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# TRANSPORTATION OF FLUID-PRESERVED NATURAL HISTORY SPECIMENS STORED IN GLASS CONTAINERS: NEW SOLUTIONS TO AN OLD PROBLEM

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*Abstract.*—The Zoology Department of The Natural History Museum, London, houses one of the largest and oldest reference collections of fluid preserved biological specimens in the world. Among the many problems associated with the curation of these specimens is that of transporting the glass storage containers within and between the Museum buildings. The Health and Safety Executive (HSE), a part of the Government Department of Employment, inspected the Zoology Department and stated that the trolleys used by the curators were, in their present condition, unsafe and required modification. A solution to the problems they identified involved changes in work practices and structural modifications to the trolleys.

Most fluid preserved natural history specimens in the Zoology Department are stored in 70% or 80% Industrial Methylated Spirit (IMS). IMS contains 70% ethanol and 4% methanol. A few specimens are preserved in 4% formaldehyde solution. A variety of containers (Clark, 1992, 1993), mostly made of glass, are used to store specimens in the reference collection located in the New Spirit Building (Stearn 1981:170). Some of these glass containers stand over a metre tall (Fig. 1) and extreme care is required when transporting them around the Museum estate. Specimens are removed from their normal storage for a number of reasons which include examination, radiography, photography, and routine curation. Experienced curators are responsible for carefully moving specimens from place to place and in the long history of the Zoology Department no serious accidents have occurred. This good record can be attributed to regular reviews of work practices to ensure that they conform to the safety regulations of the day. Appropriate training of curators and regular in-house inspections are used to maintain these standards.

## TROLLEYS

Two kinds of trolleys are used for moving specimens; custom built wooden and standard metal tray trolleys.

*Wooden trolleys.*—Most of the wooden trolleys (Fig. 2) were built in the Museum between 1936–37 to the specifications of J. R. Norman (Fish Section 1921–43). They were originally made for the purpose of moving the fish collections from the Old to the New Spirit Building (Stearn, 1981:170), and are still in use today to transport fluid preserved specimens around the Museum site. These trolleys were built with perforated brass strips along the tops of the sides and across the floor so that removable partitions, secured by pegs that located in the holes

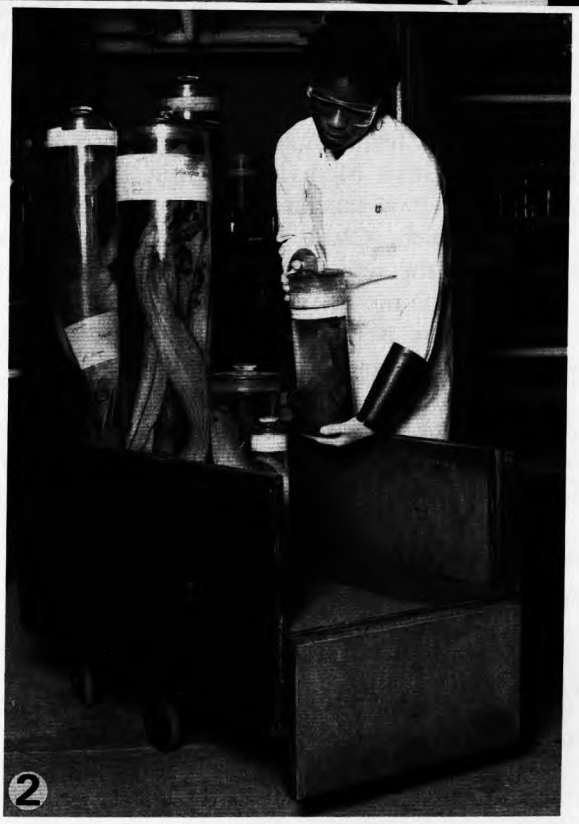

Figure 1. Fish specimens contained in ground glass stoppered jars. Some jars stand over one metre tall.

Figure 2. An original wooden trolley before modification.

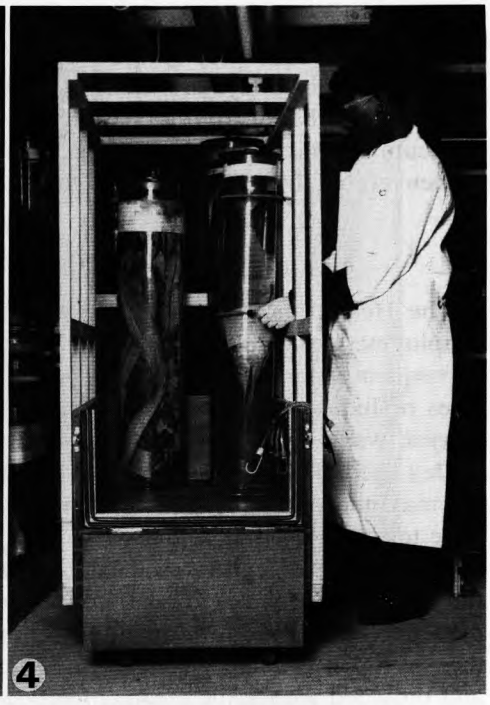

Figure 3. Preparing to move specimens on a tray trolley prior to use of plastic crates.

Figure 4. Wooden trolley after modification for transport of glass storage jars taller than 60 cm.

of these brass strips, could be inserted to divide up the carrying compartment. These partitions, no longer extant, were intended to prevent taller jars from toppling.

The trolleys were designed with 6 wheels (with solid tyres) to meet the special requirements of moving large glass specimen containers within the narrow confines of the collection storerooms. They have one swivel caster near each corner and a pair of central fixed casters, a combination which greatly enhances the maneuverability of the trolley and allows it to be turned on the spot about a central axis. Such mobility enables smooth negotiation of tight corners and easy presentation of the loading/unloading end to the destination point. The two central fixed casters are an important feature in preventing lateral movement, and in holding the trolley straight when it is being pushed forward.

The trolleys were made of oak, a dense wood which would not absorb spilled preserving fluid. Added protection against spillage was provided by varnishing the surface. The inside of the trolley was lined with cork which provided a non-slip shock-absorbent surface. A hinged flap at one end of the trolley can be lowered to facilitate loading and unloading of specimen jars.

Tall jars are always transported in these trolleys but because the centre of gravity of the containers lies above the sides of the vehicle, uneven floors need to be negotiated slowly and with great care. Two curators are normally required in such operations: one to push the trolley and the other to hold and steady the tops of the jars.

*Metal tray trolleys.*—To transport smaller bottles, open-sided metal trolleys are used (Fig. 3). These trolleys have two trays and four wheels with revolving casters. The metal trolleys are unlikely to have been constructed in-house and were probably purchased from a commercial supplier; whether from a catalogue or to Museum specifications is unknown. They are relatively light in weight and quite maneuverable, but are unsuitable for the heavier weights of large or tall storage jars.

## Health and Safety Executive Inspection

The Health and Safety Executive (HSE) of the Government Department of Employment made an official inspection of the Spirit Building and produced a subsequent report in June 1992. The HSE considered that spillage of large quantities of Industrial Methylated Spirit constituted a fire risk, and that 4% formaldehyde was a health hazard. Item 4 of the Inspector's report stated, "The hand trolley used to transfer specimen jars should be fitted with a retention lip to retain the maximum likely spillage quantity of alcohol and also provided with an outer cage to prevent jars toppling over."

A number of commercial catalogues were consulted for a trolley that satisfied the Zoology Department's curatorial requirements: the vehicle must be stable; maneuverable; smooth-running; and capable of carrying considerable loads. Further, in order to comply with the HSE directive the trolley had to be liquid-tight and provided with some means of support for jars to prevent them from toppling during transportation. None of the advertised products met all of these requirements. In addition, they were expensive and would have required extensive modification. Since the Department's existing trolleys already met curatorial criteria, curators agreed that it would be more economical to modify existing trolleys than attempt to adapt any of the products presently on the market.

## Wooden Trolley Modifications

Retention of fluid spills in the wooden trolleys were made possible by caulking the joints of the lining with a silicone compound (Sirosil-25) and installing a double-tubular rubber sealing strip (Figs. 4, 5) at the loading/unloading end. The seal is completed when the hinged flap is closed and secured by means of two 67 mm, die-cast, Fitch-pattern sash fasteners (Fig. 6). Fluid retention was tested by filling the trolley with water for twenty-four hours. There was no leakage. The tubular seal has been designed for ease of replacement.

Replacing the old partitions (see above) to provide support for taller jars in the wooden trolleys was considered impractical. This would have required the cork linings of the trolleys to be perforated, a requirement that conflicted with the need to make the trolley liquid tight. Therefore, a wooden framework was constructed (Fig. 4). The lowermost horizontal member of this framework was permanently attached to the trolley, the rest of the structure being secured with wing nuts for easy removal. The wooden framework is attached after tall bottles are placed in the vehicle. Further stability is afforded by attaching the upper parts of the bottles to the framework via elasticated straps.

While in the workshop each wooden trolley was checked and serviced. Of the nineteen trucks, which were renovated by Mansfield Wahl (specialist joiners), six were considered to have faulty wheels. In these cases the old solid tyre wheels

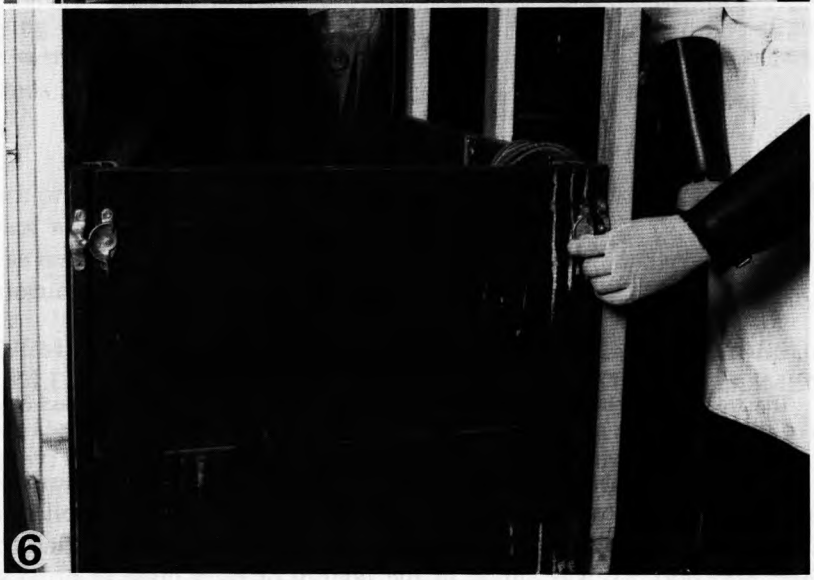

Figure 5. The hinged flap on the wooden trolley, which has been made liquid-tight by the installation of a double-tubular rubber sealing strip.

Figure 6. The hinged flap is secured by means of two 67 mm die-cast Fitch-pattern sash fasteners.

were replaced with pneumatic tyres. Although this greatly improved the smooth running of the trolley, the cost was prohibitive. Consequently, the wheels of the remaining thirteen were not changed.

## Metal Trolley Modifications

In the case of Metal trolleys, which were only intended for transporting smaller bottles, spillage containment was effected by using commercially available high

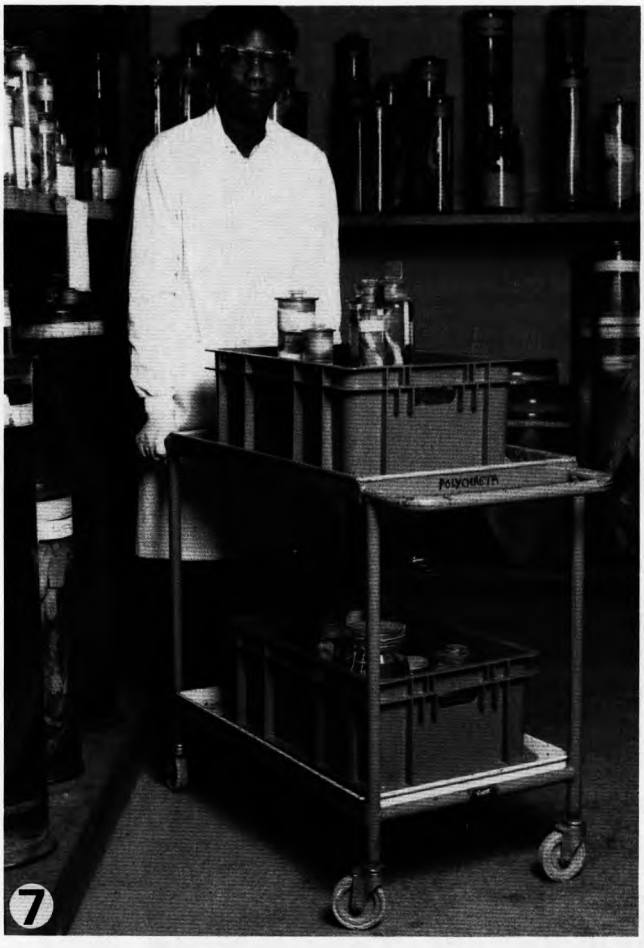


Figure 7. Tray trolley with plastic crates for stabilising jars during transportation of specimens.

density polyethylene crates measuring 60 × 40 × 22 cm (Fig. 7). The bases of the crates fit within the low walls of the upper and lower trays. As supplied, these plastic crates had a slippery and uneven floor. A piece of non-slip material, "Safestrip" (see Appendix 1), was cut to fit the bottom of each crate to provide the required even, non-slip surface. The advantages of the crates lay in their being inexpensive and readily available and, once they were installed, no further adaptation of the existing trolleys was required.

## Safety Procedures and Equipment

During 1990, the Zoology Department initiated a review of its Health and Safety procedures and equipment. Work practices reviewed included the wearing of laboratory coats, some procedures of curation, and the transportation of glass containers holding preserved specimens.

A number of "Hazardous Spill Response Kits" are available in the Spirit Building, and all storerooms housing the reference collection contain eye wash stations. When curatorial work is in progress, the wearing of safety spectacles and labo-

ratory coats is mandatory. In addition, when handling large glass storage jars, curators must wear protective leather wrist guards (Fig. 6) and nylon-supported PVC aprons (Figs. 2, 4).

Much discussion, concerning the wearing of gloves while handling glassware, took place between curators and the Departmental Safety Officer. Heavy duty gloves that would protect the hands against breaking glass were tried. However, these hampered manual dexterity to the point of causing an accident while fumbling with glass storage jars. This problem was resolved by making the wearing of wrist guards, while handling large jars, mandatory and by reviewing the method of handling glassware.

Clark (1992, 1993) reviewed the problems associated with older glassware and described some new safety features required for modern jars. Accidents can occur when handling older glassware, particularly if the jar is picked up with hands on either side of the container. Should the glass jar fracture when the hands are in this position, the lateral pressure required to grip the sides causes the glass between the hands to be crushed and the broken top section of the container falls onto the thumbs and index fingers. This type of handling accident can be avoided if the jar is carried with one hand supporting the base of the jar and the other steadying the top as seen in Figure 2. The jar should be carried at a slight angle to the vertical. If the jar fractures while held this way, the glass falls away from the hands greatly reducing the chance of cuts.

## Work Practices: New Policies and Procedures

Work practices for the transportation of glass specimen jars around the Museum complex were rewritten to incorporate the new policies and procedures. Copies of these new work practices were distributed to all Zoology Department curators. This document is reproduced below:

### SCHEMES OF WORK FOR THE TRANSPORTATION OF GLASS JARS HOLDING FLUID PRESERVED NATURAL HISTORY SPECIMENS

Each Departmental curation team in Zoology has been supplied with two types of trolley; an alloy tray trolley with a plastic crate lined with "Safestrip" and a wooden trolley modified to hold a wooden cage.

#### Tray Trolleys

1. Tray trolleys have been supplied with plastic crates; these *must* be used for the transportation of glass jars holding fluid preserved specimens.
2. "Safestrip" mats *must* be present on the floor of the plastic crates to reduce sliding of glass containers during transportation.
3. Only glassware up to a height of 40 cm and/or containing a maximum of 26 litres of preserving fluid is permitted to be carried in the polyethylene crates. Glassware exceeding these specifications must be carried in a wooden trolley.

#### Wooden Trolleys

1. During transportation of glassware the flap *must* be closed forming an effective seal to contain spillages.

2. The wooden cage *must* be used when the glass container exceeds the height inscribed on the side of the trolley and containers should be secured to the frame when necessary.
3. When using the wooden cage the glass jars should be loaded onto the trolley first, then the wooden cage *must* be fitted to the horizontal bars on the outside of the vehicle.
4. When off-loading, the cage *must* be removed first.
5. The maximum volume of preserving fluid to be carried in the wooden trolley, is inscribed on the side. This volume *must* not be exceeded.

## Protective Clothing

1. Laboratory coats *must* be worn at all times when transporting specimen containers.
2. Safety spectacles *must* be worn when loading and unloading glass containers.
3. Wrist guards and nylon supported PVC aprons are mandatory when handling large glass containers (volume equal to or greater than 5 litres) and/or over 80 cm tall.
4. Hazardous Spill Response Kits should be made available in work areas.
5. Major breakages which result in preserving fluid being spilt into the trolley or crate must be dealt with promptly.

## Maintenance

Pneumatic tyres must be maintained at the correct pressure, failure to comply could render the trolley unstable. A foot pump is located in room C3, second floor of the Spirit Building.

## Conclusion

By renovating the existing trolleys the two requirements of the Health and Safety Executive directive were met in full by the Zoology Department of The Natural History Museum. Structural modifications made to the trolleys prevent the glass bottles from toppling over and any spillage resulting from breakages would now be contained in the liquid-tight body of the vehicles. Work policies and procedures governing the transport of fluid collections have been modified to accommodate the structural changes to the trolleys and related health and safety issues. The successful solutions to the transportation problems were arrived at after consultation between curators and the staff of a joinery company. The exchange of information and experience was thought to be a valuable aid to reaching our goal.

## Acknowledgments

We wish to thank Derek Adams (Figs. 2–7) and Paul Lund (Fig. 1) of The Natural History Museum Photo Unit for taking the photographs reproduced in this paper. Also, Alwyne Wheeler for the information concerning the origins of the wooden trolleys and Miranda Lowe and Amanda Banner for their patience during the photographic session. Paul Mansfield and Michael Harding of Mansfield Wahl added the final polish by transforming our ideas into practical solutions. Wise council was given by Nick Evans, Zoology Department Safety Liaison Officer and his Deputy, Andy Warlow.

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Clark, P. F. 1993. Museum storage containers: Back to the future. Pp. 347–359 in *Contributions on the Status, Preservation, and Conservation of Natural History Collections* (F. Palacios, C. Martinez and B. Thomas, eds.). International Symposium and First World Congress on the Preservation and Conservation of Natural History Collections, Madrid, xi + 426 pp.

Stearn, W. T. 1981. *The Natural History Museum at South Kensington. A History of the British Museum (Natural History) 1753–1980.* Heineman, London, xxiii + 414 pp.

## Appendix 1

Safestrip is a non-slip material manufactured from high clarity, glass clear, PVC material. Sole manufacturers:

Copely Developments Ltd
Thurmaston Lane
Leicester LE4 7HR
England

Tel. 0533 765881/2/3
(International) 44 533 765881/2/3
Fax 0533 460117
(International) 44 533 460117

# SPIRIT COLLECTIONS: A PRELIMINARY ANALYSIS OF SOME ORGANIC MATERIALS FOUND IN THE STORAGE FLUIDS OF MAMMALS

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*Abstract.*—This paper presents preliminary data from the analysis of organic materials that were leached from specimens into their liquid storage media. Fluid samples were taken from the storage media of selected holotypes of mammal specimens. Fluids were analyzed for fatty acids and lipids using gas chromatography and gas chromatography-mass spectrometry, and for the presence of individual amino acids using amino acid analysis. A variety of lipids and their constituent fatty acids were found. $C_{12}$, $C_{14}$, $C_{16}$ and $C_{18}$ fatty acids were present, as well as a number of $C_{20}$, $C_{22}$ and $C_{24}$ acids. Furthermore, some palmitic to stearic acid (P/S) ratios were high (like those found in plants) in comparison to the low ratios normally associated with animal fat. Amino acid profiles indicated that peptides and amino acids also were being leached into the storage fluids. These profiles indicate general protein loss, including some structural proteins. Structural protein loss is characterized by a higher than expected glycine (in the 30% range) and alanine content. These results form the baseline data from which to study the deterioration of the specimens themselves.

For hundreds of years biological specimens have been stored in a variety of fluids in order to preserve them for later study (see Williams and Hawks, 1987). For instance, Down (1989) cites references from the middle of the 17th century which extol the preservative qualities of "spirit of wine." The famous chemist Robert Boyle (1629–91) is credited with promoting this preservation method in 1662. Thus, ethyl alcohol has one of the longest histories of use as a preservative. The use of glycerol as a preservative and formaldehyde as a fixative were not introduced until more than 100 years later when it became possible to identify and synthesize these compounds (see Williams and Hawks, 1987:43). The practice of preserving biological specimens in fluid grew rapidly over the years, and these collections have become a major source of preserved material used for a wide range of ecological, biological, anatomical, taxonomic and biochemical studies. Even today, ethyl alcohol solutions serve as the primary fluid for the preservation and storage of specimens. These fluid preserved specimens constitute a significant portion of the record of our earth's documented biodiversity. Estimates of world-wide fluid collections indicate that the specimens stored in this manner currently number about 1.5 billion and are growing at a rate of about 50 million per year (Peake, 1989).

To date, no one has studied the interaction between storage fluids and the stability of the specimens preserved in them from a modern chemical perspective, so that any new information generated by a partial chemical survey as reported here was thought to be beneficial, even though numerous variables, some of which are discussed later, remain uncontrolled.

To begin an assessment of the stability of specimens stored in ethyl alcohol, an analysis of the storage fluids themselves was made. It was felt that an initial study should attempt to identify dissolved material in a typical ethyl alcohol and water solution rather than try to follow any changes in the specimens themselves. The

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material leached from the specimens by alcohol and water should provide some clue concerning the preservation state of the specimens and indicate what classes of compounds could be monitored in the specimens in order to study changes that have occurred. Basic deterioration reactions, such as hydrolysis, that normally are progressing in the specimens, would be difficult to identify in the whole animal. However, once these reactions proceed to the point of producing compounds that are soluble, the resulting compounds would migrate into the solution where they could be analyzed. Inferences could then be drawn about the type of deterioration producing the soluble compounds. In addition, information gathered in studies of deteriorating fluid-preserved material could then be used as a base to begin designing (if necessary) more effective preservation and storage methods.

The current study represents a preliminary investigation of two major classes of organic materials (fats and fatty acids, and amino acids) using some of the techniques available for investigating these materials. For instance, no attempt was made to directly determine the presence of peptides that may have remained intact in the storage fluid, to analyze the fluids for their inorganic content, or to determine the presence and composition of pigments or carbohydrates that might have migrated to the fluid. As a result, the compounds reported here are some of the organic materials originally present in mammals that are soluble in an alcohol and water solution and are capable of being removed from the specimen by the storage solution.

## Materials and Methods

This study drew its samples from holotypes of mammal specimens that were collected between 1852 and 1895 and preserved in fluid. They are housed in the Division of Mammals at the National Museum of Natural History, previously known as the U.S. National Museum (USNM), and have USNM catalog numbers. The specimens are stored in a 70% ethyl alcohol and water solution which is inspected on a semi-annual schedule and "topped" as necessary to replace any evaporative losses. No data exist which would indicate whether the fluids were ever changed completely, so that it is assumed they have only been "topped," and that the fluids used for this study are about 100–140 years old (Robert Fisher, personal communication). Further, no attempt was made to investigate the effect of "topping off" on the extractive ability of the storage medium, nor how the use of the specimens or the storage environment, such as light, heat, and relative humidity, affected the results. A more rigorous and controlled set of long-term experiments would be required in order to study these variables.

The fluid samples themselves were taken from nine containers housing type specimens whose names, collection locations and dates, and USNM catalog numbers are presented in Table 1.

Approximately 50 ml of fluid was taken from each storage jar and used for analysis. First, the raw samples of fluid were visually assessed for color of solution. This description is not quantitative, but serves to indicate whether there is a correlation between solution color and the other data reported here. Their colors were graded into five arbitrary categories from transparent clear, pale yellow, medium yellow, dark yellow, to transparent orange. The pH of each solution also was measured using wide-range pH paper. It is recognized that pH paper will not provide accurate and precise results in alcohol solutions, so the pH values reported here represent only semi-quantitative results. The original 50 ml sample then was split into two 25 ml aliquots. One of these was added to a weighed polyethylene beaker and dried under vacuum in the presence of silica gel. The beaker was weighed regularly, and when the weight remained constant, the value was recorded as the dried sample weight. The sample was then extracted twice with 0.1 molar HCl and used directly for amino acid analysis. The second aliquot was extracted with 2 ml of methylene chloride three times to remove lipid components. The volume was reduced to about 0.5 ml under a stream of dry nitrogen gas, and the sample gas was transferred to a vial for analysis by gas chromatography (GC) and combined gas chromatography-mass spectrometry (GC-MS), either directly or as the methyl esters. Fatty acids that were present in the initial methylene chloride extracts were derivatized to their methyl esters for GC and GC-MS

Table 1. The names, USNM catalog numbers, and collecting location of the specimens whose fluids were used in this study.

| Specimen name | Collecting location | Collection date | USNM catalog number |
|---|---|---|---|
| *Myotis keenii keenii* bat | Graham Island, British Columbia | 1894 | #072922 |
| *Myotis lucifugus lucifugus* brown bat | Sebastian County, Arizona | 1861 | #005342 |
| *Rhogeesa gracilis* bat | Puebla, Mexico | 1894 | #070694 |
| *Tardarida brasiliensis intermedia* bat | Chiapas, Mexico | 1895 | #078488 |
| *Neotoma bryanti* rat | Baja California, Mexico | — | #186481 |
| *Peromyscus leucopus texanus* field mouse | Mason County, Texas | 1852 | #002559 |
| *Clethrionomys gapperi carolinensis* vole | Mitchell County, North Carolina | 1887 | #186490 |
| *Microtus tshuchytshurum* vole | Bering Strait, Siberia | 1866 | #008419 |
| *Neofiber alleni* muskrat | Brevard County, Florida | 1883 | #014065 |

analysis by treating them with boron trichloride ($BCl_3$) in methyl alcohol at 60°C for 5–9 minutes, extracting the mixture with hexane, drying the hexane over anhydrous $Na_2SO_4$, and injecting the resulting solution into the gas chromatograph (Anon., 1991). A portion of the original extract also was first treated with 10% KOH in methyl alcohol overnight at room temperature, extracted with methylene chloride, then treated with $BCl_3$ as described above.

Gas chromatographic analysis was conducted using a Hewlett Packard 5790 series gas chromatograph. The separating column consisted of a 0.32 mm i.d. × 30 m long silica column whose inside surface was bonded to silicone oil, which served as the separating medium (J&W DB-1), and a flame ionization detector was used for detection. Hydrogen was employed as the carrier gas.

Gas chromatography-mass spectrometry was carried out on a Finnigan MAT model 8230 combined GC-MS. This is a magnetic sector instrument which is connected as the detector for a Carlo-Erba Mega series gas chromatograph. A DB-1 column was used as before with hydrogen as the carrier gas, and the column effluent was fed directly into the mass spectrometer source for sample ionization. The source was operated in the electron impact mode at 70 kv. Reconstructed total ion chromatograms were recorded, and individual spectra were collected and examined for structural information.

Amino acid analysis was conducted on a specially constructed high-pressure liquid chromatograph. A 2 mm i.d. × 150 mm long stainless steel column filled with 3 μm diameter cation exchange resin beads was connected to a series of buffer chambers via high pressure tubing and an Eldex brand high pressure piston pump. The chambers were maintained under $1.7 \times 10^5$ pascals (25 psi) helium pressure. The pump drew buffers from the chambers in a timed sequence as determined by a Micrometrics brand sample changer/controller. Four of the separating buffers consisted of 0.25 M sodium citrate of differing pH, each containing 1 g/liter of ethylenediaminetetraacetic acid (EDTA) as a chelating agent for interfering divalent cations. The final buffer was a 0.2 M boric acid solution containing 1 g/liter sodium chloride. Buffers entered the column in a sequence of ascending pH: pH 2, pH 3.25, pH 4.5, pH 6.5 and pH 10. The column effluent then was mixed with a stream of *ortho*-phthalaldehyde and 2-mercaptoethanol (OPA, 2 g/liter, 2-mercaptoethanol, 0.5 g/liter, in pH 10 borate buffer) as the detecting agent. OPA reacts with amino acids containing primary amines (most amino acids) to produce an OPA/mercaptoethanol/amino acid product which fluoresces under UV light, is detected, acquired and recorded by a data system. However, two common amino acids contain secondary amines in a ring structure (the imino acids proline and hydroxyproline), and do not react under these conditions, and are not detected (Benson and Hare, 1975).

Table 2. A compilation of color, solids weight, and pH of mammal holotype storage fluids.

| Sample name | Color | Weight | | pH |
|---|---|---|---|---|
| | | g | g/liter | |
| *Myotis keenii keenii* | med. yellow | 0.0111 | 0.55 | 5.7 |
| *Myotis lucifugus lucifugus* | clear | 0.0018 | 0.09 | 7.2 |
| *Rhogeesa gracilis* | clear | 0.0027 | 0.14 | 6.5 |
| *Tardarida brasiliensis intermedia* | light yellow | 0.0221 | 1.11 | 6.1 |
| *Neotoma bryanti* | clear | 0.0025 | 0.12 | 6.4 |
| *Peromyscus leucopus texanus* | light yellow | 0.0027 | 0.13 | 7.3 |
| *Clethrionomys gapperi carolinensis* | light yellow | 0.0019 | 0.09 | 6.6 |
| *Microtus tshuchytshurum* | clear | 0.0005 | 0.02 | 7.2 |
| *Neofiber alleni* | med. yellow | 0.0076 | 0.38 | 5.8 |

## Results and Discussion

The results of the examination of sample color and pH, along with the weight of any nonvolatile material remaining after evaporation of the fluid, are presented in Table 2. As may be seen, the storage solutions varied in color from clear to medium yellow. No samples were cloudy, and no samples fell within the darkest two categories. The weight of nonvolatile material which was dissolved in the medium and remained after evaporation varied considerably and did not correlate well with the color of the solution. In fact, the solution from *Tardarida brasiliensis intermedia* was lighter in color than that from *Neofiber alleni,* yet the *Tardarida brasiliensis intermedia* solution contained almost three times the amount of dissolved material as did that from *Neofiber alleni.* Given similar storage conditions for each sample, it might be expected that those solutions containing more organic material would be darker. The observed colors, instead, may be due to mixtures representing differing degrees of unsaturated fatty acid or aromatic amino acid oxidation and/or degraded pigments, leading to the formation of colored products. Further study using a larger number of samples and more controlled conditions would be required to clarify questions concerning the cause of observed color differences and to determine whether variation in dissolved material (including ratios of the amount and type of organic to the amount and type of inorganic components) can be related to taxa, or to titratable acidity, formaldehyde concentration, or other measurable quantities. Table 2 also indicates that solution pHs at the time of this study are reasonably consistent, and exist within a range of 1.6 units centering around pH 6.5, i.e., 6.5 $\pm$ 0.8. This can be considered moderately acid for a 70% v/v solution of ethyl alcohol and water. As mentioned previously, pH paper provides relatively inaccurate results when used to measure the acidity or alkalinity of ethyl alcohol solutions. However, the technique can serve to indicate the general degree of consistency in pH among the storage fluids. The fluid from only one specimen used in this study, *Peromyscus leucopus texanus,* had its pH recorded previously. In 1986 the pH of this specimen was recorded as being 6.8; now the fluid has a pH of 7.3, so that it has changed about 0.5 units in the intervening time (Robert Fisher, personal communication).

The amount and type of fatty acids present in the sample were studied by conducting a mass spectral analysis of the methylene chloride extract of underivatized sample from the bat *Tardarida brasiliensis intermedia.* The total ion chro-

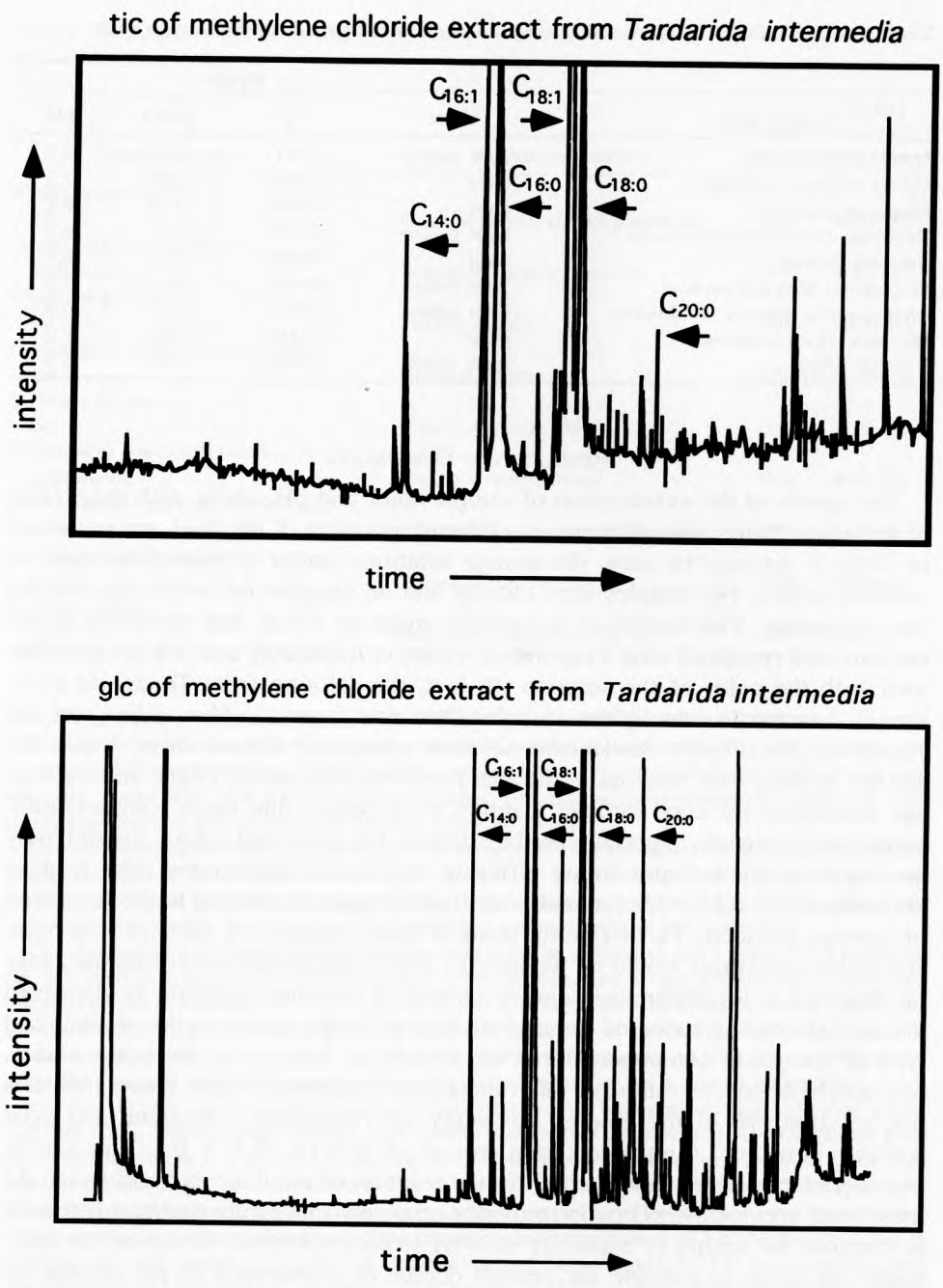


Figure 1. A comparison of the mass spectral total ion chromatogram and gas chromatographic recording of the fatty acids found in fluid surrounding *Tardarida brasiliensis intermedia*. $C_{12:0}$ = lauric acid; $C_{14:0}$ = myristic acid; $C_{16:0}$ = palmitic acid; $C_{16:1}$ = palmitoleic acid; $C_{18:0}$ = stearic acid; $C_{18:1}$ = oleic acid; $C_{20}$ = fatty acid of carbon length 20. Acids of longer chain length are to the right of $C_{20}$.

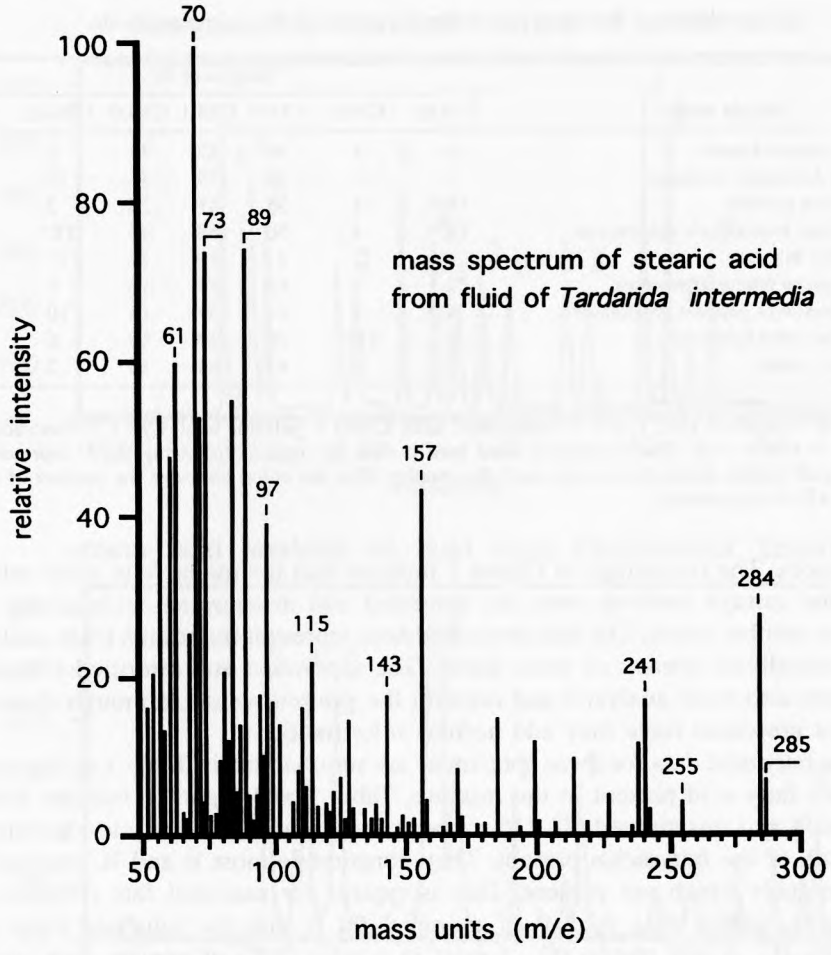


Figure 2. An electron impact mass spectrum of stearic acid, the second largest peak found in an extract of fluid surrounding *Tardarida brasiliensis intermedia*. $M^+$ = the molecular ion at m/e (mass/charge) 284; M − 43 = the molecular ion minus the fragment $[C_3H_7]^+$ at m/e 241; most of the remainder of the abundant ions in the spectrum belong to the series $[(CH_2)_nCOOH]^+$ for all values of n except the highest, corresponding to M − 15; e.g., m/e 157 is indicative of the fragment $[(CH_2)_8COOH]^+$.

matogram (TIC) for the separated fatty acids recorded on the mass spectrometer is compared with the gas chromatographic analysis of the same sample in Figure 1. Here it may be seen that a variety of fatty acids were found in the storage fluid. The identity of the acids in Figure 1 were determined by examining the individual spectra of the relevant peaks in the TIC; the spectrum used to identify one of the major peaks in the TIC as being free stearic acid is presented in Figure 2. On the basis of spectral identification of the rest of the major peaks in the TIC, assignments were made for the peaks found in the GC trace illustrated in the lower half of Figure 1 (Oldham and Stenhagen, 1972). Since the GC was operated in exactly the same manner for this set of analyses, assignment of the peaks in one GC trace allowed the identification and direct comparison of peaks in other

Table 3. A comparison of the major fatty acids in mammal holotype storage fluids.

| Sample name | Fatty acid % | | | | | | |
|---|---|---|---|---|---|---|---|
| | C14:0 | C16:1 | C16:0 | C18:1 | C18:0 | C20–22 | P/S |
| *Myotis keenii keenii* | 2 | 3 | 36 | 27 | 11 | 2 | 3.3 |
| *Myotis lucifugus lucifugus* | 2 | 3 | 29 | 35 | 16 | 12 | 1.8 |
| *Rhogeesa gracilis* | TR* | 2 | 35 | 41 | 28 | 2 | 1.3 |
| *Tardarida brasiliensis intermedia* | TR* | 4 | 36 | 68 | 44 | TR* | 0.8 |
| *Neotoma bryanti* | 4 | 2 | 31 | 19 | 14 | 17 | 2.2 |
| *Peromyscus leucopus texanus* | 2 | 3 | 25 | 37 | 18 | 5 | 1.4 |
| *Clethrionomys gapperi carolinensis* | 4 | 2 | 24 | 37 | 14 | 10 | 1.7 |
| *Microtus tshuchytshurum* | 2 | TR* | 28 | 20 | 19 | 6 | 1.5 |
| *Neofiber alleni* | 2 | 2 | 43 | 33 | 13 | 2 | 3.3 |

TR* = A non-measurable trace amount.

C14:0 = myristic acid; C16:1 = palmitoleic acid; C16:0 = palmitic acid; C18:1 = oleic acid; and C18:0 = stearic acid. The convention used here is that the number following the C represents the number of carbon atoms in the fatty acid; the number after the colon indicates the number of double bonds which are present.

GC traces. The recordings in Figure 1 indicate that the major fatty acids released into the storage medium were the saturated and unsaturated acids of the even carbon number series. The data presented here represent the results from analyzing the unmodified extracts of these fluids. The saponified and esterified aliquots of solution also were analyzed and confirm the present results, although these data are not presented since they add no new information.

The fatty acid data for these specimens are summarized in Table 3 as the percent of each fatty acid present in the mixture. Table 3 and Figure 1 indicate that the saturated and unsaturated $C_{16}$ (16 carbons long) and $C_{18}$ fatty acids account for the bulk of the fatty acids present. They constitute almost ⅓ and ⅔, respectively, of the acids which are present. This is typical for mammal fats (Hilditch and Williams, 1964). Also typical of mammal fat is that the saturated fatty acids palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) exist in a ratio (P/S) of greater than one (cf. Hilditch and Williams, 1964:127, 131). In plants, the ratio typically is less than one (cf. Mills and White, 1987:28). In the example of *Tardarida brasiliensis intermedia* above, the P/S ratio is seen to be more typical of plants, that is, less than one (see Table 3). Either this is an anomaly that bears further investigation, or the stomach contents of this insect eating bat contain sufficient plant lipids that these have become the major components in this analysis.

Except for the results from *Tardarida brasiliensis intermedia,* Table 3 indicates that the fatty acid contents of these specimens vary somewhat, but are also similar in that each contains high proportions of palmitic ($C_{16:0}$), oleic ($C_{18:1}$) and stearic ($C_{18:0}$) acids, and have P/S ratios greater than one. The fatty acids, their variation and distribution are typical for mammal fat (Hilditch and Williams, 1964). Of interest is that the dehydrating, hydrolytic, and solvent powers of the storage medium which lead to a mobilization (and redistribution) of lipids from their normal locations within these mammals, and finally, to the loss of lipid components into the storage fluid, also implies that the shape of the specimen has changed—the most likely result being a shrinkage of the specimen. This effect would be most severe in those specimens containing the highest proportion of fatty tissue.

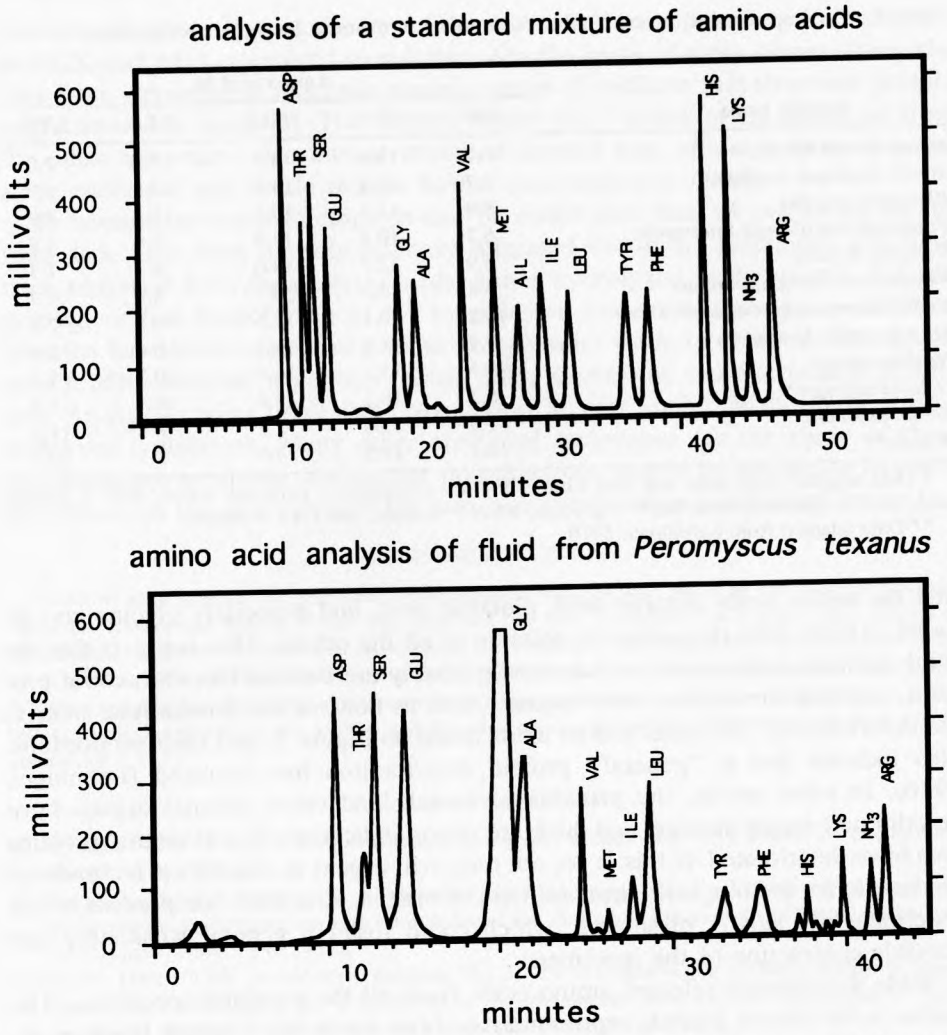


Figure 3. A comparison of the results from analyzing a standard amino acid mixture, and the amino acids found in the fluid surrounding *Peromyscus leucopus texanus*. The legend for the amino acids is: ASP = aspartic acid; THR = threonine; SER = serine; GLU = glutamic acid; GLY = glycine; ALA = alanine; VAL = valine; MET = methionine; AIL = allo-isoleucine; ILE = isoleucine; LEU = leucine; TYR = tyrosine; PHE = phenylalanine; HIS = histidine; LYS = lysine; $NH_3$ = ammonia; and ARG = arginine.

The final technique used in this study to describe the soluble organic components found in storage fluids is that of amino acid analysis. Using this technique, we can estimate the amount and type of proteins which are being hydrolyzed within the stored specimen and are diffusing to the surface from the interior, or are being lost because the surface skin itself is deteriorating. Figure 3 illustrates the results from analyzing a standard amino acid mixture and a sample of fluid taken from *Peromyscus leucopus texanus*. Here it can be seen that the sample of fluid contains amino acids representative of proteins or peptides that have leached into the storage medium (see Von Endt, 1980). In this example, the figure indicates

Table 4. A comparison of selected amino acids found in mammal holotype storage fluids.

| Sample name | Amino acid % | | | | |
|---|---|---|---|---|---|
| | ASP | GLU | GLY | ALA | LYS |
| *Myotis keenii keenii* | 10.7 | 12.6 | 18.7 | 11.2 | 2.0 |
| *Myotis lucifugus lucifugus* | 6.7 | 10.4 | 14.9 | 7.8 | 2.7 |
| *Rhogeesa gracilis* | 8.9 | 12.2 | 19.3 | 11.1 | 1.9 |
| *Tardarida brasiliensis intermedia* | 6.7 | 10.4 | 14.9 | 7.8 | 1.8 |
| *Neotoma bryanti* | 5.9 | 12.2 | 19.0 | 9.3 | 1.5 |
| *Peromyscus leucopus texanus* | 9.8 | 13.3 | 30.10 | 8.9 | 1.7 |
| *Clethrionomys gapperi carolinensis* | 10.2 | 10.1 | 13.5 | 10.1 | 2.8 |
| *Microtus tshuchytshurum* | 9.9 | 12.8 | 16.7 | 10.4 | 1.4 |
| *Neofiber alleni* | 10.1 | 13.1 | 16.4 | 10.1 | 1.1 |
| *Gelatin/collagen** | 6.2 | 10.2 | 28.8 | 11.8 | 3.8 |
| *Serum albumin*** | 9.0 | 15.6 | 2.9 | 8.8 | 11.3 |
| *Myosin*** | 10.1 | 18.5 | 4.6 | 9.3 | 10.1 |

* Data adapted from Rose and Von Endt, 1984, for comparative purposes, where ASP = aspartic acid, GLU = glutamic acid, GLY = glycine, ALA = alanine, and LYS = lysine.

** Data adapted from Lehninger, 1970.

that the amino acids aspartic acid, glutamic acid, and especially glycine and alanine exist in high proportion in relation to all the others. This suggests that the fluid contains amino acids which most probably are derived from structural proteins, and that the skin or other organs (such as bone or the intercellular matrix) are deteriorating. The other amino acids listed on Figure 3, and their proportions, also indicate that a "general" protein deterioration has occurred (Lehninger, 1970). In other words, the proteins of muscle and other internal organs have deteriorated during storage, and there are strong indications that structural proteins also have deteriorated. If this is so, not only will organs at some time be rendered not usable for histological purposes, but the organic structural components of the specimens are slowly dissolving, which could foster a general weakening and possible contraction of the specimen.

Table 4 compares selected amino acids from all the mammal specimens. The amino acids chosen include representatives from the acidic fraction [such as aspartic (ASP) and glutamic acids (GLU)], the neutral fraction [such as glycine (GLY) and alanine (ALA)], and the basic amino acids [such as lysine (LYS)]. Table 4 indicates that, in addition to the example of *Peromyscus leucopus texanus* discussed above, proteins found in all the specimens are being solubilized and being lost to the surrounding fluid.

The major mammalian structural protein, collagen, exists in a helix as a long, rod-shaped molecule, with great strength along the long axis. To achieve this shape and strength, many of the amino acids composing this protein are small in size (to allow helix formation) and have non-polar side chains. This accounts for the high percentage of GLY and ALA (about 40%) found among the amino acids of collagen, and typically, an amino acid analysis exhibiting these characteristics is indicative of this molecule. Collagen also contains a high percentage (in the 10% range) of ASP and GLU. The amino acids of serum albumin and the muscle protein myosin are included in Table 4 to illustrate a "more typical" amino acid distribution representing these and other nonstructural proteins. Both myosin and serum albumin contain a sizable proportion of ASP and GLU, as do many other

proteins present in nature. However, these proteins have relatively low amounts of GLY and ALA compared to collagen. On the basis of these observations, the data from *Peromyscus leucopus texanus* seems to indicate that structural protein degradation has occurred. The slightly higher GLY levels of the solutions from the other specimens also hint at structural protein loss, although these data are more equivocal and would require further experiments to clarify.

To summarize these findings, it can be stated that fats, as evidenced by the lipid data, have been hydrolyzed, have migrated within the specimens, and have been removed from them. Fatty acids, either hydrolyzed in the animal and migrating, or fats hydrolyzing in the solution are present in the storage medium. Proteins have deteriorated as evidenced by amino acids in solution. The amino acid profiles indicate a general protein loss, as well as some structural protein loss. As stated earlier, these data provide only an entry point for the study of fluid preserved collections. Many other analytical techniques for the study of these specimens are available, and further investigations should be devised to examine the effects on the specimens of the numerous variables not addressed here.

## Acknowledgments

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# EVALUATION OF THE EFFECTS OF DIFFERENT PRESERVATIVE AND FIXATIVE FLUIDS ON AQUATIC INVERTEBRATES FROM INTERSTITIAL WATERS

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*Abstract.*—Ethyl alcohol has long been used as a general fixing and preserving agent for small aquatic invertebrates. It is recognized that there is no all-purpose fixative, but some fixatives are effective for large numbers of taxonomic groups. The objectives of this study were to determine whether or not it is better to fix samples from interstitial waters in the field, immediately after collection, and to determine which fixatives are best suited for aquatic invertebrates. We tested 70% ethyl alcohol, 5% formaldehyde, Angelier's fluid, and Koenike's fluid on oligochaetes, ostracods, copepods, syncarids, isopods, amphipods, molluscs and water mites. Sixteen possible fixative/preservative combinations were tested. It is not possible to make generalizations about fixing samples from interstitial waters in the field. The taxonomic groups studied differed as to preferred fixatives and preservatives.

One of the main problems that arises when planning a limnological sample collecting expedition of several days in length is choosing which fluid to use as a fixative.

There is some controversy about which fluids can be considered fixing agents and which cannot. The majority of researchers use the definition of Stoddard (1989), which considers ethyl alcohol a pseudo-fixing agent or denaturing agent. According to Stoddard (1989), the effect of this agent in tissue is not to establish cross-links between the proteins (as happens with true fixing agents) but to fundamentally alter hydrogen bonding and disorder protein, resulting in a sort of macromolecular tangling of protein, carbohydrates and nucleic acids. This tangling traps other small molecules.

Nevertheless, ethyl alcohol has long been used as a general fixing agent for small aquatic invertebrates (Lincoln and Sheals, 1979) because the general appearance of the specimens is excellent following the fixing process (even after long periods of time) and the specimens can be easily manipulated for taxonomic work afterwards (e.g., clearing, dissection, or microscope observation). Our personal experience with syncarids, amphipods and isopods has clearly supported this conclusion. When an aldehyde fixing agent is used for these taxa, we have observed that the tissues are more rigid. This rigidity makes manipulations of the specimens difficult, more time is needed for the clearing of the specimens, and the cuticle tears when the animal is dissected. Thus, we considered ethyl alcohol to be a fixing agent in this study.

It is generally recognized that there is no all-purpose fixative, but some fixatives, alone or in combination, are effective for large numbers of taxonomic groups. The following criteria are often used when selecting a fixative: (1) it must be effective for a large number of taxonomic groups; (2) it must be effective in small quantities to save weight and space; (3) it must be economical to use; (4) it must not be dangerous to handle (toxic, flammable or caustic); (5) it must not

chemically react with certain synthetic materials such as clothing, sample vials, backpacks, etc.

Samples from the interstitial waters associated with rivers are generally collected with a large amount of sand, which not only retains some water, but also causes abrasion of the organisms in the sample during transport back to the laboratory. The dilution of the fixative is not too great a problem over a short period of time, as this type of sample usually contains only small amounts of organic matter, and there is little danger that the fixative will not be effective. However, abrasion can cause irreversible damage to the animals, making specific identifications impossible and limiting their value in scientific collections. It is not known whether the effects of abrasion are less harmful when the animals are alive and consequently more flexible and able to move, or when they are fixed and thus hard and rigid. This constitutes a further problem when choosing the best fluid for fixing samples.

We should keep in mind that samples from interstitial waters can be kept alive for five or six days if refrigerated. There is little danger that predators will affect the sample, because population density in the samples is usually quite low.

This study had two objectives: to determine whether or not it is better to fix samples from interstitial waters in the field, immediately after collection; and to determine which fixative is best suited for the groups of aquatic invertebrates that most often appear in this environment (oligochaetes, ostracods, copepods, syncarids, molluscs, isopods, amphipods, and water mites). We chose the two most commonly used fixative and preservative solutions in limnological studies (70% ethyl alcohol and 5% formaldehyde) and two others, Angelier's fluid and Koenike's solution (also called Viet's solution), which are generally unknown in limnological practice (García-Valdecasas and Baltanás, 1989). We also analyzed which are the most appropriate action intervals for each fluid, and finally, we evaluated the effectiveness of all possible combinations of these four fixatives as preservatives over time.

General studies on fixation and preservation techniques (e.g., Martoja and Martoja, 1970; Galigher and Kozloff, 1971; Mahoney, 1973; Lincoln and Sheals, 1979) have recognized that there is no all-purpose fixing agent, and that no mixture of fixatives is entirely suitable as a preservative. They also pointed out that a flaw in fixation cannot be subsequently remedied.

The purpose of fixation is to preserve the specimens in a state as similar to the living state as possible. In order to do this we must: (1) protect the specimens from bacterial attack; (2) prevent autolysis of the basic constituents of the cells; (3) render insoluble the cell constituents to be examined; (4) prevent distortions and contractions; (5) not chemically alter the organism (e.g., by dissolution of calcium carbonate in the shell); and (6) prepare the structures for subsequent treatment.

For morphological studies, on which we shall focus our attention, investigators seek above all to preserve proteins, which are the main constituents of cell structures. It is useful to know the rate at which a fixing agent penetrates and the degree of hardening it causes in the tissues, as well as whether changes in volume of the organism occur that distort the cell structures.

According to Baker (1956), the chemical combinations of compound fixatives were arrived at empirically. From a chemical standpoint, they are true monstros-

Table 1. General features of the four fixing agents.

| Fixative | Price | Quantity per sample | Flammable | Toxicity | Reactions with other materials |
|---|---|---|---|---|---|
| Ethyl alcohol | Expensive | High | Yes | Non-toxic | None |
| Formaldehyde | Inexpensive | Low | No | Toxic | None |
| Angelier's fluid | Inexpensive | Very low | No | Non-toxic; causes skin lesions | Damages plastics and clothing |
| Koenike's solution | Inexpensive | Low | No | Non-toxic; causes skin lesions | None |

ities, combining acids and bases, reducing and oxidizing agents, etc. However, some of these mixtures, for example Angelier's fluid for aquatic mites, are very useful. In this solution, the animals soften, which facilitates the separation of the anatomical pieces when dissecting the specimens. This dissection is an essential procedure for the specific determination of the specimen.

Table 1 shows the general features of the four fixing agents used. Table 2 lists the properties of the four fixatives, according to Baker (1956).

We have commented on the general effects of some anesthetics, but we have not included an evaluation of anesthetics here, because our objective was to evaluate only fixatives and preservatives.

According to the literature, the most commonly used fixative is 70% ethyl alcohol, followed by 5% formaldehyde, although it is recommended that formaldehyde be replaced by 70% ethyl alcohol as a preservative as soon as possible. However, these two fixatives (particularly formaldehyde) cause excessive hardening in certain animal groups, such as water mites, making it nearly impossible to dissect them for identification at the species level.

For each animal group there appears to be an optimal fixative that maintains the animal in the best possible state for observation, specific identification and preservation. Table 3 shows which fixatives are best for each group, according to the literature. In general studies, a different fixing agent is not used for each taxonomic group, but rather ethyl alcohol or formaldehyde is used to the benefit of some groups and the detriment of others.

In samples taken from interstitial waters, fixed with 70% ethyl alcohol or 5% formaldehyde in the field, the groups that can be fixed and preserved with the best results, according to our own experience and that of other researchers (A. García-Valdecasas, N. Coineau, and J. Notemboom, personal communication) are copepods, syncarids, isopods, and amphipods. Oligochaetes contract and in many cases become very rigid; ostracods almost always die with their valves tightly closed, and when formaldehyde is used, the valves disintegrate; gastropods completely withdraw into their shells if ethyl alcohol is used, and the shells disintegrate almost entirely if formaldehyde is used; water mites harden and contract in both fixing agents.

## Materials and Methods

For this study we chose the eight taxonomic groups that most often appear in interstitial waters: oligochaetes, ostracods, copepods, syncarids, isopods, amphipods, molluscs and water mites. In an effort to determine whether or not it is useful to fix samples in the field at the time they are collected, we set up two models:

Table 2. Chemical properties of the four fixing agents (Baker, 1956).

| Fixative | Action | Precipitates proteins | Dissolves lipids | Causes contraction | Hardens tissues |
|---|---|---|---|---|---|
| 70% ethyl alcohol | Reducing agent | Yes | Yes | Yes | Yes |
| 4% formaldehyde | Reducing agent | No | No | No (can cause contraction in many animals) | Yes |
| 3–5% acetic acid | Oxidizing agent | Yes | Yes | No | No |
| Chromic acid | Oxidizing agent | Yes | | No? | No |

*Model 1.* Samples collected and fixed unsorted in the field.

*Model 2.* Samples kept alive and taken to the laboratory (4–5 days in refrigeration); taxa separated; each taxon fixed separately in the laboratory.

*Model 1* (Fig. 1).—We obtained four equivalent samples (a, b, c, and d), each of 90 filtered liters, using the Karaman-Chappuis method (Camacho, 1987), from the interstitial waters of the Jarama River, in the Pontón de la Oliva (Patones, Madrid). Each of the samples was fixed in one of the following fixing agents:

A. 70% ethyl alcohol
B. 5% formaldehyde (unbuffered)
C. Angelier's fluid (1% acetic acid; 1% chromic acid; 98% distilled water), 1 ml per 100 ml of sample (Valdecasas and Baltanas, 1989)
D. Koenike's solution (3 parts glacial acetic acid, 11 parts glycerol, 6 parts distilled water)

The amount of fixing agent used in samples a, b and d was based on our own and other researcher's experience, as many factors are involved (the amount of sand, the amount of organic matter in the sample, and the volume of water retained by the sample).

Once fixed, each of the samples was divided into three equivalent groups (numbered 1 to 3).

Group 1 of each sample was washed in the laboratory after 24 hr in the fixative. Specimens were sorted into eight taxonomic groups. Each taxonomic group was then divided into four equivalent parts. Each part was placed in a small container with one of the four fixing agents, which then served as a preserving agent. Thus, all 16 possible fixative/preservative combinations were obtained.

Group 2 was washed three weeks after being placed in the fixative, and the same steps were followed as with Group 1.

Group 3 was washed six weeks after being placed in the fixative, and the same steps were followed as with Group 1.

The specimens in each group were evaluated after 24 hr in fixative and then weekly for 12 weeks in preservative.

*Model 2* (Fig. 2).—A very large sample (150 filtered liters) was collected from the same location as the sample for Model 1, using the same collecting method.

The sample was kept alive and washed immediately upon arrival in the laboratory. The specimens were then sorted into eight taxonomic groups. The specimens of each taxon were divided into four equivalent parts (a, b, c, and d) and each part was fixed in one of the four fixing agents; however, there were not enough specimens in all taxonomic groups to evaluate all four fixing agents. The first evaluation of the condition of the specimens took place 24 hr after they were placed in the fixative.

After two weeks, the specimens in each fixative were divided into four equivalent parts. Three were removed from the container, washed, and placed in one of the three remaining fixing agents, which then acted as a preservative. The fourth part was left in its original fixative, and that fixing agent was then considered a preservative. Thus, the same 16 fixative/preservative combinations as in Model 1 were obtained.

The observation intervals were the same as for Model 1.

## RESULTS

*Comparison between Models 1 and 2.*—Figure 3 shows in detail the effects of the four fixatives on the eight taxonomic groups studied. Note that not all of the

Table 3. Best anesthetics, fixatives and preservatives for each of the groups analyzed, according to the literature (Pennak, 1953; Angelier, 1953; Knudsen, 1966; Mahoney, 1973).

| Taxonomic group | Anesthetic | Fixative | Preservative |
|---|---|---|---|
| Aquatic oligochaetes | 30% ethyl alcohol with chloroform, 0.8% nitric acid or 1% chromic acid | 70% ethyl alcohol or 4% formaldehyde with 5% glycerine added | 70% ethyl alcohol or 4% formaldehyde with 5% glycerine added |
| Ostracods | Crystals of urethane to keep valves open | 5% formaldehyde or Bouin's fluid | Place in 70% ethyl alcohol after 24 hours in fixative |
| Copepods | Diluted ethyl alcohol | 5% formaldehyde, Bouin's fluid, 95% Heidenhain's suse | 4% formaldehyde buffered or 70% ethyl alcohol |
| Syncarids<br>Isopods<br>Amphipods | Diluted ethyl alcohol | 70% ethyl alcohol or 4% formaldehyde. Formaldehyde hardens the animal, making it difficult to render translucent and to dissect. Furthermore, isopod and amphipods tend to loss appendages because of contraction | 70% ethyl alcohol |
| Aquatic molluscs | Thermal shock, menthol, urethane or magnesium sulfate | 70% ethyl alcohol or 4% formaldehyde | 70% ethyl alcohol or a mixture of equal parts of 4% formaldehyde and glycerol |
| Water mites | | Angelier's fluid or Koenike's solution | Angelier's fluid or Koenike's solution, keep the structures soft and flexible, facilitating dissection |

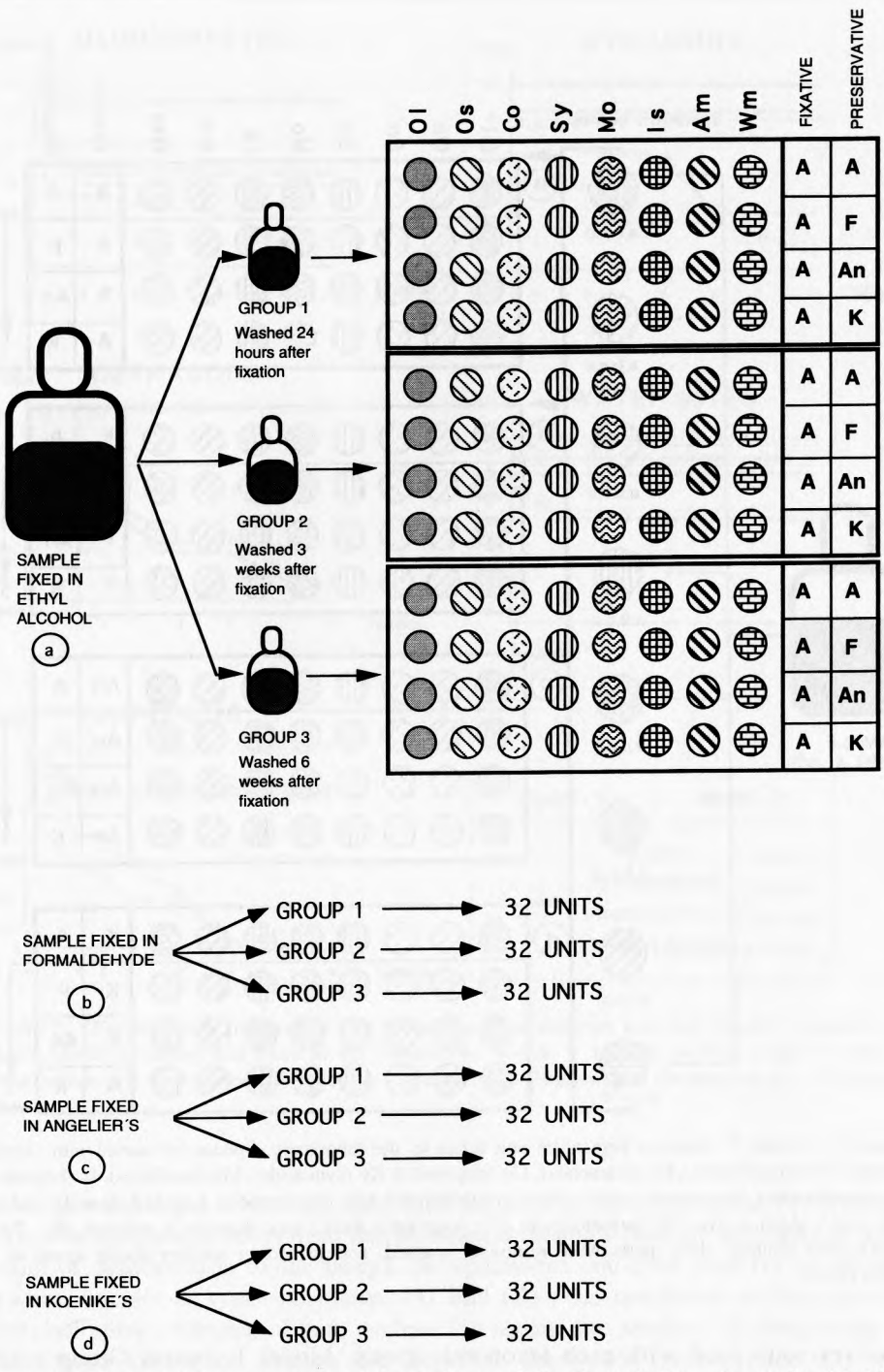


Figure 1. *Model 1.* Four equivalent samples (a, b, c and d) of aquatic invertebrates fixed in 70% ethyl alcohol (A); 5% formaldehyde (F); Angelier's fluid (An); Koenike's solution (K). Group 1 washed 24 hr after fixation; Group 2 washed three weeks after fixation; Group 3 washed six weeks after fixation. Specimens sorted into faunal groups: Ol (oligochaetes), Os (ostracods), Co (copepods), Sy (syncarids), Mo (molluscs), Is (isopods), Am (amphipods), Wm (water mites). Each faunal group divided into four parts and placed in a different preserving agent.

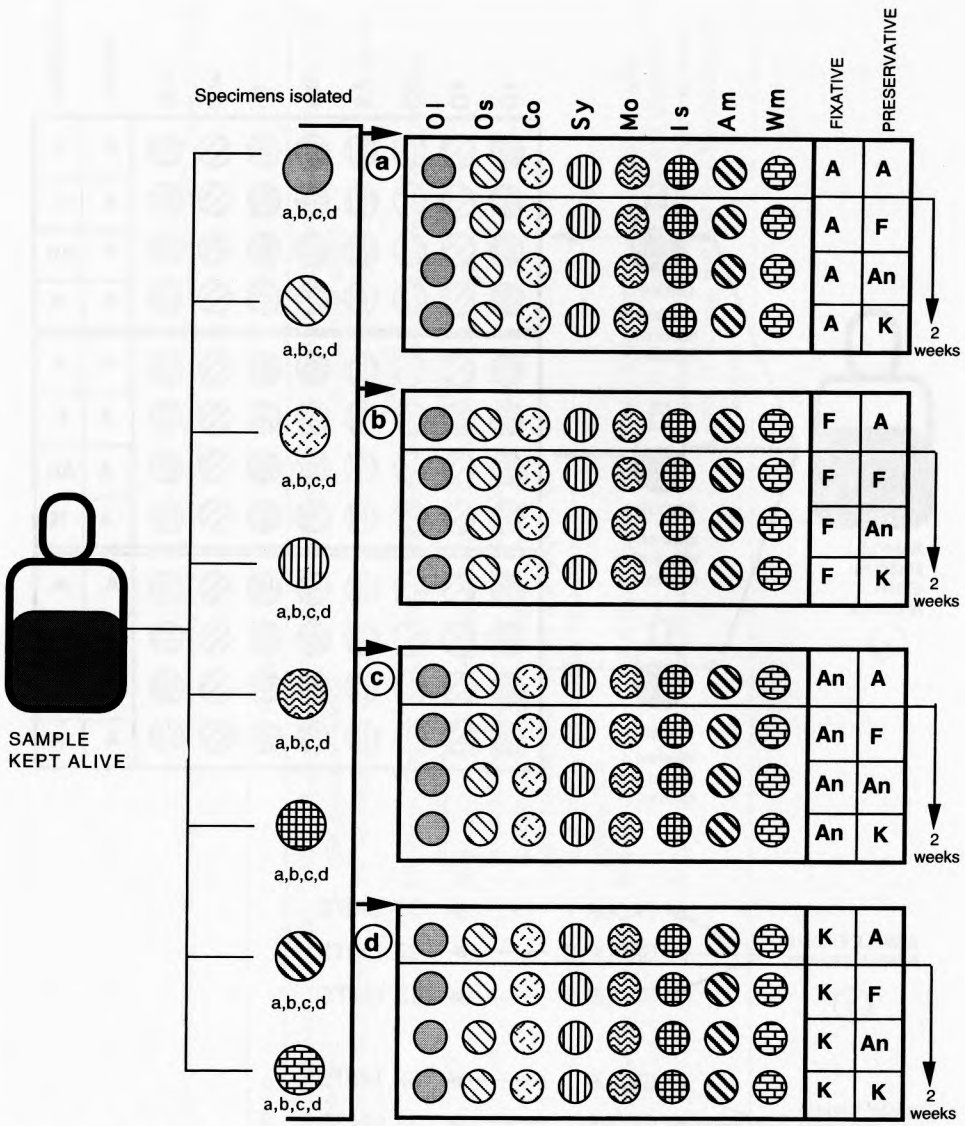


Figure 2. *Model 2.* Samples kept alive and taken to the laboratory. Specimens sorted into faunal groups: Ol (oligochaetes), Os (ostracods), Co (copepods), Sy (syncarids), Mo (molluscs), Is (isopods), Am (amphipods), Wm (water mites). Each group divided into four parts (a, b, c and d) and fixed in 70% ethyl alcohol (A); 5% formaldehyde (F); Angelier's fluid (An); Koenike's solution (K). Two weeks after fixation, three parts were removed, washed, and placed in another fixing agent as a preservative.

fixatives were used with each taxonomic group. Model 1 depicts Group 1 (in fixative for 24 hr). For both models, the first evaluation (at 0 weeks) took place after 24 hr in a fixing agent.

The preservation status of the specimens was evaluated qualitatively as: (1) Very good (Vg)—specimens had not suffered any changes, they could be used as type specimens; (2) Good (G)—the specimens suffered slight changes (loss of

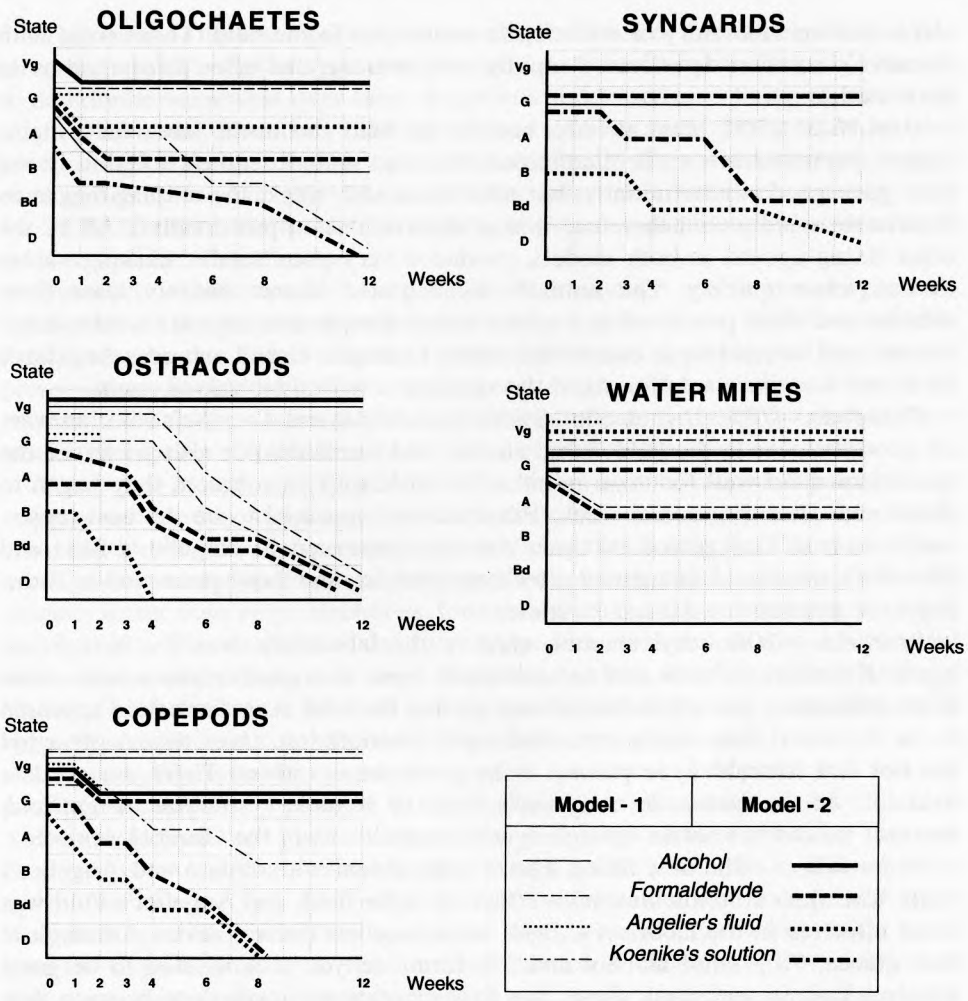


Figure 3. The effects of fixatives on five faunal groups at different intervals. Model 1 (fixed in the field), Model 2 (sorted and fixed in the laboratory). X-axis = time in weeks; Y-axis = state of preservation: Vg—very good; G—good; A—average; Bd—beginning to disintegrate; D—disintegrated.

color, general slight softening), they could be used as type specimens; (3) Average (A)—the specimens underwent morphological changes (loss of appendages, softening or deterioration of the body), the specimens could be used for taxonomic studies, but not as type specimens; (4) Bad (B)—the specimens suffered severe morphological changes, almost useless for taxonomic studies; (5) Beginning to disintegrate (Bd)—the animals had begun to decay; (6) Disintegrated (D)—the animals had disintegrated.

Due to an error in the dilution of the Angelier's fluid (an excess of more than 1 ml to every 100 ml of sample was used), we did not include the effects of this fixing agent on Group 1.

*Oligochaetes.*—The best-preserved animals were those fixed with 70% ethyl

alcohol in the laboratory or with 5% formaldehyde in the field. Those fixed with Koenike's solution deteriorated rapidly and disintegrated after three months in both models.

*Ostracods.*—70% ethyl alcohol, both in the field and in the laboratory, maintained specimens in excellent condition. Fixation with Koenike's solution in the field gave good results initially, but after the fourth week, the animals began to deteriorate rapidly, and therefore it was not useful as a preservative. All of the other fixing agents, in both models, produced very poor results, causing valves to deteriorate quickly. The animals disintegrated almost entirely after three months, and those preserved in Angelier's fluid disappeared after six weeks. Ethyl alcohol and formaldehyde caused the valves to remain closed, whereas Angelier's fluid and Koenike's solution fixed the specimens with their valves open.

*Copepods.*—70% ethyl alcohol, 5% formaldehyde, and Koenike's solution were all good fixatives in the field. Ethyl alcohol and formaldehyde also preserved the specimens quite well for three months, but in Koenike's solution, they began to deteriorate after the second week. Ethyl alcohol appeared to be the best preservative over a long period of time. Animals preserved in Angelier's fluid and Koenike's solution disintegrated after three months, and those preserved in formaldehyde presented a damaged cuticle.

*Syncarids.*—70% ethyl alcohol, used in the laboratory, was the best fixing agent. Koenike's solution and formaldehyde were also good fixatives when used in the laboratory, and ethyl alcohol was good in the field. Angelier's fluid appeared to be the worst fixative, as it caused rapid deterioration. Over time, only ethyl alcohol and formaldehyde proved to be good preservatives. There are no data available on formaldehyde, Angelier's fluid, or Koenike's solution in the field, because we did not obtain enough syncarid specimens in the Group 1 sample.

*Water mites.*—The best fixing agents were Koenike's solution and Angelier's fluid. Koenike's solution was more effective in the field, and Angelier's fluid was more effective in the laboratory. Both were excellent preservatives. Although at first glance, 70% ethyl alcohol and 5% formaldehyde also seemed to be good fixatives and preservatives, these two fixing agents are inadequate because they fix muscular structures, which makes it extremely difficult to observe diagnostic features in the cuticle (A. García-Valdecasas, personal communication).

### Comparison of the Different Fixative/Preservative Combinations

*Oligochaetes.*—The best strategy is to let Angelier's fluid act as a fixative for six weeks and then use any one of the four agents as a preservative, because they seemed to have similar effects, at least for the period of this study. Formaldehyde was an excellent fixative and preservative for oligochaetes, but it contracts the specimens if the animals have not been relaxed with some anesthetic agent before the fixing process.

*Ostracods.*—The best fixing agent seemed to be ethyl alcohol (which was also the best preservative), however, it did cause the valves to remain tightly closed. If the specimens were fixed in Angelier's fluid or Koenike's solution and then washed after 24 hr and placed in ethyl alcohol as a preservative, the valves remained open and the specimens were well preserved. It was noted that Angelier's fluid caused the animals to deteriorate (first the valves and then the soft parts) more quickly than Koenike's solution.

*Copepods.*—The best option is to use ethyl alcohol or formaldehyde as a fixative and ethyl alcohol as a preservative, because formaldehyde causes the animals to deteriorate somewhat over time. Angelier's fluid used as a fixing agent for up to six weeks, and Koenike's solution used as a fixing agent for up to 24 hr, also produced good results, though the specimens should be washed as soon as possible and preserved in ethyl alcohol. It was noted that Koenike's solution used for 24 hr rendered the specimens slightly translucent, facilitating their treatment prior to specific identification.

*Syncarids.*—Unfortunately, we did not obtain enough specimens of syncarids to observe all the possible fixative/preservative combinations. With the available data, it can be concluded only that ethyl alcohol was a very good fixative and preservative. Formaldehyde also gave good results. The specimens should be washed and sorted as soon as possible, because those in Group 2 (washed three weeks after being collected) were in worse condition than those in Group 1.

*Molluscs.*—It was noted that all of the fixing agents caused the animals to contract and harden, and therefore it is better to take the sample to the laboratory alive and anesthetize the specimens before fixing them. Angelier's fluid and Koenike's solution used as fixatives for very short periods (24 hr at most) leave the animals softer than ethyl alcohol or formaldehyde, but they also attack the shell very quickly. The condition of the microsculpture and the radula was not observed when these two fixatives were used. Over long periods, both Angelier's fluid and Koenike's solution cause the shell and the animal to disintegrate completely, leaving only the periostracum. This might be useful in certain cases.

*Isopods.*—The samples contained very few specimens of isopods, so it was not possible to compare all possible fixative/preservative combinations. Ethyl alcohol and formaldehyde are good fixing agents, and ethyl alcohol was also the best preservative. Another good combination was Koenike's solution as a fixative for up to four weeks, and then ethyl alcohol or formaldehyde as a preservative. Koenike's solution renders the specimens slightly translucent, which might facilitate the steps leading up to specific identification. Furthermore, it was observed that these animals are extremely delicate and easily lose their appendages, but they lose fewer appendages when fixed in Koenike's solution than when fixed in other agents.

*Amphipods.*—As with isopods, very few specimens were obtained. Ethyl alcohol and formaldehyde appear to be the best fixatives, as well as the best preservatives. Angelier's fluid (for up to 24 hr) and Koenike's solution (for up to a maximum of four weeks) are suitable for fixation, provided that the animals are subsequently washed and placed in ethyl alcohol as a preservative. As with isopods, these two fluids soften the structures and render them translucent, which might facilitate the subsequent study of the animals.

*Water mites.*—As stated above, ethyl alcohol and formaldehyde are good fixing agents, but cause excessive hardening of the animals. It is best to use Angelier's fluid or Koenike's solution to fix and preserve water mites.

## CONCLUSIONS

It is impossible to make any generalizations about whether or not it is more advantageous to fix a sample from interstitial waters in the field. The choice depends on many factors, such as the taxonomic groups being studied, the length

Table 4. Optimal fixative/preservative combinations and action intervals for each taxonomic group studied.

| Taxonomic group | Best fixative | Action interval | Best preservative | Alternative fixative |
|---|---|---|---|---|
| Aquatic oligochaetes | Angelier's fluid | 45 days | All others | 70% ethyl alcohol or 4% formaldehyde |
| Ostracods | Angelier's fluid<br>Koenike's solution | 15 days<br>1 day | 70% ethyl alcohol | 70% ethyl alcohol but it leaves the valves closed |
| Copepods | 70% ethyl alcohol<br>Angelier's fluid | Not a factor<br>(45 days maximum) | 70% ethyl alcohol | |
| Syncarids | 70% ethyl alcohol<br>5% formaldehyde | Not a factor<br>Not a factor | 70% ethyl alcohol | |
| Molluscs | Angelier's fluid<br>Koenike's solution | 1 day<br>1 day | 70% ethyl alcohol | 70% ethyl alcohol |
| Isopods | Koenike's solution | 30 days | 70% ethyl alcohol<br>5% formaldehyde | 70% ethyl alcohol |
| Amphipods | 70% ethyl alcohol<br>5% formaldehyde | Not a factor<br>Not a factor | 70% ethyl alcohol | Koenike's solution a<br>(maximum of 30 days) |
| Water mites | Angelier's fluid<br>Koenike's solution | Not a factor<br>Not a factor | Angelier's fluid<br>Koenike's solution | |

of the sampling trip, field conditions, and the type of study being carried out. However, certain conclusions can be drawn for each taxonomic group examined:

*Oligochaetes.*—Best fixed in the laboratory with ethyl alcohol or Angelier's fluid. If they must be fixed in the field, ethyl alcohol or formaldehyde should be used.

*Ostracods.*—Fix in the field or in the laboratory with ethyl alcohol or Koenike's solution. If Koenike's solution is used, the sample should be washed within six weeks and preserved in ethyl alcohol.

*Copepods.*—Fix in the field or laboratory with ethyl alcohol or formaldehyde.

*Syncarids.*—Fix in the laboratory with ethyl alcohol. If specimens must be fixed in the field, ethyl alcohol or formaldehyde should be used.

*Water mites.*—Good results when fixed in the field or in the laboratory with Angelier's fluid or Koenike's solution.

We did not obtain enough specimens of the other groups (molluscs, isopods, and amphipods) to make comparisons. However, from other observations of isopods, we can say that it is best to fix them with ethyl alcohol in the laboratory, because they lose fewer appendages when transported live. It has also been observed that females carrying fertilized eggs should be anesthetized with diluted ethyl alcohol or crystals of menthol in water before being fixed, as the contractions they undergo when placed in contact with the fixative cause them to lose the embryos (J. Notemboom, personal communication).

Table 4 summarizes the conclusions concerning the most suitable fixative/preservative combination for each taxonomic group, as well as the best action interval for each (Model 1).

## ACKNOWLEDGMENTS

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# GUIDELINES FOR THE CARE OF NATURAL HISTORY COLLECTIONS

SOCIETY FOR THE PRESERVATION OF NATURAL HISTORY COLLECTIONS

## I. Premise

**A. Inherent value of specimens:** Specimens in natural history collections are preserved to document presence in given localities at a given time, to validate past research, and to be available for research and other educational purposes. Specimens are collected as a sample of a region's natural and cultural environment (past and present), then are often prepared in some fashion so as to make them useful for research, exhibition, or educational purposes. Subsequent preparation, sampling, or destructive analysis may be necessary to fulfill the goals of research or legitimate educational uses. Research enhances the value of specimens.

**B. Balance between use and preservation:** Associated with the responsibility of ongoing research and educational use is the obligation of the institution to maximize the value of each specimen for future use. This applies not only to the data associated with each specimen, but also to the physical and chemical integrity of the specimen. Thus, it is critical that the demands placed on natural history specimens for current research and educational uses are balanced with the need for preservation of the specimens for future uses.

**C. Caring for collections of specimens:** Most natural history collections contain thousands, if not hundreds of thousands, of individual pieces that require care. An individual specimen may contain hundreds of related pieces. Thus guidelines for collection management and care must take into consideration the reality of large quantities of specimens and numerous pieces per specimen.

**D. Inherent value of documentation and archival records:** Evidence of the identification, condition, history, or scientific value of a specimen, artifact, or collection when recorded in a permanent manner enhances the value of the specimen. These records may actually have to substitute for the specimen or artifact should the specimens themselves deteriorate or be destroyed.

**E. Context of the institutional mission and resources:** An institution's program for managing and caring for collections exists within the context of the institution's mission and resources.

## II. Objectives

A. Management and care of collections of natural history materials should be governed by respect for the scientific, historic, physical, cultural, and aesthetic integrity of the specimen or artifact and its associated data. Concern for its future should include protection against unnecessary damage, loss, or alteration that might affect its future research, educational, or exhibition potential.

B. Collection management and care should meet the highest professional standards; it must be compatible with and enhance access to collections

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for the intended scientific and educational uses of the specimens or artifacts.

C. All processes for collecting, preparing, and sampling, as well as the maintenance and curation of specimens or artifacts, should be analyzed relative to the goals of use and preservation to insure that techniques and materials are thoroughly documented, follow sound preservation practices, and fulfill the desired objectives for the specimen's intended use.

D. Every effort must be made to minimize the level of risk facing specimens and artifacts as a result of storage and use (e.g., by using appropriate storage units, providing adequate security, carefully screening on-site users and borrowers, and employing conservation standards for methods and materials used in packing and shipping).

E. Conservation and preservation treatment should meet the highest professional standards. Generally, the preferred approach for research specimens or artifacts will involve preventive conservation. Physical or chemical modifications to a specimen may adversely affect its analytical potential. Since it is not possible to anticipate uses of specimens that may become possible with advances in technology, methods that alter specimens as little as possible are preferred. Techniques and materials selected should be those that are the most stable and have the greatest longevity. In addition, many treatments must be monitored over time to understand more fully their effects on specimens and artifacts. Added materials should be removable whenever possible. Exceptions must be fully justified and documented.

F. Documentation should meet the highest professional standards and follow recommendations of relevant professional societies (e.g., Fitzgerald, 1988; Garrett, 1989). Media used for documentation should be preserved according to professional archival standards.

G. It is unethical to modify or to conceal the true nature of a specimen or artifact through restoration. The presence and extent of restoration should be detectable, although it need not be conspicuous. Methods and materials used must be fully documented.

H. Destructive sampling of specimens or artifacts must be justified by the quality and quantity of the information to be gained, evidence that the information is available only through the proposed sampling, and evidence that the investigator has the necessary expertise to extract that information. Procedures should be established to prevent unnecessary sampling. Sampling must be fully documented and approved in advance by individuals delegated with such authority (Cato, 1993).

**III. Responsibilities for the Institution**

A. A museum has the ethical and legal responsibility to ensure that collections in its custody are "protected, secure, and unencumbered, cared for, and preserved" (American Association of Museums, 1992). Any institution holding collections of value to the scientific community has an obligation to endorse this code. To fulfill this responsibility, it is essential that institutions take steps to mitigate the use of scientifically unsound preparation and other treatment techniques, poor environmental conditions, and negligent handling in order to protect the physical and chem-

ical integrity of specimens and artifacts for present and future needs. Guidelines for professional management and care should be applied not only to research collections, but also to education and exhibit collections. Institutions should implement systems that ensure preservation both of documentation and of specimens and artifacts.

B. Each institution should develop collections policies and procedures that provide a written framework for collection management, care, and use. It is essential that each institution also provide the resources (e.g., time, money, qualified personnel, appropriate space, and facilities) needed for the long-term preservation and documentation of the collections under its responsibility, or make alternative arrangements for collection management and care with an appropriate allied institution.

C. Each institution should establish priorities for the management and care of the institution's collections as a whole, in addition to setting priorities for the care and treatment of individual specimens and artifacts of particular research, historical, aesthetic, or educational value. Values of individual specimens differ and resources are generally limited, resulting in the need to prioritize management and care activities. This can be accomplished through a holistic risk management approach. With this approach, the magnitudes of risks from all sources, as they affect each collection, are considered together. Limited resources can then be targeted to the mitigation of risks to effect the greatest possible reduction in overall rate of damage to the institution's collections.

D. Collection care is an institutional responsibility that is shared by all staff. The governing authority retains the ultimate responsibility for collection care, but the director and staff must have sufficient authority and resources to implement appropriate measures. The assignment of direct individual authority and responsibility for various components of collection care is dependent on an institution's infrastructure, but these assignments must be clearly stated in the institution's collections policy and appropriate job descriptions. It is the institution's responsibility to provide sufficient resources to pursue actively continuing education opportunities for collection staff and adequate training for volunteers.

## IV. Staff Responsibilities

A. Collection care is principally the responsibility of staff members (regardless of job titles) directly involved with specimens and artifacts: curators, collection managers, curatorial assistants, conservators, registrars, preparators, and technical assistants in these areas. Many collections care activities do not require professional conservators for implementation (Duckworth *et al.,* 1993). Other departments (e.g., education and exhibit) are also responsible for the care of specimens and artifacts that are used for education or exhibition purposes. Preventive conservation is the responsibility of all staff including, for example, building and grounds, security, and those responsible for receptions and development functions.

B. Collection care personnel should have appropriate training to understand fully all aspects of collection work (e.g., legal, ethical, environmental conditions, management, security, health and safety), the limitations of

their own expertise and authority, and the consequences of any decisions and/or actions they may take or recommend. Every effort must be made to consult with appropriate specialists to ensure that all aspects of management, preservation, and use are considered before authorization for actions is given.

C. There should be a cooperative dialogue among curators, collection managers, registrars, conservators, and collection users concerning all aspects of collection care. If only one individual is responsible for all collection care activities, every effort should be made to build a network of associates and consultants to broaden the base of available expertise.

D. Treatments should reflect the most recent, scientifically substantiated conservation information, and the development of new techniques based on sound scientific methodology should be encouraged. Treatments should be undertaken only by qualified personnel, within the limits of their area of expertise and facilities. Interventive treatments should be performed only with the consent of an objective, informed individual or individuals so authorized by the institution, and may require consultation with conservation experts outside the institution. Conflicts of interest must be avoided.

E. It is the responsibility of knowledgeable staff to identify clearly specimens and artifacts that are inherently hazardous or have been made so through preparation or fumigation practices. Staff should implement appropriate safety precautions.

F. Documentation is the responsibility of all individuals who use, prepare, manage, or care for specimens or artifacts. All techniques and materials used in collection management, care, and conservation must be fully documented. Training to develop expertise in the development and management of documentation and archival records promotes a better collection management and care system.

G. Curation is the responsibility of individuals with sufficient disciplinary expertise and knowledge of recent scientific literature to provide reliable identifications and information.

H. Collection management is the responsibility of individuals trained in museum philosophy, theory and practices, including those processes defined within these guidelines: collection, preparation, sampling, preventive conservation, maintenance, and documentation. Responsible staff should have training in a relevant disciplinary specialty but are not necessarily taxonomic or subject specialists. Training to develop expertise in the management of personnel, facilities, records and information systems promotes better collection management.

I. Conservation is the responsibility of trained conservators. Conservation and preservation personnel should have appropriate training and experience to undertake conservation and preservation procedures. Conservators should meet professional training requirements and should adhere to professional ethics and guidelines such as those defined by International Institute for Conservation–Canadian Group and Canadian Association of Professional Conservators (1989) and American Institute for Conservation (1993, draft).

J. All collection staff should keep abreast of the most recent literature and upgrade their skills in their areas of responsibility according to the highest professional standards for collection management and care.

**V. Use of Collections**

A. Use of collections should be carried out in ways that are compatible with preservation objectives and concerns held by indigenous peoples, whenever possible. Certain specimens or artifacts may be considered too rare, fragile, culturally sensitive, or significant for exhibition or loan (e.g., type specimens, specimens of extinct species, historically significant specimens, or specimens in poor condition).

B. Research objectives may necessitate intervention or destructive sampling, but this should be allowed only when the potential for gaining knowledge by such means justifies sacrifice of the specimen or artifact, and when the knowledge will be shared with the scientific community. These procedures must be undertaken in a controlled manner with approval by an authorized, qualified individual or individuals. Original data, documentation, and records of specimens that have undergone destructive sampling should also be preserved.

C. Conditions under which specimens are exhibited must be compatible with their long-term preservation. Appropriate collection care staff must be active members of exhibit planning and production teams.

D. Educational programming that uses specimens and artifacts should convey to the general public the need for managing and caring for the items according to professional standards.

E. Some specimens and artifacts in natural history collections are inherently toxic or have been made hazardous through preparation or fumigation techniques. Specimens and artifacts should be used in a manner that protects the health and safety of staff, researchers, volunteers, and visitors.

**VI. Definitions**

A. Accessioning—formal process used to accept legally and to record a specimen or artifact as a collection item (Malaro, 1979); involves the creation of an immediate, brief and permanent record utilizing a control number or unique identifier for objects added to the collection from the same source at the same time, and for which the institution accepts custody, right, or title.

B. Archives—non-current records of an organization or institution preserved because of their continuing value.

C. Artifact (human)—a human-made item, often manufactured or created from naturally-occurring materials and made for use in a cultural context.

D. Cataloging—creating of a full record of information about a specimen or artifact, cross-referenced to other records and files; includes the process of identifying and documenting these objects in detail.

E. Collecting—the process of sampling the natural and cultural world using a variety of techniques that are dependent on (1) the organism or material being obtained and (2) the intended use for the sample or the research methods likely to be applied.

F. Collection—(1) a group of specimens or artifacts with like characteristics or a common base of association (e.g., geographic, donor, cultural); (2) an organizational unit within a larger institutional structure (e.g., a collection within a university biology department).

G. Collection Care—the responsibility and function of an institution with collections that involves developing and implementing policies and procedures to protect the long-term integrity of specimens and artifacts, as well as their associated data and documentation, for use in research, education and exhibits.

H. Collection Management—the responsibility and function of an institution that fosters the preservation, accessibility, and utility of their collections and associated data. The management process involves responsibilities for recommending and implementing policy with respect to: specimen acquisition, collection growth, and deaccessioning; planning and establishing collection priorities; obtaining, allocating, and managing resources; and coordinating collection processes with the needs of curation, preservation, and specimen use. These responsibilities may be shared by collection managers, subject specialists, curators, and other institutional administrators.

I. Conservation—the application of science to the examination and treatment of museum objects and to the study of the environments in which they are placed (Duckworth *et al.,* 1993). This involves activities such as preventive conservation, examination, documentation, treatment, research, and education (American Institute for Conservation, 1993 draft).

J. Curation—the process whereby specimens or artifacts are identified and organized according to discipline-specific recommendations using the most recently available scientific literature and expertise; a primary objective of this process is to verify or add to the existing documentation for these objects, and to add to knowledge.

K. Deaccession—the formal process used to remove a specimen permanently from the collection, with appropriate transfer of title (Malaro, 1979).

L. Deterioration—change in an object's physical or chemical state. "Damage, on the other hand, is the consequent loss of attributes or value: aesthetic, scientific, historic, symbolic, monetary, etc." (Michalski, 1992).

M. Documentation—supporting evidence, recorded in a permanent manner using a variety of media (paper, photographic, etc.), of the identification, condition, history, or scientific value of a specimen, artifact, or collection. This encompasses information that is inherent to the individual specimen and its associations in its natural environment as well as that which reflects processes and transactions affecting the specimen (e.g., accessioning, cataloging, loaning, sampling, analysis, treatment, etc.). Documentation is an integral aspect of the use, management, and preservation of a specimen, artifact, or collection.

N. Maintenance—routine actions that support the goals of preservation of and access to the collection such as monitoring, general housekeeping,

providing appropriate storage and exhibition conditions, and organizing a collection.

O. Object—a material, tangible item of any kind; an inclusive, non-specific term for specimen, artifact, etc.

P. Preparation—the procedures used in the field or in the institution to enhance the utility of an organism, object, or inorganic material for a specified use. The resulting specimen may represent only a portion of the original organism or material or may be otherwise altered from its original state. Procedures should be compatible with intended uses and conservation objectives, and should be documented.

Q. Preservation—those aspects of conservation that involve preventive measures, such as maintenance procedures and correcting adverse environmental conditions; in natural science conservation, preservation also includes treatments carried out initially to prepare specimens.

R. Preventive Conservation—actions taken to minimize or slow the rate of deterioration and to prevent damage to collections; includes activities such as risk assessment, development and implementation of guidelines for continuing use and care, appropriate environmental conditions for storage and exhibition, and proper procedures for handling, packing, transport and use. These responsibilities may be shared by collection managers, conservators, subject specialists, curators, and other institutional administrators.

S. Registration—(1) the process of assigning an immediate and permanent means of identifying a specimen or artifact for which the institution has permanently or temporarily assumed responsibility; one facet of documentation; (2) as an institutional function, includes the logical organization of documentation and maintaining access to that information.

T. Repository—a collection administered by a non-profit public or private institution, that adheres to professional standards for collection management and care (e.g., Alberta Museums Association, 1990; Lee *et al.*, 1982; American Society of Mammalogists, 1974) to ensure that specimens acquired will be professionally maintained and remain accessible for future use.

U. Sampling—selecting a portion as a representative of the whole; in natural science collections, sampling refers more specifically to the process of removing a portion of a specimen or artifact for analysis. The analysis may be destructive to the sample.

V. Specimen—an organism, part of an organism, or naturally-occurring material that has been collected, that may or may not have undergone some preparation treatment. It may exist in its original state, in an altered form, or some combination of the two. A specimen may be comprised of one piece or many related pieces. It may be composed of one physical or chemical component or represent a composite of materials.

W. Stabilization—treatment of an object or its environment in a manner intended to reduce the probability or rate of deterioration and probability of damage.

X. Treatment—actions taken, physically or chemically, to stabilize or make accessible a specimen or artifact; includes, for example, techniques such

as preparation, cleaning, mending, supporting, pest eradication, and consolidation.

Y. Voucher—a specimen and its associated data that physically document the existence of that organism or object at a given place and time. This definition is more broadly based than that put forth by Lee *et al.* (1982) in recognition of the potential for specimens held in a collection for use as substantiating evidence.

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*These guidelines were endorsed by the SPNHC Council, May 15, 1994, and reflect the result of input by numerous professionals over a three year period. It has been particularly gratifying that the review and comments have involved individuals from all of the professions associated with the use and care of natural history collections: collection managers, curators, conservators, administrators, research scientists, registrars, archivists, etc. This document is meant to serve as a tool for institutions and their staffs to continue to elevate the standards of managing and caring for natural history collections.*

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Paisley S. Cato, Co-Chair
Sessional Committee on Common Philosophies and Objectives

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*General.*—It is strongly recommended that, before submitting a paper, the author ask qualified persons to appraise it. The author should submit three copies of the manuscript either typewritten or printed on letter quality printers. **All parts of the manuscript must be double spaced** with pica or elite type on 8½ × 11 inch (21.6 by 27.9 cm) or A4 paper and at least one inch (2.5 cm) margins on all sides. Manuscripts should not be right justified, and manuscripts produced on low-quality dot matrix printers are not acceptable.

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Jones, E. M., and R. D. Owen. 1987. Fluid preservation of specimens. Pp. 51–64 in *Mammal Collection Management* (H. H. Genoways, C. Jones, and O. L. Rossolimo, eds.). Texas Tech University Press, Lubbock, 219 pp.

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**Articles**