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# OBSERVATIONS ON FORMALIN-INDUCED DARKENING OF HERPETOLOGICAL SPECIMENS

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*Abstract.*—Samples of freshly-killed specimens of desert grassland whiptail lizards (*Cnemidophorus uniparens*) were fixed in either 10% or 20% formalin and then subjected to one of six treatments: no immersion in formalin; or immersion in the same formalin concentration as that used for fixation for one day, one week, one month, three months, or six months. Specimens were then transferred to 55% isopropanol and their preservation condition was evaluated 31 months after fixation. Observable differences were detected among the samples in the extent of color pattern conservation and specimen darkening. These differences can be attributed to the formalin concentration used and the amount of time immersed in a formalin solution.

Color pattern characteristics are extremely important in studies of many taxonomic groups. For amphibians and reptiles, color pattern is often used with meristic and mensural data in species identification and systematic studies (e.g., recent studies of variation in *Cnemidophorus* by Hendricks and Dixon, 1986, and Taylor and Buschman, 1993). Thus, the extent to which color pattern features are conserved can be as important as the preservation of other anatomical features in determining the utility of a museum specimen.

The use of formalin for fixation of amphibian and reptile specimens is standard practice for herpetologists (Anderson, 1948; Duellman, 1962; Pisani, 1973; Pisani and Villa, 1974; Simmons, 1987; McDiarmid, 1994). The loss of certain soluble pigments (e.g., carotenoids) is generally an unavoidable result of the preservation process (Smith, 1955; Pisani, 1973). Many workers have observed that some herpetological specimens darken appreciably soon after fixation (e.g., Slevin, 1927; Simmons, 1993). This darkening can often obscure or obliterate characteristics of color pattern not normally lost during preservation (e.g., the distribution of melanin in the integument).

Although formalin has long been known to cause darkening of some specimens, few studies have addressed this phenomenon. Taylor (1977) observed that fish specimens stored in formalin buffered with sodium borate (borax) may either clear or darken, depending on pH of the solution. Freezing prior to formalin fixation may also result in darkened specimens (Scott and Aquino-Shuster, 1989). Similar darkening may occur when freshly-killed specimens are fixed with non-buffered formalin solutions (personal observation). Factors that may cause or contribute to this darkening include prolonged storage of specimens in formalin, the use of strong (>10% concentration) formalin solutions for fixation or storage, temperature fluctuations, or formalin pH (Simmons, 1993; personal observation).

Published recommendations for how long herpetological specimens should be immersed in formalin are often vague, e.g., "at least 48 hours" (Duellman, 1962: 40) and "several days to two weeks at a minimum" (Simmons, 1987:16). For logistical reasons, it is not uncommon for field collections to remain in formalin

for weeks or months before they can be transferred to alcohol. Adequate fixation time varies from specimen to specimen and is at least partly dependent on many variables, including size of specimen, permeability of tissues, extent of autolysis (specimen "freshness"), and concentration of formalin used. Although a 10% formalin solution is used for most herpetological applications, stronger solutions (20–50% concentration) have been recommended for fixation of very large or frozen specimens (Johnston and Parker, 1980).

In this study I examined the effect of formalin on the conservation of color pattern in specimens of lizards by (1) leaving specimens immersed in formalin over a range of times, and (2) using two different formalin concentrations in specimen fixation: 10%, a concentration used in most herpetological preservation; and 20%, a concentration that may be appropriate for some material (Johnston and Parker, 1980). The goal was to determine if differences in immersion time or formalin concentration affected the quality of color pattern conservation.

The desert grassland whiptail (*Cnemidophorus uniparens*), the species used in this study, is a parthenogenetic, all-female species in which populations consist of genetically identical individuals. Variation in color pattern among adults from the same population is nearly non-existent, and ontogenetic change in pattern is minimal. In addition, members of the genus seem to be prone to post-fixation darkening (personal observation). These characteristics make *C. uniparens* an ideal subject for examining the effects of different preservation methods on color pattern of specimens.

#### MATERIALS AND METHODS

A sample of 36 desert grassland whiptails (53–78 mm snout–vent length [SVL]) was collected in central Sierra County, New Mexico over a six-week period during the summer of 1991. The lizards were euthanized in the laboratory within 24 hours after capture by an injection of ca. 0.4 ml of a 14% solution of sodium pentobarbital (Nembutal) and water in or near the heart. After death, SVLs were measured to the nearest millimeter and a numbered tag (made of acid-free paper) was tied to each specimen. Notes on color pattern were recorded for each specimen before fixation. The ventral surfaces of the tail and limbs on each specimen were perforated with a needle or razor blade to allow penetration of fixative, and the body cavity was injected with 2.0–3.0 ml of either a 10% (one part commercial grade formaldehyde solution to nine parts deionized water) or 20% solution of formalin. Formalin stocks were buffered with 3.6 g monobasic (dihydrate) and 3.6 g dibasic (anhydrous) sodium phosphate per liter of solution, a buffer ratio originally recommended by Markle (1984) for ichthyoplankton fixation and which has been used in the Museum of Southwestern Biology for general herpetological applications since the mid-1980s. The pH of these stocks was 6.6 for 10% and 6.2 for 20%. Specimens were positioned in a covered plastic tray on unprinted, unbleached paper towels saturated with the same formalin solution used for injection and were allowed to fix for 6 hours in darkness.

After fixation, specimens were randomly assigned to one of six treatments (Tables 1 and 2). In the first treatment (T1), specimens were removed from the fixing tray after 6 hours, rinsed with tap water for 2–3 minutes, and placed in 55% isopropanol for storage. In the remaining five treatments (T2–T6), the specimens were transferred from the fixing tray to a jar containing formalin of the same concentration that was used for fixing. The specimens remained immersed for the following lengths of time: one day (T2), one week (T3), one month (T4), three months (T5), and six months (T6). Upon removal from formalin, all specimens were rinsed with tap water for 2–3 minutes and transferred to 55% isopropanol for storage. Specimens were kept in the dark, at approximately 22°C, and in bail-top glass jars (ca. 5 specimens per liter of fluid) during both formalin immersion and subsequent preservation in isopropanol.

Specimens were examined at 7, 19, and 31 months post-fixation. Detailed evaluations of color pattern clarity, discoloration, rigidity of limbs and tails, etc., were recorded at the 31-month exami-

Table 1. Summary of six immersion treatments for both 10% and 20% formalin concentrations. All specimens were allowed to harden in fixing tray for 6 hours before being assigned to a treatment; all were rinsed for 2–3 minutes in tap water upon removal from formalin and before placement in 55% isopropanol.

Treatment	Procedure
T1	Removed from tray; rinsed; immersed in isopropanol.
T2	Removed from tray; immersed in formalin for 1 day (24 hours); rinsed; immersed in isopropanol.
T3	Removed from tray; immersed in formalin for 1 week (7 days); rinsed; immersed in isopropanol.
T4	Removed from tray; immersed in formalin for 1 month (30 days); rinsed; immersed in isopropanol.
T5	Removed from tray; immersed in formalin for 3 months (ca. 91 days); rinsed; immersed in isopropanol.
T6	Removed from tray; immersed in formalin for 6 months (ca. 182 days); rinsed; immersed in isopropanol.

nation. All specimens and treatment documentation are deposited in the Museum of Southwestern Biology, University of New Mexico (MSB 56604-639).

#### RESULTS AND DISCUSSION

Conservation of color pattern in specimens varied among the different formalin treatments (Fig. 1; Table 3). Many of these differences, such as extent of dark coloration on venter and clarity of dorsal pattern, were apparent at 7 months post-fixation; little or no additional change was evident when specimens were re-examined at 19 months and 31 months post-fixation. Because detailed notes were recorded only at 31 months post-fixation, the following results and discussion consider only the final examination. Results are summarized by formalin concentration and treatment in Table 3.

*10% formalin.*—Dorsal pattern remained distinct in T1–T3; stripes were still clear and the mottled pattern on the hind limbs was discernible. The T1 and T2 specimens had much of the brownish dorsal coloration seen in life even 31 months post-fixation. The T4 and T5 specimens were blackish dorsally and the stripes were still discernible albeit less clear than in the T1–T3 series. The T6 specimens were almost uniform black dorsally; the stripes were very faint and hind limb mottling was obscured.

Table 2. Numbers of formalin-fixed *Cnemidophorus uniparens* used in six immersion treatments at two different formalin concentrations. See Table 1 for explanation of treatments.

Treatment	Immersion time in formalin	Formalin concentration		
		10%	20%	Total
T1	0 (fixed only)	3	2	5
T2	1 day	4	2	6
T3	1 week	4	3	7
T4	1 month	4	2	6
T5	3 months	5	2	7
T6	6 months	3	2	5
Total		23	13	36

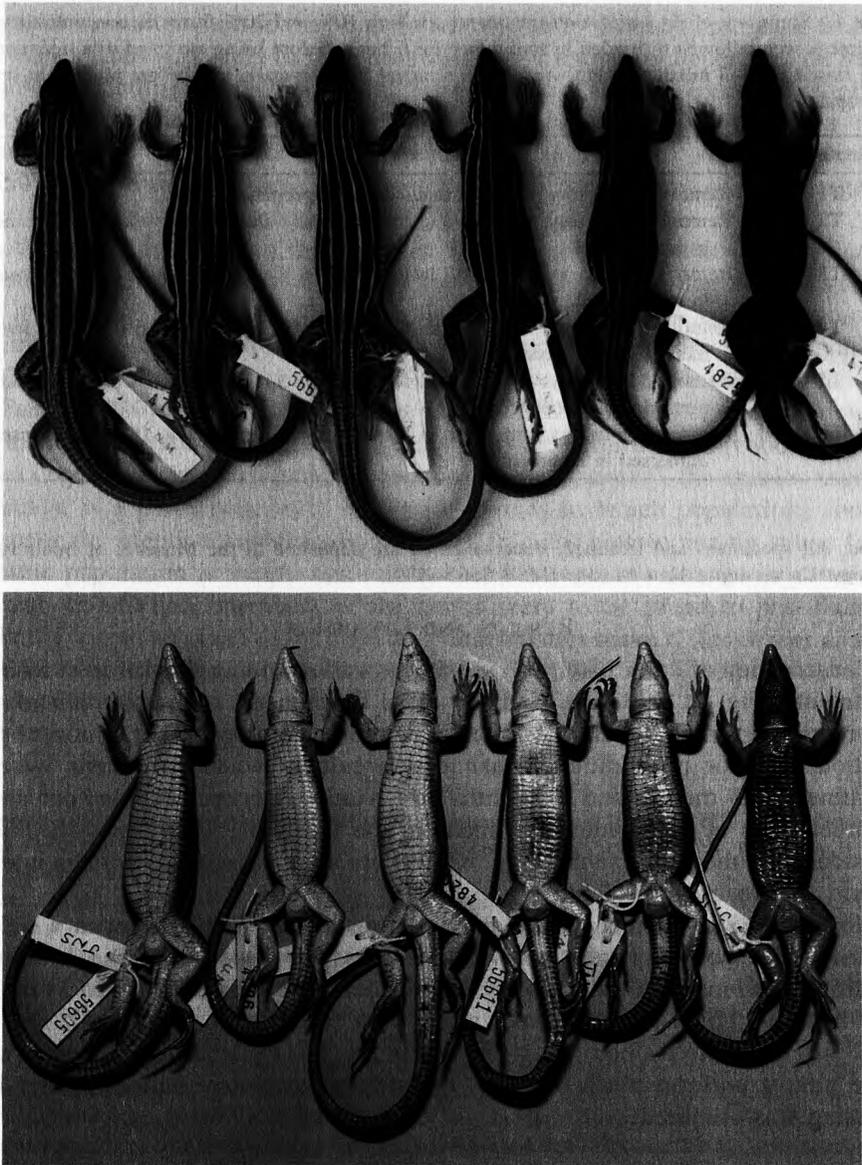


Figure 1. Dorsal (*top*) and ventral aspects of representative specimens of *Cnemidophorus uniparens*, approximately 31 months after fixation in 10% formalin. Left to right: Treatments T1 through T6. See Table 1 for explanation of treatments.

The ventral coloration of these specimens varied much like the dorsal. The T1–T3 specimens were slightly discolored by yellow and light blue blotching but the T4 and T5 material had acquired an almost uniform blue color to the torso and gular region. The venters of the T6 specimens were black.

*20% formalin.*—These specimens showed essentially the same pattern of darkening as the 10% formalin samples except that darkening occurred with shorter

Table 3. Notes on color pattern for specimens fixed in 10% and 20% formalin based on examination at 31 months post-fixation. Characteristics of dorsal and ventral coloration and clarity of dorsal pattern are summarized for all specimens within each treatment category.

Treatment	Formalin conc.	Color pattern notes*
T1	10%	Dorsum deep brown; stripes and hind limb pattern white and distinct; venter yellowish.
	20%	As previous except venter bluish.
T2	10%	Dorsum dark brown to black; stripes and hind limb pattern white and distinct; venter yellowish or light bluish.
	20%	As previous except venter bluish.
T3	10%	Dorsum dark brown to black; stripes and hind limb pattern white and distinct; venter mostly yellowish with some light bluish color.
	20%	Dorsum black; stripes and hind limb pattern slightly reduced in clarity; venter mottled with black and blue.
T4	10%	Dorsum black; stripes and hind limb pattern slightly less clear than T3; venter bluish.
	20%	Dorsum black; stripes and hind limb pattern faded; venter bluish-black.
T5	10%	Dorsum black; stripes and hind limb pattern faded and intermittent; venter mottled with blue.
	20%	Dorsum black; stripes and hind limb pattern almost invisible; venter dark bluish to black.
T6	10%	Dorsum black; stripes and hind limb pattern almost invisible; venter black.
	20%	Dorsum black; stripes and hind limb pattern almost invisible; venter black.

\* Color pattern characteristics for all specimens recorded prior to fixation in formalin are as follows: dorsal background color deep brown; dorsal stripes distinct and white to light yellow in color; light mottled pattern on dorsal surface of hind limbs distinct; ventral surface of body immaculate and white with faint tint of brown.

immersion times (Table 3). Although the T1 and T2 material looked essentially the same as the specimens exposed to the same treatments using 10% formalin, the T3 specimens preserved in 20% appeared darker than those preserved in 10%. Also, the T4 specimens preserved in 20% were as darkened as any of the T5 or T6 specimens preserved in 10%. Thus, the critical point when formalin immersion began to significantly alter color pattern appearance was at or near the T3 stage (one week post-fixation).

Specimens from all treatments and both formalin concentrations appeared to be about equally well-fixed; no tissue softness or scale sloughing was evident in any of the specimens when examined. Some deformation (curling) of the tail in the T1 (10%) specimen used in Figure 1 was evident after being photographed under hot lights for ca. 5 minutes. This loss of rigidity might be attributable to the short fixation time of this specimen because the other photographed specimens were not affected.

Differences in pattern conservation among specimens in the T1–T3 series fixed in 10% formalin were very minor. This observation suggests that immersion in formalin for up to a week will not cause greater pattern loss than occurs within a day; however, fixation for only several hours (as in the T1 specimens) may not

allow formalin to penetrate all areas of a specimen. Parts of the anatomy with thick integument or a high surface area:volume ratio (e.g., digits and tail), which can not be easily injected, may require more time in formalin to fix fully. The results suggest that adequate fixation and prevention of specimen darkening probably can be achieved in most cases by using only 10% buffered formalin, closely monitoring the fixation process, and then removing the specimen from formalin after 3–7 days. For very large or frozen specimens, other methods may be necessary (Johnston and Parker, 1980).

Post-fixation treatments that may prevent formalin-induced darkening include soaking specimens in water to remove the formalin (discussed in Simmons, 1993) or opening the body cavity after fixation to allow displacement of injected formalin with alcohol (W. G. Degenhardt, personal communication). The former method, however, may have unwanted side-effects such as osmotic damage to tissues (Simmons, 1993), specimen swelling (N. J. Scott, Jr., personal communication), and "reverse fixation" or removal of the formalin-created cross-links between protein chains (Taylor, 1977; McDiarmid, 1994). The importance of formalin pH and buffering compounds in specimen darkening has yet to be determined.

Reptilian taxa that seem to be most prone to post-fixation darkening are whip-tails (*Cnemidophorus* spp.), skinks (e.g., *Eumeces* spp.), garter snakes (*Thamnophis* spp.), and (to a lesser extent) iguanid lizards (W. G. Degenhardt, personal communication; personal observation); however, some darkening may occur in formalin-fixed specimens of any taxon. In extreme cases, the specimen becomes black and the color pattern is completely obscured, often making the specimen unidentifiable to species and essentially useless for most research applications.

#### CONCLUSION

Slevin (1927), an early critic of using formalin as a fixative, recommended that herpetological specimens should be removed from formalin as soon as possible after fixation due to the chemical's tendency to cause darkening or discoloration. Almost seventy years later, Slevin's advice still appears to be valid. Despite more than a century of use, little published information is available on the effects of formalin fixation on specimen conservation. Indeed, it is still unclear whether formalin fixation is an improvement over alcohol preservation alone for most specimen uses (Simmons, 1993). Additional study of formalin and its effects on specimens, along with a reevaluation of its use in herpetological collections, is clearly needed.

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# EFFECT OF OSTEOLOGICAL PROCESSING TREATMENTS ON DIMENSIONS AND MOISTURE ABSORPTION POTENTIAL OF RODENT SKULLS

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*Abstract.*—Moisture absorption and anisotropic response of bone are detectable by change in weight and dimension of the bone as it acclimates to different levels of relative humidity (RH). Because of this sensitivity to RH fluctuation, it was speculated that osteological processing treatments also may induce weight and dimensional change. Because considerable variation in processing treatments exists among institutions and time periods, questions are raised about skeletal material used for systematics research. To examine dimensional change of bone attributed to processing treatment, a series of skulls was divided into four groups. Each group was subjected to one treatment: no fluid processing (control); soaking 24 hours in distilled water at ambient temperatures; soaking 24 hours in an ammonia solution at ambient temperatures; or soaking one hour in 90°C distilled water. Size reduction was noted for most dimensions of skulls subjected to fluid treatments; in some cases, dimensional change was significant when compared to the control group. The moisture absorption potential of bone was also influenced by processing treatment. A discussion follows on the influences processing treatment may have on morphometric analyses in systematics research.

“At the lowest level bone can be considered to be a composite material consisting of a fibrous protein, collagen, stiffened by an extremely dense filling of calcium phosphate” (Curry, 1984). The qualities provided by the organic collagen and inorganic calcium phosphate complement one another to make bone a unique material. The loss or destruction of either component could impact the nature of bone itself. Associated with undegraded bone are lipids and various kinds of tissues, including cartilage, nerves, blood vessels, and connective tissues, such as tendons and ligaments; a major component of many of these tissues is collagen (Wainwright *et al.*, 1976). Studies utilizing bone often require the removal of these extraneous tissues.

Laboratories processing osteological materials have developed various methods of removing non-osseous tissues from bone. Methods of choice have evolved with time and changing needs. Currently, there are several methods in practice. In most cases, little or no documentation exists about processing treatments and their effects on preserving stability and research value of bone. Based on the literature (Hangay and Dingley, 1985; Hildebrand, 1968; Knudsen, 1966; Russell, 1947; Wagstaffe and Fidler, 1968; Williams *et al.*, 1977), the removal of extraneous tissues and subsequent cleaning of bone typically involves the use of heat, alkaline chemicals, enzymes, organisms, or a combination of these methods. The success of any method is based on its ability to degrade and solubilize collagen. For instance, when collagen is heated to temperatures exceeding 65°C, peptide bonds are subjected to hydrolytic or oxidative degradation. This causes the molecule to

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collapse, leaving a soluble, amorphous, gelatinous mass which, when dried, has adhesive qualities (Gustavson, 1956; Haines, 1987). Collagen solubility and denaturation can also be achieved by subjecting the protein to alkaline conditions exceeding a pH of 9.0 (Gustavson, 1956; Whitaker, 1980). Solubilized proteins can be washed away from the bone with reasonable ease. Unfortunately, the same mechanisms that destroy extraneous tissues also act on the organic matrix of bone.

Because bone is readily responsive to different relative humidity (RH) levels (Lafontaine and Wood, 1982; Williams *et al.*, 1993), it is difficult to anticipate how any treatment, treatment deviation (i.e., duration or concentration), or treatment combination (i.e., boiling with chemicals), might affect the integrity of bone with respect to stability and research value. Based on current knowledge of how bone responds to factors such as moisture, alkaline chemicals, and high temperatures (Hall and Russell, 1933; Knudsen, 1966; Williams, 1991; Williams *et al.*, 1993), it is expected that size and shape of bones will be affected by treatment. Such changes might affect morphometric analyses of systematics studies based on osteological materials.

Mammalian systematists have used cranial dimensions for assessing natural variation (i.e., individual, sex, age, seasonal, and geographic differences) among and between population and for species determination (DeBlase and Martin, 1981; Hall, 1981; Jones and Manning, 1992; Swanepoel and Genoways, 1979). To assess natural variation, morphometric studies avoid incorporating procedural variation. As systematists diligently and meticulously record measurements, possibly from hundred of specimens collected during the past two centuries, variation derived from processing treatment is rarely considered. In view of the importance of cranial dimensions in the current understanding of mammalian systematics, there is a need to evaluate the influence of various processing techniques on bone dimensions.

In this study, we used crania of ground squirrels (Family Sciuridae; *Spermophilus tridecemlineatus*) to examine four basic methods of cleaning bone. The methods examined are currently used at various institutions in North America and other parts of the World. The results of this study provide information relevant to research integrity of specimens, as well as specimen stability.

#### METHODS AND MATERIALS

An independent study of seasonal variation in thirteen-lined ground squirrels (*Spermophilus tridecemlineatus arenicola*) afforded an unique opportunity to select a series of skulls from over 100 specimens collected and processed under uniform conditions. To document changes resulting from different processing techniques, natural variation had to be minimized. Twenty-four specimens, all females from Lubbock County, Texas, collected in March and April of 1990, were selected. These specimens had skull lengths ranging from 38.0 to 41.0 mm, and based on similarities of molar tooth-wear, were adults of the same age. Also, these specimens had not received treatments that would be considered intrusive (i.e., chemical applications for processing and pest control). All specimens were processed with dermestid beetles followed with mechanical cleaning as described by Williams (1992). Skulls were stored in flint-glass vials, sealed with polyethylene caps; vials were kept in closed storage cases since 1990, except during examination.

Cranial measurements were taken with an image capture system described by Meacham (1993). This system incorporated an imaging board (PCVISIONplus) and software package (MorphoSys) with a 386 microcomputer and computer monitor (Meacham and Duncan, 1990). By incorporating a video camera (Cohu model 4815-2000), viewing stage (Vari Quest 100), and viewing monitor (Sony Triniton), the image of a skull placed on the viewing stage was transmitted by camera and converted to

pixels on the viewing monitor. Cursor movement and programming instructions, with respect to the enlarged image on the viewing monitor, were performed by the user with the microcomputer and a separate monitor. This set-up allowed the user to precisely mark measurement parameters which were interpreted by the computer in terms of pixels. The output was as a measurement with an accuracy level of 0.001 mm.

Selection of measurements for the study were influenced by several factors: ability to effectively replicate measurements; balancing the number of dimensions representing length and width; and using dimensions that have relevance to typical morphometrical studies. Six cranial measurements, three representing length and three representing width (Fig. 1), documented in the study are described below:

- A) *greatest length of skull*—greatest distance between the posteriormost part of the skull above the foramen magnum and the anteriormost part of the nasal bones.
- B) *length of diastema*—shortest distance between the alveoli of the upper premolar and upper incisor.
- C) *length of maxillary tooththrow*—distance between the anteriormost margin of the right upper premolar and the posteriormost margin of the right upper third molar.
- D) *zygomatic breadth*—greatest distance across the inner margins of the zygomatic arches (near the jugal-squamosal suture), perpendicular to the long axis of the skull.
- E) *breadth across auditory bullae*—greatest distance across the anterior projections of the ear canals.
- F) *breadth across upper molars*—greatest distance across the outer margins of the third upper molars.

Weights, measured with an analytical balance (Mettler H8, accurate to 0.001 gm), were recorded for all specimens. Following initial documentation of measurements and weight, the 24 specimens were divided into four groups of six specimens each. All groups received preliminary cleaning by dermestid beetles and mechanical techniques (Williams, 1992). Group 1 (control) received no further treatment. Skulls in Group 2 each were soaked in 100 ml of distilled water for 24 hours and air-dried under ambient conditions (35% RH; 23.3°C). Treatment of Group 3 simulated described procedures involving the use of ammonia (Hildebrand, 1968:23; Hoffmeister and Lee, 1963). Under ambient conditions each skull was soaked in 100 ml of "full strength" ammonia solution (= ammonium hydroxide, NH<sub>3</sub>, 28–30%; Fisher Reagent Grade, A.C.S., NA2672; A669-212) for 24 hours, rinsed with distilled water, and air-dried under ambient conditions. Simulating treatments involving heated water (Hangay and Dingley, 1985; Hildebrand, 1968; Wagstaffe and Fidler, 1968; de Wet *et al.*, 1990), each skull of Group 4 was soaked in 23°C distilled water for one hour (for moisture acclimation), placed in 25 ml of 90°C distilled water for one hour, and air-dried under ambient conditions. During this treatment, water (1–2 ml) was periodically added to maintain levels of 25 ml; temperature was maintained with a Fisher Dry Bath Incubator (Catalog number, 11-718-2) and monitored with a thermometer accurate to 0.5°C.

Following treatment, the skulls were allowed to acclimate to ambient conditions for five days before measurements and weights were retaken. Skulls were examined to document changes in appearance attributed to treatment.

To evaluate possible changes in moisture absorption potential, skulls were acclimated to RH levels of 25% and 85% using procedures described by Williams *et al.* (1993). Acclimation for each RH level was 20 and 15 days, respectively. Weights were taken with the analytical balance. Percent weight increase was determined for each skull.

ABstat<sup>®</sup> (Anderson-Bell, 1987) computer software was used to tabulate standard statistics and perform analysis of variance between groups. Standard statistics (mean, range, standard deviation, and coefficient of variation) were calculated for pre- and post-treatment dimensions, as well as for weight changes associated with the moisture absorption tests. Analysis of variance incorporated arcsine transformations of values indicating percent (Sokal and Rohlf, 1969:386). Significance level was set at  $P \leq 0.05$ .

## RESULTS

Cranial dimensions taken before and after treatment for each group are presented in Table 1. Except for dimensions involving the maxillary tooththrow, there was a reduction in average size for all groups based on the techniques used for

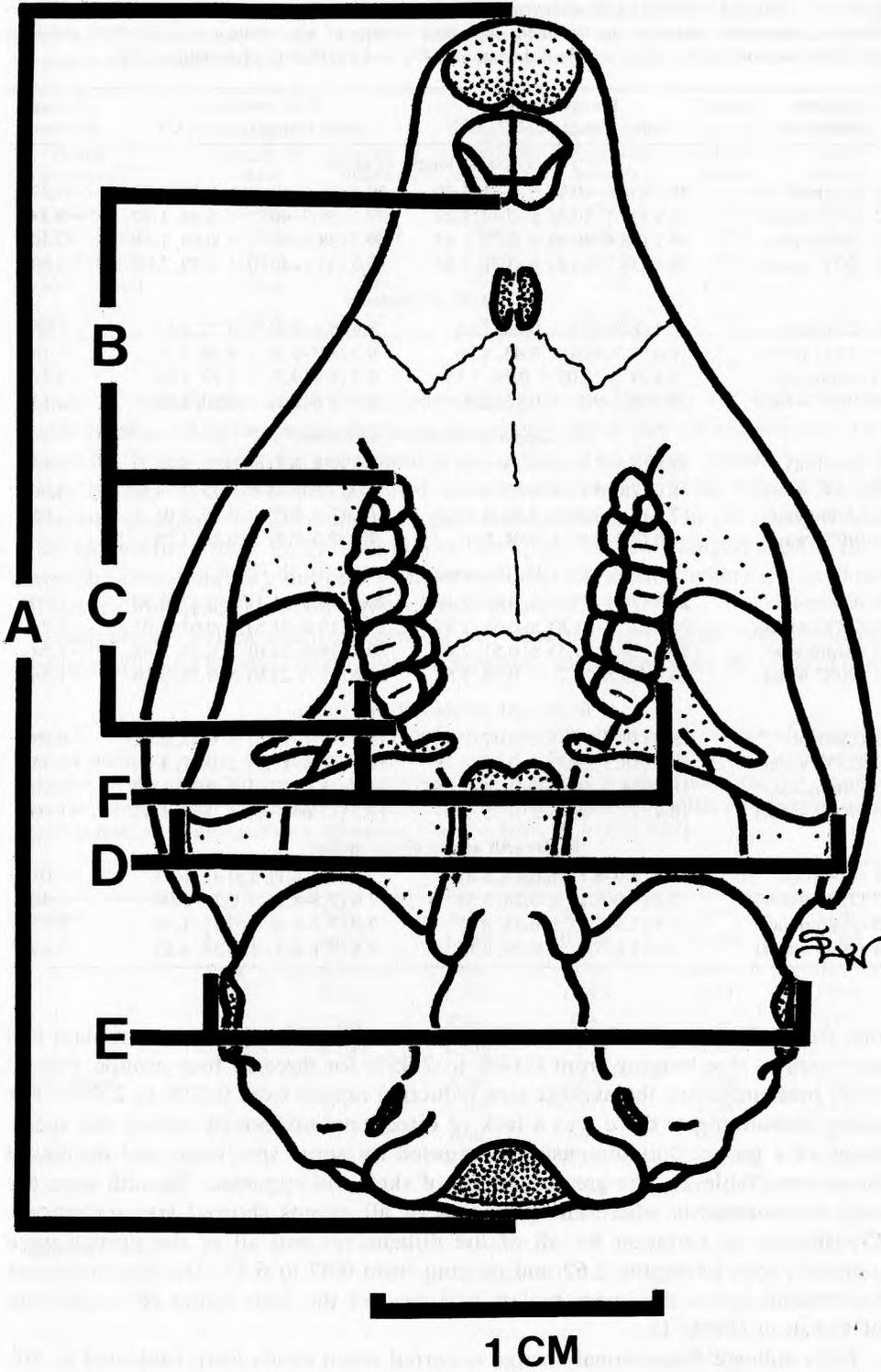


Figure 1. Ventral view of a ground squirrel cranium (measurements are described in text).

Table 1. Standard statistics of six dimensions (mm) taken from 24 crania of *Spermophilus tridecemlineatus*, separated according to treatment into four groups of six crania each. Standard statistics provided include mean, range, standard deviation (SD), and coefficient of variation (CV).

Group (treatment)	Pre-treatment mean (range) $\pm$ SD, CV	Post-treatment mean (range) $\pm$ SD, CV	Average % change
<b>A. Greatest length of skull</b>			
1 (Control)	39.7 (38.6–40.7) $\pm$ 0.71, 1.80	39.4 (38.6–40.6) $\pm$ 0.71, 1.79	-0.22
2 (23°C water)	39.9 (39.1–40.5) $\pm$ 0.49, 1.22	39.5 (38.7–40.0) $\pm$ 0.43, 1.10	-0.43
3 (Ammonia)	39.7 (38.4–40.9) $\pm$ 0.77, 1.95	39.3 (38.2–40.1) $\pm$ 0.65, 1.66	-0.40
4 (90°C water)	39.6 (38.5–40.4) $\pm$ 0.71, 1.81	39.0 (37.7–40.0) $\pm$ 0.79, 2.02	-0.59
<b>B. Length of diastema</b>			
1 (Control)	9.1 (8.9–9.5) $\pm$ 0.24, 2.66	8.9 (8.6–9.6) $\pm$ 0.32, 3.63	-1.66
2 (23°C water)	9.4 (8.7–9.9) $\pm$ 0.45, 4.76	9.3 (8.7–9.5) $\pm$ 0.30, 3.29	-1.14
3 (Ammonia)	9.4 (9.1–10.0) $\pm$ 0.29, 3.13	9.2 (8.9–9.7) $\pm$ 0.27, 2.90	-2.44
4 (90°C water)	9.1 (8.7–9.3) $\pm$ 0.26, 2.90	8.9 (8.6–9.1) $\pm$ 0.20, 2.27	-2.11
<b>C. Length of maxillary toothrow</b>			
1 (Control)	7.8 (7.6–7.9) $\pm$ 0.13, 1.66	7.8 (7.4–8.2) $\pm$ 0.26, 3.38	0.14
2 (23°C water)	8.0 (7.6–8.3) $\pm$ 0.23, 2.91	8.0 (7.8–8.2) $\pm$ 0.15, 1.91	5.96
3 (Ammonia)	7.8 (7.4–8.2) $\pm$ 0.30, 3.87	8.0 (7.7–8.2) $\pm$ 0.16, 2.01	7.05
4 (90°C water)	7.8 (7.7–7.9) $\pm$ 0.04, 0.57	7.8 (7.6–7.9) $\pm$ 0.10, 1.28	-0.19
<b>D. Zygomatic breadth</b>			
1 (Control)	20.8 (20.1–21.4) $\pm$ 0.45, 2.18	20.5 (19.8–21.1) $\pm$ 0.47, 2.32	-1.30
2 (23°C water)	21.3 (20.0–21.8) $\pm$ 0.61, 2.87	21.0 (19.6–21.5) $\pm$ 0.63, 3.01	-1.28
3 (Ammonia)	20.8 (20.1–21.5) $\pm$ 0.51, 2.45	20.5 (19.6–21.0) $\pm$ 0.54, 2.62	-1.54
4 (90°C water)	20.9 (19.8–22.2) $\pm$ 0.73, 3.51	20.6 (19.6–21.9) $\pm$ 0.73, 3.56	-1.56
<b>E. Breadth across auditory bullae</b>			
1 (Control)	18.5 (17.7–19.1) $\pm$ 0.47, 2.56	18.5 (17.7–19.0) $\pm$ 0.47, 2.56	-0.05
2 (23°C water)	18.9 (18.5–19.3) $\pm$ 0.26, 1.38	18.9 (18.4–19.2) $\pm$ 0.27, 1.44	-0.31
3 (Ammonia)	18.7 (18.3–19.0) $\pm$ 0.29, 1.56	18.5 (18.0–19.0) $\pm$ 0.37, 1.99	-0.70
4 (90°C water)	18.4 (17.7–18.8) $\pm$ 0.38, 2.04	18.3 (17.6–18.6) $\pm$ 0.33, 1.83	-1.03
<b>F. Breadth across upper molars</b>			
1 (Control)	7.6 (6.9–8.1) $\pm$ 0.45, 5.87	7.6 (6.9–8.1) $\pm$ 0.49, 6.43	-0.01
2 (23°C water)	7.8 (7.3–8.1) $\pm$ 0.28, 3.58	7.9 (7.5–8.2) $\pm$ 0.23, 2.88	1.48
3 (Ammonia)	7.5 (7.3–7.9) $\pm$ 0.23, 3.07	7.8 (7.7–8.0) $\pm$ 0.11, 1.40	4.21
4 (90°C water)	7.5 (7.1–7.9) $\pm$ 0.29, 3.81	7.8 (7.4–8.3) $\pm$ 0.34, 4.33	3.88

this study. The length of the maxillary toothrow and breadth across molars had increases in size ranging from 0.14% to 7.05% for three of four groups. For all other measurements, the average size reduction ranged from 0.22% to 2.44%. For some measurements there was a lack of directional uniformity among the specimens of a group, thus dimensions increased for some specimens and decreased for others (Table 2). The greatest length of skull and zygomatic breadth were the only measurements where all specimens of all groups showed size reductions. Coefficients of variation for all of the dimensions and all of the groups were generally low, averaging 2.62 and ranging from 0.57 to 6.43. The measurements for breadth across the upper molars had most of the high values for coefficient of variation (Table 1).

Data indicate dimensional change occurred when skulls were subjected to different processing treatments. A comparison of groups, using analysis of variance

Table 2. Summary of percentages of specimens and dimensions showing size reduction. Although there was a general trend for size reduction, some instances of size increase were noted, such as dimensions involving dentition.

Group (treatment)	Greatest length of skull	Length of diastema	Length of maxillary toothrow	Zygomatic breadth	Breadth across auditory bullae	Breadth across upper molars
1 (Control)	100.0	66.7	66.7	100.0	50.0	83.3
2 (23°C water)	100.0	66.7	50.0	100.0	66.7	16.7
3 (Ammonia)	100.0	100.0	33.3	100.0	66.7	16.7
4 (90°C water)	100.0	100.0	66.7	100.0	100.0	16.7
Totals	100.0	83.3	54.2	100.0	70.8	33.3

(Table 3), shows that some dimensions changed significantly ( $P \leq 0.05$ ) as a result of processing treatment. Because the control group did not receive any fluid treatment, it represents the most natural condition. The group treated with 90°C water (Group 4) showed the greatest dimensional change, with half of the dimensions changing significantly more than the control group. In the group treated with ammonia (Group 3), breadth across the upper molars changed significantly. Breadth across auditory bullae was the only other dimension where a significant difference occurred (between Group 2 and Group 4).

Analyses of weight changes between pre- and post-treatments was hampered because of initial balance misalignment; however, examination of the control sam-

Table 3. Summary of analyses of variance between treatments, using an arcsine transformation of percent change of cranial dimensions; significance level,  $P \leq 0.05$ ; F-values are given for comparative purposes. Codes are as follows: A = greatest length of skull; B = length of diastema; C = length of maxillary toothrow; D = zygomatic breadth; E = breadth across auditory bullae; F' = breadth across upper molars; ns = no significant difference; \* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ .

	23°C water		Ammonia		90°C water	
Control	A/ns	D/ns	A/ns	D/ns	A/**	D/ns
	4.765	0.027	1.739	0.098	11.289	0.763
	B/ns	E/ns	B/ns	E/ns	B/ns	E/*
	0.406	0.936	1.379	1.687	0.997	7.898
23°C water	C/ns	F/ns	C/ns	F/*	C/ns	F/*
	0.226	4.100	1.533	7.030	0.008	5.558
			A/ns	D/ns	A/ns	D/ns
			0.507	0.173	2.295	0.839
Ammonia			B/ns	E/ns	B/ns	E/*
			4.231	0.325	3.538	6.621
			C/ns	F/ns	C/ns	F/ns
			0.571	0.887	0.437	0.552
90°C water					A/ns	D/ns
					2.727	0.074
					B/ns	E/ns
					0.275	1.561
				C/ns	F/ns	
				2.518	0.017	

Table 4. Standard statistics (mean, range, standard deviation, and coefficient of variation) for moisture absorption tests for the four groups of six crania each. Values represented indicate weight change from 25% RH to 85% RH. Significant differences ( $P \leq 0.01$ ) were found between all group comparisons.

Group (treatment)	% Weight change		CV
	Mean (range) $\pm$ SD		
1 (Control)	2.76 (2.57–3.06) $\pm$ 0.16		5.64
2 (23°C water)	2.25 (2.20–2.31) $\pm$ 0.03		1.40
3 (Ammonia)	2.36 (2.28–2.40) $\pm$ 0.04		1.58
4 (90°C water)	2.48 (2.29–2.65) $\pm$ 0.11		4.35

ple provided an understanding of the correction factor needed to make at least general observations. Both the control (Group 1) and water-treated sample (Group 2) had less than 1% change in weight, whereas the ammonia-treated sample (Group 3) and 90°C water-treated sample (Group 4) had an average weight loss of 1.4% and 5.0%, respectively. Visual examination determined weight loss was not due to lost skull parts.

Visual inspection of individual crania identified three characters to aid in treatment recognition—coloration of bone, appearance of bone surface under magnification, and condition of non-osseous tissues between the teeth and alveolar bone. An assumption was made that untreated bone, represented by the control, would have a “natural” appearance because the bone and associated tissues had not been altered after mechanical cleaning. The coloration of Group 1 (control) and Group 2 (water treatment) were similar. Group 3 (ammonia treatment) and Group 4 (90°C treatment) were noticeably lighter. Under 7 $\times$ -magnification, the control group was noticeably different from other groups because of the presence of minute tissue fragments adhering to bone surfaces, particularly in areas protected from abrasion that may be caused by contact surfaces. The bone surface of the other groups appeared cleaner and glossier. The non-osseous alveolar tissues of groups 1 and 2 were whitish and tended to fill spaces between the teeth and alveolar bone; tissues of groups 3 and 4 were yellowed and noticeably recessed in the alveoli. Under magnification, the presence of cavities in the alveolar tissues of groups 3 and 4, suggested that tissue shrinkage and differential destruction of the non-osseous tissues had occurred.

Table 4 summarizes the analysis of moisture absorption potential, and indicates processing treatments are capable of altering the hygroscopicity of bone. The weight gain of all samples acclimated from 25% to 85% RH ranged from 2.20% to 3.06% (mean, 2.46%). Group 1 (control) showed the greatest moisture absorption (mean weight gain, 2.76%), indicating that fluid treatments contribute to a loss of moisture absorption potential. The control group also had the greatest amount of variation of all groups (coefficient of variation, 5.64). Group 2 (mean weight gain, 2.25%), representing what is considered the least intrusive fluid treatment (23°C water), deviated the most from the control group with the difference in percent weight change being highly significant ( $P \leq 0.00001$ ;  $F = 57.078$ ). The mean weight gain of Group 4 (90°C water) was 2.48% which was significantly different from the control group but at a much lower level ( $P \leq 0.01$ ;  $F = 11.697$ ). The group treated with ammonia (Group 3) had a mean weight gain

(2.36%) falling between those of Groups 2 and 4, but still significantly different ( $P \leq 0.0001$ ;  $F = 34.881$ ) from the control group.

#### DISCUSSION

Although this project incorporated sophisticated equipment, it was never the intention of this contribution to evaluate or endorse the equipment and methods used. Instead, this project utilized the equipment simply as a tool for documenting a much more important issue, that of differentially altering specimens by processing treatments.

The advantages and disadvantages of various osteological processing treatments have been recognized previously. This has led to the realization that boiling water (with or without additives) is among the most destructive treatments (Groen, 1988; Knudsen, 1966), and that the use of organisms is perhaps the least destructive method for removing non-osseous tissues (Hall and Russell, 1933; Russell, 1947; Sommer and Anderson, 1974; Williams and Rogers, 1989); however, the final cleaning of bone still involves a variety of methods including hot water, alkaline solutions, mechanical methods, or combinations of these procedures (Hildebrand, 1968; Knudsen, 1966; Williams, 1992; Williams *et al.*, 1977).

Regardless of the cleaning treatment, it would be expected that the use of water with chemicals or high temperatures could affect bone in several ways. The hygroscopic and anisotropic responses of bone and teeth to ambient moisture levels are well documented and involve obvious and irreversible structural damage (Canadian Conservation Institute, 1983; Currey, 1984; Guldbeck and MacLeish, 1985; Lafontaine and Wood, 1982; MacGregor, 1985; Williams, 1991; Williams *et al.*, 1993). These RH-induced changes combined with the removal of one of the two fundamental components of bone as a composite material, can only exacerbate the risk of damage. For example, hot water and alkaline solutions degrade proteins and lipids and contribute to their solubilization (Armstrong, 1983; Feehey, 1980; Plummer, 1987; Whitaker, 1980). It is possible for cartilage and ligaments to be destroyed, leaving bones disarticulated (Knudsen, 1966). When the embedded periodontal ligaments connecting alveolar bone with dentition (Hillson, 1986) are destroyed, loss of teeth from the skull can be expected. In extreme cases, the bone becomes porous because the only remaining component is the inorganic matrix.

It was demonstrated in this study that processing treatments affect bone in many ways. Weight loss and visible changes indicate components of the bone were lost which in turn contributed to changes in dimensions and moisture absorption potential. Any analysis based on significantly altered specimens also will be based on artificial characteristics.

Although dimensional change in *S. tridecemlineatus* skulls has been documented with different RH levels, it was not considered significant with univariate analyses (Williams *et al.*, 1993); however, patterns of moisture absorption observed with treatment groups helps in understanding the nature of osteological changes associated with processing treatments. The control group (Group 1) had the greatest weight change resulting from exposure to different RH levels; this would be expected with bone having minute remnants of non-osseous, hygroscopic tissues adhering to surfaces. The water-treated group (Group 2) had very little weight change between pre- and post-treatment, yet it deviated the most

from the control by having the lowest weight change with respect to moisture absorption potential. This suggests the treatment removed only components that would go into solution (i.e., amino acids) or suspension (i.e., blood). This caused minimal weight loss, possibly involving only components that might affect moisture absorption. We theorize that much of the absorption change can be attributed to lipids remaining in cell membranes (Wessells and Hopson, 1988), causing the organic tissues to be somewhat hydrophobic.

Treatments involving ammonia (Group 3) and 90°C water (Group 4), resulted in greater weight loss and increased moisture absorption potential compared to 23°C water treatment (Group 2). Much of the weight loss might be attributed to degradation and solubilization of proteinaceous tissues as well as saponification of lipids (Armstrong, 1983; Plummer, 1987) in marrow cavities of the bone (Curry, 1984). Although the primary components of bone are hygroscopic, it is proposed that much of the increase in moisture absorption potential is caused by the respective treatments degrading cellular lipids and making the associated tissues more hydrophilic.

Before discussing the implications of this study, it is appropriate to critically review the methods and materials used. Although very sophisticated equipment was utilized, minor discrepancies were noted when the two sets of measurements for the control sample were not exactly the same. Such deviations can be explained by measurement error resulting from specimen positioning or subtle changes in precisely redefining measurement points on the monitor (in terms of pixels). It was noted also that dimensional changes among specimens were not always in the same direction. It is not clear whether this is related to measurement error or a matter of different anisotropic responses related to individual variation. For instance, it is conceivable that individual variation would be an issue for samples involving different age groups, particularly in specimens of young mammals where bones are not fully ossified. Most of the documented variation was in dimensions that might be influenced by the non-osseous tissues. For example, the length of maxillary toothrow and breadth across upper molars were measurements having high coefficients of variation, possibly because the teeth are repositioned as fluid treatments cause the non-osseous alveolar tissues to swell or degrade. This correlation suggests that mammalian taxa with fully ossified crania (i.e., bats) might be less affected by processing treatments (except where groups of teeth are concerned).

It is evident that processing treatments can and do cause alteration of rodent skulls. These changes involve components of the bone itself, as indicated by appearance, mass, dimensions, and response to environmental conditions. These findings have far-reaching implications when the methods used in this study are compared to those in actual practice. Because cleaning procedures are dependent on so many variables (i.e., personal preference, skill level, facilities, health and safety concerns, nature of preservation, specimen size, and evolving trends among laboratories), it is difficult to fully appreciate the magnitude of the problem and its implications for research.

The limitation of the study is that the processing treatments examined barely address the broad range of other treatments that have been used for mammal skulls. For instance, a 24-hour soaking in distilled water is almost negligible compared to weeks of maceration involved with some procedures (Knudsen,

1966; Wagstaffe and Fidler, 1968). Also, immersing skulls in 90°C water for one hour is not nearly as harsh as boiling treatments that may last several hours, and may include additives such as ammonia and bleaches (Groen, 1988; Hangay and Dingley, 1985; de Wet *et al.*, 1990). If it is possible for significant differences in cranial dimensions to occur with the comparatively mild treatments used in this study, then there probably is a high risk of more significant changes being caused by more intrusive methods.

The realization of how rodent skulls might be significantly altered by processing treatments raises questions about the accuracy of previous morphometric research. It would seem that a series of artificially altered skulls may have limited value for any project involving morphometrical analyses; however, the critical issue is the magnitude of variation. For instance, it is possible for natural variation (i.e., individual, age, sex, and geographic) of a taxonomic group to be sufficiently substantial to obscure any variation that would be attributed to processing treatment. In such cases, conclusions based on univariate analyses should be unchanged; conclusions of multivariate analyses probably would be unchanged also, as long as documented dimensions do not reflect changes in shape. In taxonomic groups where natural variation is more subtle, it may be possible for variation caused by processing treatment to be emphasized, making it difficult to recognize true taxonomic affinities. It is conceivable that this may be an issue with taxa that are currently more easily recognized by biochemical or genetic methods than by more traditional methods (i.e., morphometrics).

Future investigations in systematics should evaluate the likelihood of whether variation attributed to processing treatment may influence analyses. If there is a risk, efforts should be made to use specimens that have received the same processing treatment. Ideally, this could be done by selecting specimens with a known and recorded treatment history; thus, efforts should be made to document treatments so that researchers can evaluate the quality of potential research material. Selection of specimens from specific institutions or time-periods might reduce the likelihood of incorporating variation caused by processing treatment. In such cases, it would be desirable to critically examine the appearance of bone for indications of the nature of previous treatment.

When examining skulls for signs of previous treatment, unusual whitening of the bone, absence of organic tissues, bone porosity, and disassociated parts serve as indicators of prior treatments that have the potential to contribute to dimensional change. Also, any sign of repair or restoration (i.e., teeth glued in sockets or presence of coatings or consolidants) would be equally useful in identifying questionable specimens.

#### CONCLUSIONS

In the current study, a very select group of specimens was utilized to demonstrate that processing treatments can affect dimensions of rodent skulls; however, limitations imposed on this study call for similar studies to be performed on other groups of specimens. For instance, specimens representing different age or taxonomic groups may not respond to processing treatments in the same way reported in the current study. Also, only four treatments within a rather regimented protocol were examined and compared with this study. There is a need to study how variations in these procedures (i.e., chemical concentration, duration, temperature

level, and enzyme additives) might affect the bone appearance, dimensional change, and response to different RH levels.

These findings probably have little effect on previous systematics studies where taxonomic groups were clearly recognized; however, variation attributed to processing treatment may obscure subtle diagnostic features based on dimensions, thus limiting the ability of past researchers to differentiate very similar taxa. With respect to future research, particularly where very similar taxa are involved, it would be desirable to use specimens processed in the same manner. If the analyses include those for age variation, consideration should be given to possible differential changes in shape and size of specimens resulting from processing treatment.

If research interests of systematics collections are to be served, the current study demonstrates two fundamental needs with respect to processing treatments. First, there is a desperate need for basic documentation of how individual specimens have been treated (Garrett, 1989; Society for the Preservation of Natural History Collections, 1994). Secondly, there is a need for processing treatments that involve non-intrusive methods (Williams, 1992), so that systematics investigations will not be jeopardized by incorporating artificial characteristics. Ideally, there would be standards developed among institutions to address both of these issues.

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# FIXATION AND PRESERVATION OF MUSEUM MARINE COLLECTIONS USING FORMALDEHYDE/GLUTARALDEHYDE MIXES

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*Abstract.*—A preliminary investigation into the suitability of buffered formaldehyde/glutaraldehyde mixes as general fixatives and storage solutions for marine animals in museum collections was undertaken. By comparison with material held in formaldehyde only, whole specimens in aldehyde mixes show less brittleness, better joint flexure and have undergone less colour changes; while their quality of fixation at the tissue level is at least as good. The buffers sodium orthophosphate and sodium glycerophosphate were tested for their ability to maintain pH stability. Buffer reaction products, in the form of insoluble crystalline deposits, show sodium orthophosphate to be unsatisfactory for some marine invertebrates. A mixture of formaldehyde 2.5% glutaraldehyde 1% buffered by sodium glycerophosphate 2% was found to provide the best specimen preservation results after a period of two years.

The desire for a general purpose fixative/preservative for marine animals, particularly for those caring for long-term biological collections, has increased as methods and techniques for collection preservation and care have improved over the last decade. In our approach we are mindful of the situation where curators have responsibilities for a range of phyla; in our case, intact and often large fishes, echinoids, crustaceans, molluscs, annelids and coelenterates.

Historically, museum practices in the preservation of marine specimens recommended the use of commercial formalin (containing 40% formaldehyde) diluted to strengths of 3–10% and neutralised by buffers such as calcium carbonate, borax, or even hexamine. Wagstaffe and Fidler (1955, 1968) detailed the use of formaldehyde for the preservation of fish and a variety of marine invertebrates using a two stage process—formalin fixation followed by alcohol preservation. From a museum standpoint, both Stoddard (1989) and Simmons (1991) have since produced more useful modern synopses of aldehydes as fixatives and preservatives. In relation to marine animals, the results of the Scientific Committee for Oceanic Research, working party 23, (Steedman, 1976) are likely to be more significant. Steedman (1976) noted, by way of review, that for general purposes only two of the aldehydes, formaldehyde and glutaraldehyde, deserved consideration.

The idea of examining fixatives containing a mixture of formaldehyde and glutaraldehyde was considered by Taylor (1977). In his study a small collection of marine fish held in a formaldehyde/glutaraldehyde mixture were found to be marginally better in their resistance to pancreatin clearing after one year than those in formaldehyde (buffered and unbuffered). The potential of using bifunctional, unsaturated, glutaraldehyde in combination with monofunctional, strongly hydrated, formaldehyde seems to offer complementary advantages for fixation through their selective reactivity with protein fractions and the stability of these products.

Formaldehyde bonds to various proteins, or protein fractions, can be unstable and more or less easily dissociable. Both Schauenstein *et al.* (1977) and Taylor

(1977), following Pearse (1968), note that washing specimens following formalin fixation can remove cross-linked reaction products with the possibility of tissues undergoing autolysis. The acidity of formaldehyde resulting from dissociation is also a considerable disadvantage to fixation if the pH is low enough to induce polymerization of the formaldehyde. The buffering of formaldehyde is particularly important for those marine animals which have a high susceptibility to calcium loss when stored in acidic conditions, e.g., crustacea and mollusca.

Much of the established repute of glutaraldehyde lies in its excellence in intermolecular cross-linking reactions. Habeeb and Hiramoto (1968) showed that it was very reactive towards the N-terminal amino groups of peptides, alpha-amino groups of amino acids and the sulphhydryl group of cysteine. It is also known to have a stabilizing effect on collagen and interacts with lipoprotein. Hayat (1981: 72) records that "cross-linking of histones by glutaraldehyde is irreversible." The irreversibility of cross-linked bonding by glutaraldehyde led us to try a reduction of contributory aldehyde concentration in fixative mixtures thereby providing an additional benefit for worker health. While somewhat dependent on pH and temperature, glutaraldehyde's rate of response is greater than that of formaldehyde. Sabatini *et al.* (1963) found glutaraldehyde (4–6.5%) to be the most effective of the aldehydes tested in preserving cellular fine structure.

Despite the availability of good information on buffering formaldehyde fixatives used for some marine organisms (Steedman, 1976; McLaughlin *et al.*, 1982) the choice of a reliable buffer for general use has not been particularly straightforward. Taylor (1977) and Hughes and Cosgrove (1990) specifically recommended against borax, yet it was still shown by them to have widespread use in North American fish and herpetology collections. Others, while providing favourable evidence for orthophosphates (Markle, 1984) or sodium acetate (Tucker and Chester, 1984) in relation to ichthyoplankton, have not considered examples where calcified tissue (exoskeleton) is to be fixed. Although glutaraldehyde reactivity somewhat improves with rising pH it is offset by polymerization which, above pH 9.0, extensively removes aldehyde groups. We considered it essential to buffer solutions for an optimal range (pH 6.5–8.0) as specimen damage including calcium loss, brittleness, protein contraction/swelling, gelatinization and translucency will occur outside this range (Tucker and Chester, 1984).

As well as evaluating the abilities of formaldehyde/glutaraldehyde mixes as general fixatives, the complimentary role of buffer action for various orthophosphate salts is investigated.

#### METHODS

Five aldehyde fixatives were evaluated as long-term preservatives: (A) formaldehyde 4%; (B) formaldehyde 2.5% + glutaraldehyde 0.5%; (C) formaldehyde 2.5% + glutaraldehyde 2%; (D) formaldehyde 5% + glutaraldehyde 5%; (E) formaldehyde 2.5% + glutaraldehyde 1%. Tests were conducted in commercial grade (Agee<sup>®</sup>) glass storage jars of 125 and 500 ml capacity, sealed by plastic (wadded insert) screw lids. Jars were filled to overflowing at the commencement of the trial period creating a minimal air gap, but were not topped up during test intervals.

A range of marine animals were gathered to represent different phyla (standardized by genera and species) for each trial fixative. These were examined for gross morphological condition, quality and appearance of specimens and fluids at 6 monthly intervals throughout the 24 month trial period. Solution pH was measured on a progressively increasing time interval (days, weeks, months) over a total of 24 months, using an Orion<sup>®</sup> bench-top pH/mV/temperature meter with a Ross<sup>®</sup> sure-flow glass electrode having a given accuracy of 0.03 pH.

After 24 months the histology of fish liver tissues fixed by the trial solutions were compared with long standing 20–60 year old museum specimens for which a two stage process (formalin 10% fixation; ethanol 70% or isopropanol 40% preservation) had been followed. Tissue preparation of all samples was standardized to routine thin-sectioning (disposable blades), Erlich acid haematoxylin staining for a fixed period, followed by examination and evaluation using a light microscope.

## MATERIALS

Marine animals were gathered from two coastal sites; an adjoining beach and rock reef (Tapeka, Bay of Islands) and at a mudflat (Hobson Bay, Auckland). For trials involving orthophosphate buffers a standardized group of species comprising: *Aldrichetta forsteri* (Cuvier & Valenciennes, 1846), *Patiriella regularis* (Verrill, 1867), *Turbo smaragdus* Gmelin, 1867, *Ozius truncatus* H. Milne Edwards, 1834, *Palaemon affinis* H. Milne Edwards, 1937, and *Perinereis novaehollandiae* Kinberg, 1866 were used to represent respectively fish, echinoderm, mollusc, crab, shrimp and worm. In a second set of trials using sodium glycerophosphate buffer the species mix involved: *Hyporhamphus ihi* Phillips, 1932, *Patiriella regularis*, *Zeacumantus lutulentus* (Kiener, 1841), *Ozius truncatus*, and *Palaemon affinis*. Test material (3 individuals of each species per jar) was fixed immediately after capture in their respective aldehyde solution. The fluid was not changed during the trial. A specimen to fixative ratio of 1:5 was used.

Mixed aldehyde fixatives were prepared from commercially available formaldehyde 40% and glutaraldehyde 25% stock solutions. Dilution followed the procedures given by Steedman (1976). By way of minor modifications these solutions were prepared with de-ionized water as the principal solvent, with 10% mono-propylene glycol (commercial grade) to reduce desiccation and hardening (Williamson and Russell, 1965). Three (commercial grade) buffers, 0.01 M sodium dihydrogen phosphate (monobasic salt) with 0.01 M disodium hydrogen phosphate (dibasic salt), or alternatively, 0.02 M disodium glycerophosphate (pentahydrate) were used to provide a 2% buffer to each aldehyde mix.

## RESULTS

### PH

The pH responses of trial solutions (both controls (Appendix 1) and those with specimens (Appendix 2)) generally followed known Cannizzaro and buffering reaction responses which operate to minimally lower the pH over time. Greatest end-point variation in pH after 24 months occurred in standing fractions where specimens had not been introduced.

On adding specimens to solutions (Appendix 2) there was a rapid lowering of pH, a recognized reaction response between aldehyde and proteins, within the first seven days but pH was readjusted (buffering effect) over the ensuing two months. While differing starting points (pH 6.69–7.43) are recorded for trial solutions it should be noted that they were prepared from two different batches of commercial formaldehyde. As might be anticipated from buffered systems, plateau and end point pH results are broadly similar. Stability, however, can be related to the chemical buffering agent. After the immediate lowering of pH due to specimen response, solutions A–D buffered by sodium orthophosphate 2% continued to show irregularities in pH (Fig. 1) throughout the trial period. In contrast, solution E (formaldehyde 2.5% glutaraldehyde 1.0%) buffered by sodium glycerophosphate 2% performed consistently well.

### Condition of Fluid and Specimens

In Table 1, the observable condition of fluids and specimens are noted after the 24 month standing period only. Though these results were affected by unsatisfactory buffering (crystal deposits), there is considerable variability between individual specimens in the degree of integumentary crystal formation on exo-

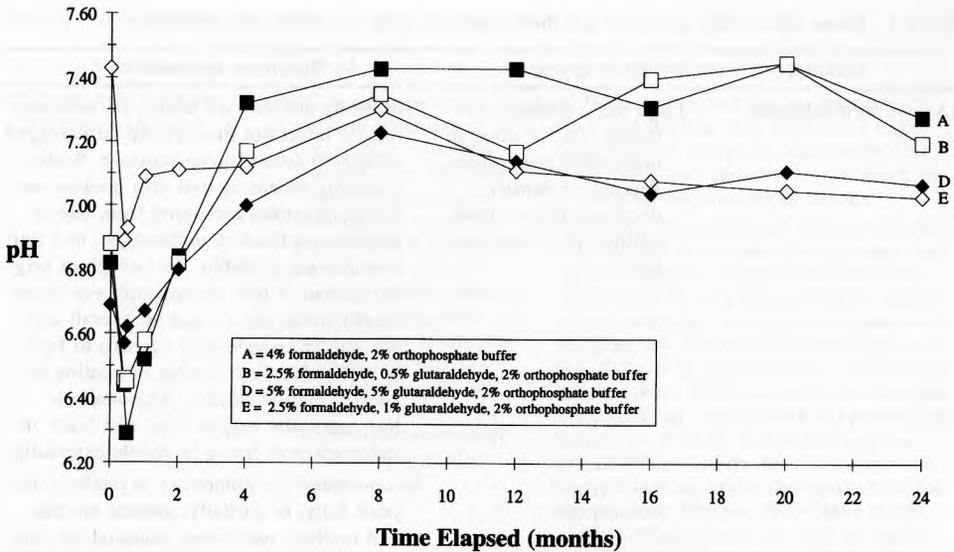


Figure 1. Mean pH variation over elapsed time for solutions with specimens.

skeletons. Having used a range of individuals allowed a somewhat more reasonable reporting of specimen condition based largely on material unaffected by these deposits.

Preservation in mildly dilute formaldehyde (solution A) rapidly decolorized pigments and stiffened tissues to the extent that brittleness led to mechanical damage during handling. Where glutaraldehyde was used (solutions B–E) pigmentary fading, at least in the yellow-red-purple regions of the spectrum, was retarded. The presence of glutaraldehyde was also observed to delay the destruction of an argenteum layer in fish material. While tissue brittleness and shrinkage is least in solutions of low aldehyde strength the best overall in relation to specimen condition is solution E (formaldehyde 2.5% glutaraldehyde 1%).

### Histology

Examination of stained thin sections of fish livers held in various fixatives and preservatives (Table 2) revealed the gross cellular organization within the tissue was retained in every case. In the measurement of "quality of fixation" independent comments (Hillary Hollaway, personal communication) relating to a seven slide series indicates that solutions of high dilution (formaldehyde 2.5% glutaraldehyde 0.5%) did not fix cellular structure particularly well. However, the adequacy of cellular fixation at the slightly higher concentration (formaldehyde 2.5% glutaraldehyde 1%) was complimentary to the determination of specimen condition in relation to solution E noted previously.

### DISCUSSION

Much of the "potential superiority" (Taylor, 1977) of formaldehyde/glutaraldehyde combination fixatives over formaldehyde alone has not altogether been fully realized by the trials under consideration. We must note some disappointing artefactual effects induced by histological technique and buffering problems as-

Table 1. Gross observable specimen and fluid condition after 24 months elapsed time.

Solution	Fluid appearance	Specimen appearance
(A) 4% formaldehyde	Fluid has a greenish-yellowish tint but clear of detractable precipitates, and colour density does not obscure readability of contents and label.	<p>Fish. Body and fins are brittle, fin structure mostly intact but fin-rays can be damaged (snapped) under forcep pressure. Scales generally absent though skin pockets are intact; examples recovered from lateral midline are flexible, undamaged, and ring formation is readable. All vestige of original colour is lost, chromatophores (fixed black) are in situ so that an overall colour pattern, purple-grey (dorsal) to buff-brown (ventral), is visible in relation to chromatophore density. Argenteum is lost, opercular region clear, and black interior gut wall lining is visible externally.</p> <p>Invertebrates. Integumentary crystalline deposit fully, or partially, present on crab and mollusc specimens. Material has lost colour and consistently has pale orange-yellow tones throughout. Crab exoskeletons are firm, partially flexible but limbs and carapace tear easily at exoskeletal joints. Shrimp carapace inflexible, limbs easily removed, abdomen and carapace separated by light contact pressure. Echinoderm spines easily separated from ambulacra but have retained good structural strength and form. Mollusc shell surfaces chalky, easily scratched. Polychaete worms fixed without marked osmotic stress; shape, skin strength and flexibility are good.</p>
(B) 2.5% formaldehyde, 0.5% glutaraldehyde	Fluid tinted bistre-yellow but clear of precipitates. All specimens have yellow-dull orange tints which are resident in both integument and internal muscle tissues.	<p>Fish. Fixation and preservation is good with fins and scales intact while body and fins are flexible. Eye cirlet and argenteum layers are partially complete, chromatophores visible but original colour is lost (reduced to grey-black).</p> <p>Invertebrates. Crystalline deposits fully, or partially, developed over crab and mollusc exoskeleton but absent from all other material. Limbs of crabs having crystal deposits are deciduate but carapace structure is reasonably strong and flexible. Shrimp ova are hemispherical with outer membrane and vitellus intact. Echinoderm spines can be dislodged from ambulacra but individual spines and tube feet are well fixed. Mollusc shells are somewhat chalky, but retain strength. Polychaete soft body intact and not ruptured by forcep pressure; parapodia flexible.</p>

Table 1. Continued.

Solution	Fluid appearance	Specimen appearance
(C) 2.5% formaldehyde, 2% glutaraldehyde	Fluid appearance has a strong yellow-green colouration, precipitation has occurred, and there is an unacceptable loss of clarity. A partial or full crystalline deposit covers most specimens; densely so on crabs, molluscs, echinoderms and polychaetes, microscopically on fish and shrimps. Material has the general appearance of yellowing in all tissues.	Fish. Body, scales and fins are intact, well fixed and flexible. Eye circling and argenteum partially complete, chromatophores intact but colour reduced to grey-black depending on chromatophore density. Invertebrates. Red and black pigmentary colours are still discernable though some fading has occurred. Opaque structures are discoloured by pale tones of bistre-yellow to yellow-orange. Limbs and carapaces of decapods are flexible, withstand light contact, but tear at integumentary joints under mild pressure. Crab carapaces are thinned, depressible and can be broken (penetration wound) by forceps. Echinoderm spines and ambulacral plates have lost structural strength and are easily damaged under forcep pressure. Mollusc shells have microscopic surface powdering, and are easily scratched and eroded by handling. Polychaetes show osmotic stress, skin surface and parapodia are torn by handling.
(D) 5% formaldehyde, 5% glutaraldehyde		Comments for Solution C apply here, but in addition specimens are generally more brittle with limbs and fins fragmenting under forcep pressure. Colour retention is improved; fish argenteum layers are more or less complete and green pigments of Echinoderms have been retained (with slight spectral changes and fading). Decapod ova and eye-stalks have strongly darkened (brown-black), this extends into the occipital region generally, in some individuals.
(E) 2.5% formaldehyde, 1% glutaraldehyde	Fluid has a pale green-yellow tint, colour density does not impede readability of contents and label. Crystalline deposits, noted in other solutions, are absent from all specimens. Material is generally well fixed, flexible and has retained original shape and structural strength.	Fish. Muscle tissue has a very slight yellowish tint throughout. Argenteum layers, including eye circling, partially or fully complete, chromatophores present but colour reduced to grey-black tones. Invertebrates. All material withstood routine handling without damage, soft tissues have retained shape and strength without obvious dehydration. Texture, sculpturing and hardness of calcium derived exoskeletons appear to be unchanged by fixation/preservation. Colour retention is good particularly for brown, red and orange tones so that original markings (striations and blotches) are well defined. Decapod muscle has slight yellowish tones, eye-stalks and ova slightly darkened, surface markings (orange striations) still visible. Echinoderm (blue-green) and Mollusc (brown, purple) surface colours are still present, although slight fading has occurred in the echinoderm material.

Table 2. Comparative histology, based on historic Museum preservatives and fluids under trial at 24 months elapsed time.

	Solution	Comments
Slide 1	Formaldehyde 4% (unbuffered), followed by ethanol 70% (specimen held since May 1932)	Staining very pale, cellular structure organised but cannot be determined for quality because much of the tissue has been disrupted by shrinkage and/or leached by prolonged storage.
Slide 2	Formaldehyde 4% (unbuffered), followed by isopropanol 40% (specimen held since June 1970)	Tissue compression and knife marks from sectioning, some loss of cell contents and vacuolar spaces developed within the tissue. Cells regularly arranged, nuclei stained with distinct nucleoli, lipid droplets present.
Slide 3	Formaldehyde 4% (solution A)	Tissue has regular outline and cell structure but some vacuoles, good staining for nuclei and contents, lipid droplets present. Has the best fixation and overall appearance of all trial slides.
Slide 4	Formaldehyde 2.5%, Glutaraldehyde 0.5% (solution B)	Cell structure lacking clear definition some cells have a "boiled" appearance, haematoxylin washed out, nuclear staining is poor. Unusual chromatin pattern in some cells, lipid droplets present, fixation otherwise good.
Slide 5	Formaldehyde 2.5%, Glutaraldehyde 2% (solution C)	
Slide 6	Formaldehyde 5.0%, Glutaraldehyde 5.0% (Solution D)	In both slides cells are poorly defined, staining is patchy, sections are torn and somewhat compressed. A regular cellular arrangement, lipid droplets present, suggesting reasonable fixation but quality is obscured by artefacts in histology technique.
Slide 7	Formaldehyde 2.5%, Glutaraldehyde 0.5% (Glycerophosphate 2% buffer)	General image is poor, many cells are disrupted with no distinct outline, necrotic patches. Evidence of artefacts due to improper technique, unusual chromatin pattern, fixation might be inadequate.

sociated with the crystal formation by the sodium phosphate buffers. It might also be argued that the batches of mixed specimens, held under somewhat poor fluid to volume ratios, and without fluid changes, would not match "normal working circumstances." Nevertheless, given the intent of the trial objectives for the long-term use of these mixes for museum collections there are some highly significant results, not least of which is that fixation/preservation activity has been well established under rather extreme short-term conditions.

While it is implied that a general fixative should perform more or less equally for all conditions, there will inevitably be a degree of unpredictability about the chemistry of biological fixation because of different and quite specific properties between phyla, populations and even individuals. Steedman (1976) comments that additives including glycol act to reduce the polymerization of formaldehyde. The Merck index (Windholz, 1976) notes instances (high temperatures) where propylene glycol may oxidize to give products such as propionaldehyde. We have as-

sumed, however, the diluted glycol to be neutral, or to have minimal aldehyde contribution with respect to fixation and pH. While a number of buffers have been regularly used in aldehyde systems, choosing a suitable buffer is more complex. Buffers such as borax, calcium carbonate and hexamine have residual disadvantages for both pH stability and specimen clearing (Steedman, 1976; Taylor, 1977). Paradoxically the use of orthophosphates, has been favorably reported for marine animals by Markle (1984), McLaughlin *et al.* (1982), despite Steedman (1976) noting that its buffering in "seawater/formaldehyde solutions rapidly produces insoluble products." Our experiences with integumentary crystalline deposits (Table 1) simply reflect the unsuitability of orthophosphates where the animals undergoing fixation are marine invertebrates. Fluctuations in fixative pH (Appendix 2) are attributed to a chemical instability of orthophosphate buffer activity induced by these insoluble precipitates. In a similar regime, however, the use of sodium glycerophosphate (2%) as a buffer did not produce noticeable artefacts and has reliably maintained a constant fixative pH over a two-year period.

Results relating to specimen condition show that on a comparative basis mixed aldehyde fluids have been least detrimental to gross specimen morphology by way of tissue stiffening, loss of joint flexure, and shrinkage, as well as colour change. Moreover, the relatively low concentration of aldehydes carries additional advantages for cost effectiveness and worker health. Staff and visitors have been agreeably surprised to find that specimens from mixed fluids could be handled without the obvious and objectionable properties normally experienced with formaldehyde alone. We attribute this to low aldehyde concentrations and the possibility of interaction or bonding between the two aldehydes, with glutaraldehyde depressing the vapor phase of formaldehyde. Inevitably the extent to which aldehyde concentrations may be lowered, while still achieving satisfactory fixation, is of particular interest. Histology results (Table 2) indicated a partial necrosis of fish liver tissue when whole animals (TL ca. 75mm) were held in formaldehyde 2.5% and glutaraldehyde 0.5%. At this concentration the aldehyde components are considered to be marginally low.

Solutions containing glutaraldehyde 4% (Taylor, 1977) produce undesirable, but not detrimental, brown discolouration in tissues and yellow staining of lipids. We found that yellow discolouration was not particularly discernable at levels of glutaraldehyde 1.0% and 0.5% in formaldehyde mixes. However, because fresh material was held (fixed & preserved in the same fluid) continuously there was a noticeable carry over of blood pigments and body fluids which affected solution clarity. In the context of solution contamination, Moore (1989) notes that dissociation and leaching of lipids from cell contents will, through oxidation, lead to rancidity and bacterial decay. There were no free-floating fat globules and oil droplets in any of the fluids. The implication that lipids are still bound within cell structures is supported by the histology results.

At a gross cellular level the general evidence from fish liver histology (Table 2) shows that fixation has been almost equally effective for all regimes of treatment. Closer examination distinguishes those specimens preserved in alcohol from aldehydes. They were distinguished by interstitial vacuoles, empty cells and poor staining. Moore (1989) gives similar undesirable qualities for formalin fixed, ethanol preserved, material held over a period of twenty years. This is an important factor in our preference for long-term aldehyde storage over the use of alcohol

preservatives. Amongst aldehyde treated tissues, formaldehyde has the best appearance and quality, while those from formaldehyde/glutaraldehyde solutions rate poorly. Some of the poor mixed solution ratings can be attributed to artefacts of technique, glycol interference, blade cutting and the need for altered processing and staining protocols for glutaraldehyde (Hillary Hollaway, personal communication) by way of explanation. In these circumstances the solutions cannot be effectively rated for quality, except as noted earlier for slide 7, where aldehyde concentration is marginally low.

#### CONCLUSIONS

Evidence from this investigation supports the contention that for general use, buffered aldehydes have distinct advantages over alcohol preservatives. Some of these include good specimen morphology, colour and tissue histology, as well as features applying to fluid changes (rancidity, evaporation) and fire risk. Solutions of mixed aldehydes, using glutaraldehyde 1–2% in conjunction with formaldehyde, confer even greater improvements in the above aspects of specimen condition. Taylor (1977) has already shown, in relation to glutaraldehyde solutions, that there is less damage to bone structure and specimens are resistant to denaturing by proteolytic enzymes. Additionally, fixation products of glutaraldehyde are unlikely to be reversible in dilute storage solutions or by washing specimens. Sodium glycerophosphate 2% was found to be compatible with marine organisms and a reliable buffer for general storage involving aldehydes. This cumulative evidence indicates a superiority of formaldehyde/glutaraldehyde solutions over formaldehyde alone for long-term storage of marine animals. These initial results have suggested areas for future research and the need for more sophisticated testing in some areas.

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Appendix 1. Mean pH changes for elapsed time – solutions without specimens.

Elapsed time	A	B	C	D
Initial	6.82	6.88	6.76	6.67
1 week	6.90	6.81	6.74	6.69
2 weeks	6.90	6.81	6.79	6.72
1 month	6.98	6.91	6.81	6.73
2 months	6.91	6.86	6.71	6.72
4 months	6.97	6.85	6.67	6.63
8 months	7.08	6.93	6.74	6.37
12 months	6.89	6.68	6.43	6.25
16 months	6.94	6.57	6.30	6.21
20 months	6.95	6.56	6.26	6.12
24 months	6.91	6.50	6.17	6.00

A = 4% formaldehyde, 2% orthophosphate buffer.

B = 2.5% formaldehyde, 0.5% glutaraldehyde, 2% orthophosphate buffer.

C = 2.5% formaldehyde, 2% glutaraldehyde, 2% orthophosphate buffer.

D = 5% formaldehyde, 5% glutaraldehyde, 2% orthophosphate buffer.

Appendix 2. Mean pH for elapsed time – solutions with specimens added.

Elapsed time	A	B	C	D	E
Initial	6.82	6.88	6.76	6.69	7.43
1 week	6.44	6.46	6.50	6.57	6.89
2 weeks	6.29	6.45	6.38	6.62	6.93
1 month	6.52	6.58	6.53	6.67	7.09
2 months	6.84	6.82	6.68	6.80	—
4 months	7.32	7.17	7.03	7.00	7.12
8 months	7.43	7.35	7.20	7.23	7.30
12 months	7.43	7.17	7.03	7.14	7.11
16 months	7.31	7.40	7.01	7.04	7.08
20 months	7.45	7.45	7.12	7.11	7.05
24 months	7.28	7.20	6.79	7.07	7.03

A = 4% formaldehyde, 2% orthophosphate buffer.

B = 2.5% formaldehyde, 0.5% glutaraldehyde, 2% orthophosphate buffer.

C = 2.5% formaldehyde, 2% glutaraldehyde, 2% orthophosphate buffer.

D = 5% formaldehyde, 5% glutaraldehyde, 2% orthophosphate buffer.

E = 2.5% formaldehyde, 1% glutaraldehyde, 2% glycerophosphate buffer.

# SPNHC: THE FIRST TEN YEARS

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*Abstract.*—In 1985, the Society for the Preservation of Natural History Collections (SPNHC) was created to meet the concerns of a growing number of individuals involved with the development, management, and care of natural history collections. The Society is unique among natural history professional organizations because of its international scope and multidisciplinary approach to collections management and care. The ten-year history of this organization is impressive, particularly with respect to increasing awareness of the value and requirements of collections, collaborating nationally and internationally with other organizations, and contributing to new knowledge, resources and standards for the natural history profession. In celebration of the Society's ten year anniversary, the history and accomplishments of SPNHC during the last decade are documented.

## INTRODUCTION

The Society for the Preservation of Natural History Collections (SPNHC) is a "multidisciplinary organization, including persons within the fields of anthropology, botany, geology, paleontology, zoology, and others who are interested in the development and preservation of natural history collections" (SPNHC, 1991). The organization's international and multidisciplinary scope makes SPNHC unique among professional natural history organizations. Its diverse disciplinary representation provides strength through numbers and broad based knowledge.

The purpose of SPNHC is "a) to provide for and maintain an international association of persons who study, care for, work with, or are interested in natural history collections; b) to encourage studies about the essential requirements for preservation, management, storage, research, and display of natural history collections; c) to publish at least a newsletter and encourage the dissemination of information about natural history collections in journals, bulletins, proceedings, and wherever such information is appropriate; and d) to hold regular meetings and encourage conferences, symposia, workshops, and other meetings about natural history collections in order to facilitate exchanges of ideas and information" (SPNHC, 1991).

## THE BEGINNING

Some say that the "seeds" of SPNHC were planted in 1981, when D. J. Faber and G. R. Fitzgerald of the National Museum of Natural Sciences (now Canadian Museum of Nature) organized the first *Workshop on the Care and Maintenance of Natural History Collections* (Faber, 1983). This workshop demonstrated that the multidisciplinary approach to addressing management and care issues of collections was important. However, it was not until the summary of the second workshop, held at the Royal Ontario Museum in May 1985 (Waddington and Rudkin, 1986), that ideas of an organization for individuals involved with natural history collections was formally discussed. During the open discussion, moderated by D. J. Faber, there were different opinions about the direction and scope that such an organization should have.

In October 1985, D. J. Faber distributed a 26-page pamphlet to workshop at-

tendees in an effort to keep the idea of a collection-oriented organization viable. This publication became the first issue of *Collection Forum*. It proposed that the organization be called the "Society for Scientific Collections," and it proposed an organizational structure (Faber, 1985). It also serves as an important record by identifying the 67 individuals who attended the second workshop. This record, however, is not considered a listing of charter members because it does not include others who were involved when the organization was actually started.

SPNHC became a reality on November 6, 1985, at a meeting hosted by the Buffalo Museum of Science. The individuals at this meeting were S. S. Albright, D. J. Faber (Chair), D. Laub, C. Romero-Sierra, C. L. Rose, W. Y. Watson, and S. L. Williams (Faber, 1986a). This meeting established the present name of the organization and identified its primary membership as being represented by an international and multidisciplinary audience. The operating structure of the organization was discussed, but not formalized, at that time.

In May 1986, the National Museum of Natural History (Washington, D.C.) hosted the first annual meeting of SPNHC. Participants at this meeting elected the first officers of the organization—D. J. Faber, President; C. Romero-Sierra, President-Elect; and S. S. Albright, Secretary. M. Rankin was the first appointed Treasurer (Faber, 1986b), however, he was replaced the following year by J. P. Cuerrier (Faber, 1987a).

#### THE BYLAWS

The organization's goal of serving an international and multidisciplinary membership presented challenges in creating a set of bylaws that would satisfy all parties involved. Representatives from Canada and the United States were key participants during the developmental stages. The Bylaws addressed membership, meetings, officers, council, committees, fiscal details, amendment procedures, the SPNHC seal, and dissolution (SPNHC, 1991).

With the Bylaws in place by May 1988, independent efforts in Canada and the United States were initiated to obtain tax-free status as a non-profit organization. C. Romero-Sierra was primarily responsible for obtaining the Canadian Letters Patent on May 2, 1989. S. B. McLaren assumed responsibility for obtaining federal non-profit status in the United States in November 1988 and Articles of Incorporation (in Pennsylvania) on May 27, 1989.

The original Bylaws accepted by the membership provided a strong framework for the developing organization. A growing number of standing rules, policies, and guidelines assisted the organization in its operations. As the organization matured and the operations and services were established, it became evident that the Bylaws would require revision to meet changing needs and times. These changes included membership and financial growth, operation refinements, and expanding collaboration among organizations. In May 1994, the membership approved the revision of the Bylaws that are in effect today. At the same time, the Canadian corporation of the Society for the Preservation of Natural History Collections established a separate Board of Directors and approved a separate set of revised Bylaws; thus, SPNHC in Canada and the United States became two distinct organizations.

Table 1. SPNHC Officers during the first 10 years.

Inclusive years	Individuals/Offices		
	President-Elect	President	Past-President
1986–1988	C. Romero-Sierra	D. J. Faber	
1988–1990	S. L. Williams	C. Romero-Sierra	D. J. Faber
1990–1992	G. R. Fitzgerald	S. L. Williams	C. Romero-Sierra
1992–1994	C. L. Rose	G. R. Fitzgerald	S. L. Williams
1994–1996	G. W. Hughes	C. L. Rose	G. R. Fitzgerald
	Secretary	Treasurer	Managing Editor
1985–1986			D. J. Faber
1986–1987	S. S. Albright	J. P. Cuerrier	D. J. Faber
1987–1988	S. S. Albright	J. P. Cuerrier	D. J. Faber
1988–1989	S. S. Albright	S. B. McLaren	P. S. Cato
1989–1990	S. S. Albright	S. B. McLaren	P. S. Cato
1990–1991	J. B. Waddington	S. B. McLaren	P. S. Cato
1991–1992	J. B. Waddington	S. B. McLaren	P. S. Cato
1992–1993	J. B. Waddington	S. B. McLaren	P. S. Cato
1993–1994	J. B. Waddington	S. B. McLaren	J. E. Simmons
1994–1995	M. E. Taylor	J. Golden	J. E. Simmons
1995–1996	M. E. Taylor	J. Golden	J. E. Simmons

#### THE COUNCIL

SPNHC leadership is provided by the Council which consists of six officers and six Members-at-Large. The officers include the President, Past-President, President-Elect, Treasurer, Secretary, and Managing Editor. The positions of President-Elect, Secretary, and Members-at-Large are elected offices with specific terms; the Treasurer and Managing Editor are appointed for one- to two-year terms, with the provision of being renewed repeatedly by mutual agreement. Individuals elected to the presidential positions accept a six-year commitment, but serve in three different capacities, separated into two-year increments (President-Elect, President, and Past President). The Members-at-Large hold the longest term of three years; each year two Members-at-Large are retired and replaced (Table 2). This structure provides consistency of direction and operations over time for the Council, as well as training for individuals who may become future officers.

During the first ten years, the individuals elected to the presidential positions were D. J. Faber, C. Romero-Sierra, S. L. Williams, G. R. Fitzgerald, C. L. Rose, and G. W. Hughes; the Secretaries have been S. S. Albright, J. B. Waddington, and M. E. Taylor; the Treasurers have been J. P. Cuerrier, S. B. McLaren, and J. Golden; the Managing Editors have been D. J. Faber, P. S. Cato, and J. E. Simmons (Table 1). The Members-at-Large and their respective terms are given in Table 2.

Council meetings are held during the annual meetings of the Society. In 1987, the Council found it useful to have meetings that preceded and followed the General Business Meeting. This practice is still used for Council operations. In 1989, significant restructuring of the Council meeting was initiated to increase efficiency and to actively involve and familiarize the membership with the operations of the Society. Since that time, it has been standard practice for committee

Table 2. SPNHC Members-at-Large during the first 10 years.

Inclusive years	Individuals
1987-1988	I. U. Birker and S. L. Williams (subsequently became President-Elect and was replaced by C. L. Rose).
1987-1989	F. J. Collier and W. Y. Watson.
1987-1990	J. Golden and J. B. Waddington.
1988-1991	I. U. Birker and C. L. Rose.
1989-1992	J. P. Angle and G. R. Fitzgerald (subsequently became President-Elect and was replaced by C. J. Bossert in 1990).
1990-1993	C. A. Hawks and J. E. Simmons.
1991-1994	G. W. Hughes and C. L. Rose (subsequently became President-Elect and was replaced by J. Gisbert in 1992).
1992-1995	L. Barkley and R. R. Waller.
1993-1996	G. Brown and S. Y. Shelton.
1994-1997	P. S. Cato and S. B. McLaren.
1995-1998	E. E. Merritt and A. Pinzl.

chairs, selected representatives, and incoming officers to attend all Council meetings.

#### THE COMMITTEES

Much of the success of SPNHC is directly related to activities and accomplishments of the Standing and Sessional committees. The Standing Committees continue from the term of one President to the next, whereas the Sessional Committees exist at the discretion of the President in office.

Initially, the committee structure accommodated operational functions as well as broad interests of the membership. The first official committees and respective chairs (in parentheses) included Annual Conference (C. Romero-Sierra), Bibliography (S. Teraguchi), Bylaws (C. Romero-Sierra), Colleges and Universities (W. Y. Watson), Conservation (C. L. Rose), Liaison (J. Golden), Membership and Directory (S. S. Albright), Publications (D. J. Faber), and Supplies and Equipment (L. Marhue) (Faber, 1986b). The following year, three additional committees were added—Ballot-Counting (J. Danis and P. A. Doepke), Finance (J. P. Cuerrier), and Nominations (F. J. Collier and W. Y. Watson). The Liaison Committee was renamed as Publicity and Liaison (E. Benamy); the Sessional Committees included Bibliography (J. Kaylor), Computer-use (I. Hardy), Conservation (C. L. Rose), Incorporation (D. J. Faber), Supplies and Equipment (C. Kishinami and L. Marhue), and Education (M. A. Dancey) (Faber, 1987b).

In 1987, a review of SPNHC operations indicated that various functions and services of the Society were being duplicated or overlooked with the existing Committee structure. Also, the direction and scope of some committees were inconsistent and occasionally too restrictive to effectively serve the total membership. As a result, the committee system was reorganized in 1988, so that a holistic structure would be in place to address the needs of the Society and its membership.

#### STANDING COMMITTEES

The 1988 reassessment initially identified 11 committees—four were critical in fulfilling the functions of the organization (Bylaws, Election, Executive, and Fi-

nance committees) and seven were essential in providing important services to the general membership (Archives, Conference, Conservation, Membership, Publications, Publicity and Liaison, and Resources committees). Subsequent committee restructuring and identification of societal needs resulted in the formation of three additional service committees (Awards and Recognition, Documentation, and Education and Training) and the merging of the Publicity and Liaison Committee with the Membership Committee. Details about some committees have been presented by Rose (1993). The following briefly describes the history, activities, and individuals associated with the current committees (listed alphabetically).

*Archives Committee.*—The Archives Committee is responsible for collecting and maintaining written and photographic documentation of the Society. The Committee has been chaired by J. Kaylor (1988–1989) and J. P. Angle (1989–1995). In 1990, the Society accepted an offer by the Smithsonian Institution Archives to serve as a permanent repository for the organization's archives.

*Awards and Recognition Committee.*—The Awards and Recognition Committee is responsible for developing and exercising standards and protocols so that the Society may recognize and award deserving individuals for exceptional achievement. Activities related to the Committee began as an honorary membership subcommittee of the Elections Committee. The Awards and Recognition Committee became a Sessional Committee (1991–1992) under the Williams presidency, and it became a Standing Committee in 1992. Following established protocol, the Committee reviews and makes recommendations about potential recipients of the SPNHC Award, President's Award, Faber Award, and Honorary Membership to the Executive Committee which is responsible for the final decision. Honorary Membership is extended to individuals who have significantly contributed to exemplary activities that are consistent with the goals of the Society. The SPNHC Award recognizes individuals for life-time accomplishments that serve the Society's goals. The President's Award recognizes members for exceptional service to the Society. The Faber Award is a competitive grant for projects that will help to advance the management and care of natural history collections.

Since the creation of the Awards and Recognition Committee, M.-L. Florian has received the SPNHC Award in 1993, and S. B. McLaren and P. S. Cato have received the President's Award in 1993 and 1995, respectively. This Standing Committee has been chaired by C. J. Bossert (1992–1994) and S. L. Williams (1994–present).

*Bylaws Committee.*—The Bylaws Committee is responsible for the continued development of the Society Bylaws and Standing Rules as needed, as well as the monitoring of Society activities to ensure agreement with the Bylaws. C. Romero-Sierra (1986–1988) chaired the committee that was responsible for the first accepted version of the Bylaws. Because the President works directly with the Bylaws over an extended period of time, the Committee has been chaired by the Past-President since 1988.

*Conference Committee.*—The Conference Committee is responsible for soliciting invitations from institutions to host the annual meeting of the Society. The Committee is chaired by the President-Elect because appropriate Society repre-

Table 3. Annual meetings of SPNHC during the first 10 years.

Year	Host and location (date)
1986	National Museum of Natural History, Washington, D.C. (21–22 May).
1987	Redpath Museum and McGill University, Montreal, Quebec (31 May–03 June).
1988	Carnegie Museum of Natural History, Pittsburgh, Pennsylvania (30 May–03 June).
1989	Tyrrell Museum of Palaeontology, Drumheller, Alberta, and University of Calgary, Calgary, Alberta (23–28 July).
1990	Field Museum of Natural History, Chicago, Illinois (07–11 May).
1991	Canadian Museum of Nature, Ottawa, Ontario (06–11 May).
1992	University of Nebraska State Museum, Lincoln, Nebraska (02–06 June).
1993	Royal British Columbia Museum, Victoria, British Columbia (07–12 June).
1994	Missouri Botanical Garden, St. Louis, Missouri (11–15 May).
1995	Royal Ontario Museum, Toronto, Ontario (02–06 June).

sentation may be required, and the President-Elect must be familiar with events that will occur when related responsibilities shift to those of the President (Table 1).

The Committee serves as a conference facilitator by making recommendations, as needed, to the Local Committee of the host institution. The host institution is responsible for the logistics and finances of the meeting, thus protecting the tax-exempt status of the Society. In recent years, the number of participants at any single meeting has been around 175 individuals.

Historically, the annual meetings of SPNHC have alternated between Canada and the United States (Table 3). It is anticipated that this pattern will change due to the challenge of finding new host institutions.

*Conservation Committee.*—The Conservation Committee is responsible for acquiring, developing, and distributing to the Society information that will promote the long-term preservation of natural history specimens and associated materials. Activities and membership interest in this Committee have made it one of the two largest committees in the Society. The Committee accommodates its large membership by dividing activities into several subcommittees which currently include Conservation Research, Cooperative Purchasing, Preservation Posters, and Wet Collection Assessment. Some of the previous subcommittees (for example, Documentation and Education subcommittees) subsequently evolved into standing committees of the Society.

The Conservation Committee has been directly or indirectly involved with producing standards and publications (Fitzgerald, 1988; Garrett, 1989; Rose and Torres, 1992; Rose *et al.*, 1995). The Committee has been chaired by C. L. Rose (1987–1992), C. L. Rose and C. G. Leckie (1992–1993), C. G. Leckie and C. A. Hawks (1993–1995), and B. P. Moore (1995–present).

*Documentation Committee.*—The Documentation Committee is responsible for promoting the permanence and quality of all documents associated with the management and care of natural history specimens. The Committee started as a subcommittee of the Conservation Committee (1989–1993), evolved into a Sessional Committee (1993–1994) under the Fitzgerald presidency, and became a Standing Committee in 1994. The Committee has been co-chaired by S. M. Woodward (1992–1993), J. Golden and S. B. McLaren (1993–1994), and S. Kraft and J. Zak (1994–present).

*Education and Training Committee.*—The Education and Training Committee

Table 4. Special training topics provided at annual meetings during the first 10 years.

Year	Topic
1988	Health Hazards Associated with Natural History Museums.
1989	Storage Design and Material for Natural History.
1990	Exhibiting Natural History Materials.
1991	Practical Approaches to Preventive Conservation.
1992	Pest Management.
1993	Archival Concerns of Natural History Museums.
1994	Risk Assessment.
1995	Managing the Modern Herbarium.

is responsible for developing and providing educational and training opportunities for the SPNHC membership. The Committee started as a subcommittee of the Conservation Committee (1988–1989), evolved into a Sessional Committee (1989–1991) under the Romero-Sierra presidency, and became a Standing Committee in 1991.

Since 1988, there have been eight highly successful workshops to enhance the professional knowledge and skills of participating individuals (Table 4). The quality of these workshops is due in part to obtaining outside funding. The Bay Foundation and the Canadian Conservation Institute have been important contributors in helping to make these opportunities worthwhile for diverse audiences. The Committee also has played a major role in developing written material for the SPNHC publications.

Initially, the Seasonal and Standing committees for Education and Training were chaired by L. Barkley (1989–1993). When the planning and organization for each workshop started exceeding one year, the structure was changed to co-chairs. The co-chairs of the Committee have been L. Barkley and E. E. Merritt (1993–1994) and S. C. Byers and E. E. Merritt (1994–present).

*Election Committee.*—The Election Committee is responsible for coordinating the nomination and election of Council members. In the past, there were two committees, Nominations and Ballot Counting, with F. J. Collier, W. Y. Watson, J. Danis, and P. A. Doepke serving in chair positions. In 1988, these became subcommittees under the current Election Committee; Honorary Membership was also a subcommittee. These subdivisions no longer exist, and Honorary Membership is now part of the Awards and Recognition Committee. The Standing Committee has been chaired by F. J. Collier (1988–1991) and J. C. Price (1991–present).

*Executive Committee.*—The Executive Committee, consisting of the SPNHC officers, is responsible for carrying out the day-to-day business of the Society. As a committee, the members may conduct meetings and develop proposals for Council. In some instances, it may be necessary for the Committee to act as a group to address specific needs of the Society. The Committee is chaired by the current President (Table 1).

*Finance Committee.*—The Finance Committee is responsible for monitoring the Society's financial accounts, handling reserve funds, developing fiscal policies, and attending to other financial matters required by Council. SPNHC began with very limited resources, as is common to many new organizations, but it was not

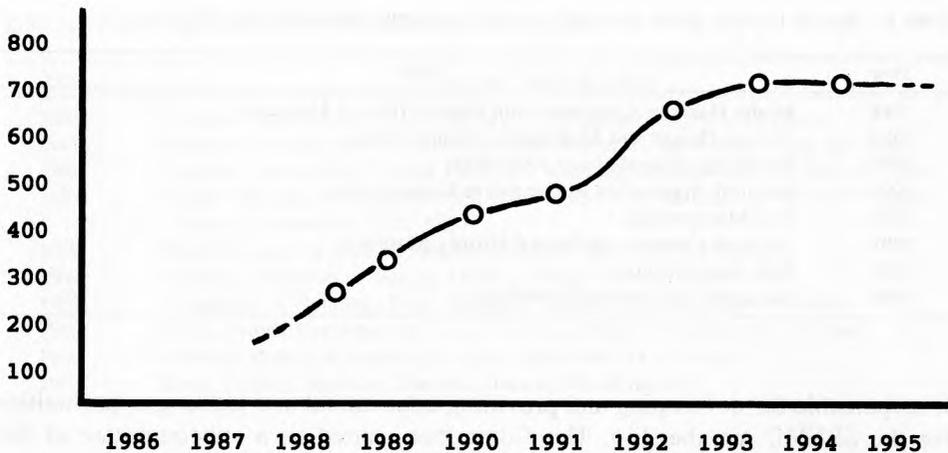


Figure 1. Total number of members of the Society for the Preservation of Natural History Collections from 1986 to 1995.

long before the financial structure stabilized and began to grow. A solid financial base has allowed the Society to undertake special committee projects and to take advantage of new opportunities, such as federal grants. The Committee continues to carefully monitor and develop the financial resources of the Society to anticipate and accommodate future activities.

The SPNHC Treasurer has always served on, if not chaired, the Finance Committee. The Committee has been chaired by J. P. Cuerrier (1987–1988), S. B. McLaren (1988–1989), H. H. Genoways (1989–1990), S. B. McLaren (1990–1993), J. Golden and S. B. McLaren (1993–1995), and S. B. McLaren (1995–present).

*Membership Committee.*—The Membership Committee promotes membership growth and encourages retention of existing members. Because some of its activities overlapped with those of the Publicity and Liaison Committee, the two committees were merged under Membership in 1992. As a result, the Committee also is responsible for publicizing Society activities and functions, and for obtaining pertinent information about related organizations and publications. The Membership Committee has been chaired by S. S. Albright (1987–1988), S. L. Williams (1988–1989), D. S. Chaney (1989–1990), E. E. Merritt (1990–1992), and A. Pinzl (1992–present).

Growth of SPNHC has been rapid, and in recent years membership has stabilized at around 700 members (Fig. 1). Although dominated by members from Canada and the United States, at least 23 other countries are represented in the current membership (J. Golden, personal communication).

*Publications Committee.*—The Publications Committee is responsible for developing and maintaining publication policies to provide regular publications that reflect the Society's purpose. SPNHC currently publishes a spring and fall issue of *Collection Forum* and a winter and summer issue of the *SPNHC Newsletter*. A membership directory is published about every other year. The Committee also assists other committees with the production of special publications, such as *Storage of Natural History Collections: Ideas and Practical Solutions* (Rose and Torres, 1992) and *Storage*

of *Natural History Collections: A Preventive Conservation Approach* (Rose et al., 1995). The Committee is currently developing another publication series, *SPNHC Leaflets*, for disseminating technical and procedural information.

The journal of the Society, *Collection Forum*, was first published in October 1985, about a month before the organization was actually formed. In 1986, separate spring and fall issues were published; these were followed by a joint spring and fall issue in 1987. Early issues of *Collection Forum* attempted to maintain a balance between contributions of a societal nature with those representing original work. An assessment of professional resources for natural history collections made it apparent that there was a niche that SPNHC could fill with a professional journal specializing in information on the management and care of natural history collections (Cato, 1988).

In the summer of 1987, the first issue of the *SPNHC Newsletter* was published. Its purpose was to provide an outlet for much of the societal information previously published in the journal. In the fall of 1988, the format and operations of *Collection Forum* were restructured to make it an outlet for peer-reviewed contributions that had direct relevance to the profession. Allen Press of Lawrence, Kansas, was selected as the publisher of *Collection Forum* because of their reputation for producing professional quality products for a reasonable cost.

The Managing Editor serves as an appointed officer of the Society and as the chair of the Publications Committee (Table 1). The SPNHC Newsletter Editors have been P. S. Cato (1987–1988), J. Golden (1988–1993), and P. M. Sumpter (1993–present). Currently, the Committee is co-chaired by the Managing Editor and Newsletter Editor to facilitate information exchange and newsletter coverage of Council meetings.

*Resources Committee.*—The Resources Committee is responsible for developing and maintaining pertinent information about resources that would be useful to the general membership of the Society. This Committee is one of the two largest committees of the Society. Current subcommittees include Bibliography, Supplies and Equipment, and Cooperative Purchasing. The Committee has been chaired by C. A. Hawks (1988–1992) and I. Hardy (1992–present).

The Committee has been successful in providing resource information to members and non-members. Examples of the Committee's contribution to the Society include *A Preliminary List of Conservation Resources for the Care of Natural History Collections* (Hawks and Rose, 1987), Resource Center (Madrid meeting), and the *Supplies and Materials for Museum Collections* traveling exhibit (developed by C. Kishinami).

#### SESSIONAL COMMITTEES

As previously stated, some Standing Committees originated as Sessional Committees (Awards and Recognition, Documentation, and Education and Training). However, some Sessional Committees were formed to address specific short-term needs of SPNHC. Examples of Sessional Committees and their respective activities are as follows:

- The Committee for Evaluating Collection Support Positions (P. S. Cato, Chair; 1989–1991) resulted in an important demographic profile of collection-related professions (Cato, 1991).

- The Committee for the SPNHC Logo (C. Romero-Sierra, Chair; 1989–1990) was instrumental in developing the distinctive logo that represents the Society.
- The Committee assisting with the development of the 1992 Madrid meeting (C. Romero-Sierra, Chair; 1988–1991) was critical in establishing the SPNHC role at the International Symposium and First World Congress on the Preservation and Conservation of Natural History Collections. This Committee subsequently evolved into the Sessional Committee for Liaison with the World Council for Collection Resources (C. L. Rose, Chair; 1994–present) (see below for further details).
- The Committee for Common Philosophies and Objectives (P. S. Cato, Chair; 1993–1994) published the Society's *Guidelines for the Care of Natural History Collections* (SPNHC, 1994).
- The Committee for Long-Range (Strategic) Planning (G. W. Hughes, Chair; 1994–present) is currently addressing the goals and direction of the Society for the future.

#### COOPERATIVE PROJECTS

The 1992 International Symposium and First World Congress on the Preservation and Conservation of Natural History Collections, commonly referred to as the "Madrid meeting", was the most ambitious endeavor of the Society since its formation. The idea for the Madrid meeting was presented in 1987 by J. Gisbert at the SPNHC Annual Meeting in Montreal. In 1988, the Council made a formal decision for SPNHC to participate in an opportunity that was being coordinated by the Spanish government to celebrate the 500th anniversary of Columbus' voyage to the Americas. During the next four years, individuals and committees of SPNHC worked diligently with other natural history organizations to develop a program that would appeal to all members of the international and multidisciplinary natural history community. The Madrid meeting was exceptionally successful with 626 individuals from 75 countries expressing a unified concern for the future of natural history collections in the form of written resolutions (Palacios *et al.*, 1993a:33–37). These resolutions, which were subsequently endorsed by the SPNHC membership, were very important for providing direction for future activities and forming the World Council for Collection Resources (WCCR). However, the real success of the Madrid meeting for SPNHC was the recognition the Society received from the international natural history community as a solid and effective organization that actively addresses the interests and needs of all natural history collections (Anon., 1992). The sharp increase (28%) in membership in 1992 was a clear indication of the new recognition and support that SPNHC had received (Fig. 1).

Following the Madrid meeting, SPNHC involvement continued with participation in conference publications (Palacios *et al.*, 1993a, 1993b; Rose *et al.*, 1993) and with further development of the WCCR. On December 15–16, 1992, a meeting was held in Washington, D.C., to set up the operating structure of the WCCR. Participants of this meeting included G. M. Davis, A. R. Emery, G. R. Fitzgerald, R. Garcia-Perea, J. Gisbert, F. Palacios, C. Romero-Sierra (Chair), C. L. Rose, and S. L. Williams. In 1994, SPNHC and the Association of Systematics Collections (ASC) conducted a joint annual meeting in St. Louis, Missouri, where a

group met to continue the initiatives established at the Madrid meeting. Since then, the SPNHC leadership has been involved with the planning of the WCCR and Second World Congress on the Preservation and Conservation of Natural History Collections to be held at the University of Cambridge (UK) in 1996, under the direction of C. Collins.

The SPNHC Council has considered it to be important to serve its membership through representation in national "umbrella" organizations. For this reason, the Society has worked closely with the National Institute for the Conservation of Cultural Property (NIC) since 1988. The Society's concerns for natural history collections continue to be addressed by NIC. A product of this joint effort is the recent NIC/NSF-funded project assessing conservation needs of natural history collections (Duckworth *et al.*, 1993).

SPNHC has worked closely with the Association of Systematics Collections (ASC). The Society participated in the ASC meeting at the University of Nebraska State Museum in 1989, and both organizations met jointly at the Missouri Botanical Gardens in 1994. The two organizations have worked together on projects such as the NIC/NSF project, the Madrid meeting, and development of the WCCR. One recent cooperative effort resulted in the ASC publication, *Guidelines for Institutional Policies & Planning in Natural History Collections* (Hoagland, 1994). Currently, both organizations are encouraging email communications by co-sponsoring NHCOLL-L (Natural History Collections Listserve) which is hosted by the University of California at Berkeley. P. M. Sumpter was instrumental in obtaining this service for the Society.

The Society also is an invited representative on the External Advisory Council of the American Institute for the Conservation of Artistic and Historical Works (AIC). During annual Advisory Council meetings, methods of increasing communication and addressing natural history conservation concerns are discussed and initiated.

#### THE SUPPORTERS OF SPNHC

The success and accomplishments that the Society has attained thus far would not have been possible without strong supporters of the organization. Fundamental to the existence of the Society is a strong membership. Society operations are dependent on a growing number of dedicated individuals (members and non-members) who contribute to the Society through services to committees, publications, annual meetings, and other activities; participating members benefit in turn with professional development and networking. Many of the Society's opportunities have been a direct result of assistance from institutions, agencies, foundations, and corporations.

Institutional support usually has occurred in three forms; the most obvious is hosting of annual meetings. Less obvious is the support institutions provide individual members to attend annual meetings and to participate in leadership and service capacities within the organization. Finally, institutions have occasionally covered some of the operational costs of the Society, particularly during its early years. Institutional support provided by the Canadian Conservation Institute (CCI) has been particularly important to the Society; CCI has provided conservation training for several of the SPNHC education and training programs.

Funding from agencies and foundations has been critical to the accomplish-

ments of the Society. In particular, the Institute for Museum Services (IMS) and the Bay Foundation have repeatedly awarded grants to SPNHC for its activities. For instance, IMS funding has supported the Society's efforts to produce books that serve the entire museum community (Rose and Torres, 1992; Rose *et al.*, 1995). The Bay Foundation has supported the Society's education and training projects, such as annual meeting workshops and the Resource Center at the Madrid meeting. The Museum Assistance Program of the Department of Canadian Heritage also has supported SPNHC initiatives.

Support from corporations also has been important to the success of SPNHC, particularly with annual meetings and special projects. A growing number of diverse corporations have discovered mutually beneficial interactions with SPNHC and its members. For instance, the annual meetings provide a mechanism for educating potential customers, developing new ideas, and becoming aware of changing trends and opportunities.

It would be possible to cite other examples of support that the Society has received. However, it is the intention of this contribution to simply acknowledge how important this support has been, particularly during the first ten years of the Society's history, and to express, on behalf of SPNHC, sincere appreciation for all the support received from individuals, agencies, foundations, corporations, and other contributors.

#### CONCLUSION

This contribution documents important activities and accomplishments of SPNHC during its first ten years of existence. It is hoped that this information will help those who participated in these events to reflect on what has been achieved, *and* that this information will help new members understand how these events have been important to the history and future of the Society. This is, without a doubt, a success story of what individuals with a common cause can achieve. It is hoped that the achievements and professional development of the first ten years will be perpetuated in the future.

#### ACKNOWLEDGMENTS

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Sarasan, L. 1987. What to look for in an automated collections management system. *Museum Studies Journal*, 3:82–93.

Thomson, G. 1986. *The Museum Environment*, 2nd ed. Butterworths, London, 293 pp.

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# CONTENTS

## Articles

- Observations on formalin-induced darkening of herpetological specimens  
..... *James N. Stuart* 39
- Effect of osteological processing treatments on dimensions and moisture  
absorption potential of rodent skulls .....  
..... *Stephen L. Williams and Heather C. Smith* 46
- Fixation and preservation of museum marine collections using formal-  
dehyde/glutaraldehyde mixes ..... *A.B. Stephenson and J.L. Riley* 58
- SPNHC: The first ten years ..... *Stephen L. Williams* 69