diagnostic characters in certain groups of invertebrates will require consultation with a specialist. When organisms no longer respond to tactile stimulation they may be transferred to a fixative.

2. Fixation:

Initial fixation in 10% buffered formalin should be for a period of three days to one week due to the low penetrating qualities of the fixative. All specimens of large size and invertebrates with extensive body or gastric cavities should have these cavities injected with the fixative.

Exceptions to fixation in formalin occur in groups with delicate calcereous structures which are important for identification. One example is the Phylum Porifera (Sponges), the spicules of which will dissolve or be destroyed by even the weakest acid. This group should be fixed in 95% ETOH. If any questions arise for any group an expert should be consulted.

3. Washing:

Wash very gently in cool freshwater until the odor of formalin is significantly reduced. For small specimens this will take from one to a few hours. Larger specimens should be held for several days to reduce the formalin residue. Caution, prolonged soaking in freshwater will result in softening of specimens and bacterial decomposition. The use of screens, sieves, fine mesh netting, etc. is recommended when washing samples to avoid loss of small specimens.

4. Preservation:

When washed sufficiently specimens or samples should be transferred to 70 to 75% ETOH. If non-denatured ETOH is not available material may be preserved in 50% ISOH. The initial preservation solution should be changed after 24 hours to negate any dilution or discoloration problems. The body or gastric cavity of large organisms also should be injected with the terminal preservative. To avoid damage (abrasion, compaction, etc.) and lessen dilution problems after washing, specimens should never be crowded.

Certain invertebrate groups should be left in formalin and not transferred to an alcohol for storage. Sea anemones are best fixed and preserved in 10% buffered formalin and the gastric cavity ought to be injected adequately. Pelagic invertebrates with high water content

(i.e. siphonophores, scyphozoans, ctenophores, chaetognaths, salps, etc.) should be fixed and preserved in 5% buffered formalin. Washing should be avoided with these delicate specimens.

C. Marine Fishes

1. Fixation:

Fishes are fixed best as soon as possible after death. They may be immersed directly into 10% buffered formalin. Caution, fixation of live fish in 10% formalin causes frantic movement, wherein damage may occur, or the mouth and gills become strongly flexed.

For large fishes, in excess of 9 inches, the body cavity should be injected with 10% buffered formalin. If injection is not available, then the body cavity should be slit for several inches to allow the fixative to penetrate and act upon internal tissues. The slit should be made on the fish's right side as the left is always reserved for photographs, illustrations, counts and measurements. Flatfishes should be slit on the ventral or blind side. Smaller fishes do not have to be injected or slit unless they have been dead a long time or the weather is exceptionally hot. Fishes are normally left for 3 to 5 days in fixative. If food habit studies are to be undertakne then the stomachs of fishes should be removed and fixed separately to negate decay and further digestion.

Otoliths frequently are diagnostic for each species of fish and are important in studies of food habits and ageing as well as for their identification in sediments. If they are to be utilized in these kinds of studies they should be removed prior to fixing because they will dissolve even in buffered fixative.

2. Washing:

After fixing, wash the fishes for several days in water to reduce the fixative. A small residue of fixative may be retained in the fishes to enhance long-term preservation. Do not over wash.

3. Preservation:

Replace the wash water with either 70% ETOH or 50% ISOH; then after 24 hours replace the alcohol with new preservative. Some fishes do not preserve well in alcohol and should be retained in 5% buffered formalin after initial fixation in 10% buffered formalin. These include all fish larvae and eggs, as well as the gelatinous fishes of the snailfish family, Liparidae.

4. Warning:

Many fishes have sharp spines that occasionally may have a toxic mucus on them capable of producing intense pain. Therefore, spines should be treated with great respect, even in dead fishes. If a spine breaks off in a wound, it is important to remove it. Immersion of the wound in hot water is the best immediate course with medical attention if necessary.

IV. Documentation (Minimal Label Data)

No other facet of acquiring and maintaining collections is more important than the data that are associated with the preserved materials. A collection that can not be documented in time and space is worthless. Unless stringent safeguards are maintained, coding of any type of samples is a very risky procedure. Loss of field or laboratory records may invalidate an entire survey. Each sample or specimen lot must be labeled so that it may stand alone as an identifiable unit to anyone not associated with a given project. In all cases, a master label that includes the information listed below, should be placed in the sample container. Exterior labels must be only

supplemental as their loss risk is very high. Sample labels and field notebook pages are to be found in appendices I-III.

1. Geographic and Political Location

Exact location is essential. Sufficient information must be provided to allow someone to return to the same spot in the future. Ship stations should include latitutde and longitude as well as a radar fix or equivalent. Shore stations must include county and location of site in relation to a landmark with a stabilized name.

2. Habitat

Substratum and depth are most important. If collection is intertidal, tidal height should be estimated.

3. Method of Capture

Specific capture method is important. Indicate method of hand collection (i.e., scuba, dip net, hook and line, spear, poison and type, etc.) or identify specific oceanographic collection gear (i.e., pelagic or benthic trawls, grabs, dredges, nets, etc.).

4. Source of Material

Name of collector (initials are insufficient), or name of ship and station number. Name of agency and research project should also be identified.

5. Date of collection

The month should always be spelled out or given in roman numerals; the day of the month and the year should be arabic. The order should always be day, month, and year (including century). Acceptable examples are 6 October 1976 or 6 X 1976. A date such as 10/6/76 can be confusing and is not acceptable.

6. Preservation

The types and methods of relaxing, fixing, and preserving the specimens should be indicated.

7. Identification

Specimens identified to species level should have the complete taxonomic citation. This includes the complete and correct spelling of the genus and species, underlined, followed by the author and date. The International Codes for Botanical and Zoological nomenclature are different, so a systematist should be consulted for rules of citation if uncertainities exist. When the material has been identified to species, it must indicate the identifier (no initials are acceptable).

Responsibility for identifications lies solely with the sampling organization. Taxonomic verification and supplemental identifications may be made by specialists, recognized experts or consultants so long as the original data and all previous identifications are not modified.

8. Additional Data (Remarks or Notes)

Many curators appreciate receiving supplemental information observed in specimen capture. This information can greatly add to the scientific value of the collection. Examples include: color or color patterns in life; ecological associations; habitat preferences; behavioral observations; sexual activities; food and feeding observations; temperature; salinity; tests for heavy metals or other perameters, which may have been run; etc.

9. Writing Impliments

Experience has shown that for general field use a simple lead pencil is the preferred writing instrument. A number 2 to 3 lead is recommended as this degree of hardness will usually leave a retrievable

impression even if the label becomes smudged. Ink in the field is to be avoided. Permanence of ink in a collection depends on the type of ink, paper and fixative/preservative used. In addition, the ink must be completely dry before a label is placed with a wet sample. Even then, some ink may delaminate from the paper, leaving little or no impression. Higgins Eternal and Pelican inks are satisfactory. Typewriters using a pure carbon inked ribbon work very well on labels, but Fil Type transfer ribbons used in many electric machines will not hold up in alcohol. Ball point and felt-tip pens are never acceptable for field, laboratory or curatorial use.

V. Collection Deposition and Accessibility

We are aware that many surveys are taken in the nearshore California marine environment. The materials collected frequently are not saved. Some of the specimens taken during these surveys represent potentially significant contributions to the knowledge of coastal marine life. The failure to recognize this significance and the destruction of all the collected materials is a terrible waste of our natural resources and does not act in the best interests of scientific research. We recommend that several archival institutions be notified of all collections resulting from survey and/or monitoring activities so that the material may be evaluated for its intrinsic scientific value.

1. Archival Institutions

Archival institutions are those charged with the responsibility for long-term care and maintenance of collections of plants and animals. They have professional curatorial staffs and specialized facilities for systematic storage and study of these organisms. Their collections include two types:

those of systematic and voucher importance that are permanent collections and retained in perpetuity; and those of non-permanent status that either will be added to the permanent collections, distributed for research and teaching or discarded.

Systematic collections usually are stored in an institution by discipline in such a manner to allow quick retrieval. They are easily accessible for the user community from the collection by taxonomic category. Non-permanent collections differ from permanent ones in that they may be incompletely curated, or not incorporated into the central collection and, therefore, are not easily accessible for study. In some cases, there are collections in deep storage and may be retrieved only with great effort. Such collections contain fully-curated specimens that are not frequently utilized but important as indicators of long-term changes. Frequently there are extensive backlogs of collections waiting to be curated. These backlogs are essentially unavailable for study until they become curated.

2. Term of Deposition

We recommend that collections be retained for a minimum period of five years. We further recommend that collections be maintained by an archival institution in the region of the survey. If, however, the survey agency chooses to maintain collections for the recommended five year period, these collections must be readily accessible to qualified investigators. At the end of five years the survey agency must consult an archival institution to determine if the scientific value of the collections warrant further preservation. If the survey agency decides

to dispose of the collections before the end of the recommended five year period, the collections must be deposited at an archival institution for the remainder of the 5-year period. The location of collections generated by surveying and monitoring activities shall be specifically indicated in the published report. During the time that collections are maintained by an archival institution, the survey agency must bear partial cost of curatorial maintenance. After five years the archival institution may assume full responsibility for the further maintenance of distribution of the collections.

3. Documents of Transfer

Archival institutions should accept collections only with assurance that the specimens were legally obtained. Therefore, copies of all original permits should accompany the collection when transferred to the repository institution.

Most permit regulations require that annual lists of specimens collected under the permit be submitted to the regulatory agency. Copies of these lists also should accompany the collections.

4. Data Retrieval

We recommend that electronic data processing (EDP) be considered in the future for all subtidal and intertidal surveys to assist in the efficient management and retrieval of the data.

VI. Appendices

1. Field Labels

The following label format is recommended for field collections. Printed polypaper labels of this sort are presently available in booklet form.

2. Field Collection Notebooks

The following pages are examples of field information that is desired. Notebooks of this sort should be maintained as duplicate records.

3. Museum Labels

The following are examples of labels from various repository institutions in California. These are placed in containers with specimens in solution, which have been fully curated and permanently archived in their respective systematic collections. An environmental consulting firm or monitoring agency should consult with the repository to develop a compatible label format.