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RE-CURATION OF ALCOHOL-PRESERVED SPECIMENS: COMPARISON OF GRADUAL VERSUS DIRECT SPECIMEN TRANSFER ON SPECIMEN CONDITION AND ASSESSMENT OF SPECIMEN VALUE

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Abstract.—Two different re-curation techniques were tested for ethanol (EtOH)-preserved spiders to determine if transferring specimens from older, potentially lower concentration EtOH solutions directly into vials with higher EtOH solutions damages specimens. One technique involved transferring specimens from a low 45% EtOH solution into a graded series of solutions with increasing ethanol concentrations. The second technique involved the direct transfer of specimens from a low 45% EtOH solution into a higher 75% EtOH solution. We assessed specimen condition using a variety of visual parameters. We found no impact on specimen condition regardless of which technique was used and concluded that spider specimens in degraded ethanol can be safely re-curated directly into new solution with no fear of specimen damage.

We also developed a method for assessing the research value of specimens in a donation. This technique involved scoring different value criteria pertinent for a particular collection. Specimens can thus be assigned a value assessment score or rank. Such a value assessment technique provides a means by which re-curation of specimens can be prioritized. It also provides a means by which museums with few resources can quickly determine whether a donation should be accepted.

INTRODUCTION

The Denver Museum of Nature and Science (DMNS) established an ethanol (EtOH) preserved collection of arachnids in 1999. With the concurrent initiation of an active research program in arachnology, the collection grew exponentially in six yr from under 50 vials to over 24,000 vials. The collection currently contains 43 type specimens and is being used as a repository for voucher specimens.

In 2003, in order to consolidate the Colorado arachnids into one State collection, the curator of the Colorado State University (CSU) arthropod collection, Dr. Boris Kondratieff, transferred the bulk of the CSU arachnid collection to the DMNS. The CSU collection consisted of specimens collected mostly along the front range of the Rocky Mountains from the 1940s–1990s, most coming from the 1960s. The 2,965 vials transferred to DMNS from CSU were housed in neoprene stoppered vials with low levels of highly degraded ethanol of uncertain concentration. Kondratieff indicated (pers. comm.) that the alcohol collection had been checked "periodically" and that he and his assistants had tried to "top off" the ethanol, if necessary, once a year. The ethanol had not been completely changed in the vials since 1986. Because of the apparent deterioration of the liquid preservative combined with the age of the specimens it was decided that some testing should be done to ensure that the specimens were not damaged during re-curation.

Re-curation of fluid-preserved museum specimens often occurs in one step: either the low levels of preservative are brought up to an acceptable level by adding additional preservative or the specimen is removed from the poor preservative and placed directly into new preservative. However, the addition of more alcohol, or "topping off," does not necessarily bring the alcohol concentration up to an acceptable level of 75%–80% required for adequate specimen preservation (Cato 1990, Pickering 1997, Waller and

Simmons 2003). In addition, transferring specimens from low concentration alcohol to higher alcohol concentrations can potentially damage specimens (Moore 1989, Simmons 1995, Pickering 1997).

The purpose of this experiment was to determine if transferring older ethanol preserved specimens directly from low concentrations into new vials with 75% (acceptable) EtOH concentration will damage specimens. We compared two different re-curation techniques: 1) gradual transfer from low ethanol concentrations through a series of higher concentration solutions and 2) direct transfer from low ethanol concentration solutions to 75% EtOH concentration solutions. The results will allow us to assess how effective incremental transfer versus direct transfer of older specimens into new preservative is in alleviating specimen damage. It will also provide useful guidelines and parameters for the proper re-curation of fluid-preserved arthropod specimens that may be of use to the museum conservation community.

In addition to evaluating these two re-curation techniques on specimen preservation, we also developed a method for assessing specimen value. This method is specific to spider specimens, but a similar technique could be developed for any museum specimen or collection. Such a value assessment may be useful in prioritizing specimens needing re-curation or needing preventive measures to slow deterioration. An overall value assessment could also be used to determine whether a donation is worth accepting into a museum with limited resources for collections management.

Methods

Comparison of Re-Curation Techniques

Three sets of 50 experimental spiders were assembled. These spiders were large juveniles from the DMNS collection that were preserved initially in 75% EtOH, an ethanol concentration within the range considered ideal for preservation of small invertebrates (Levi 1966). Spiders are killed, fixed, and preserved in 70–75% EtOH. No special fixative (e.g., formalin) is used in spider curation. Although only adult spiders can be identified to species because genitalic characters are required for accurate species assessment, juveniles can easily be identified to family using such morphological characters as eye arrangement, leg positioning and length, and cephalothorax and abdomen characteristics. The spiders used represented 12 different families and were divided into three sets of one to six specimens per family in each set. All three sets had equal representation of the 12 families. One set represented the control; the second set was used for the gradual transfer treatment; the third was used for the direct transfer treatment. Each vial was numbered from 1–50 and with the letter designation C (control), G (gradual), or D (direct).

Waller and Strang (1996) found that the anti-septic, preservative effects of alcohol were maximized from 50%–80%, so our low end concentration was set just outside of this range at 45%. The control spiders were kept in 75% EtOH solution throughout the experiment. The gradual and direct treatment spiders were hydrated in a solution of 45% EtOH for 7 days. By stabilizing the gradual and direct experimental spiders at this low alcohol concentration, we maximized chances of observing effects on specimen condition when transferring them from lower to higher concentrations of ethanol.

The specimen condition of the two treatment groups was assessed after the 7 day acclimatization period. The gradual treatment specimens were then transferred from 45% EtOH to 55% EtOH and left in that solution for 2.5 hr; then the specimens were

transferred to 65% EtOH for 2.5 hrs; then to 75% EtOH. The direct treatment specimens were transferred directly from 45% EtOH into 75% EtOH. The control specimens were assessed at the beginning of the experiment and at the end of the experiment (15 days apart). Rehydration protocols, in which specimens are transferred through serial dilutions of gradually higher ethanol concentrations, typically require specimens to remain in each concentration for an hour or less (Barth 2000, Wong et al. 2004–2005, Crowley 2005, Mori et al. 2006). Leaving our specimens in each dilution for 2.5 hr was, therefore, considered more than sufficient time to bring the specimens into equilibrium with the solution.

We assessed specimen condition based on eight criteria that reflected how well preserved the specimens were. These conditions are specific to this taxonomic group as recommended by SPNHC-CC (1990, p. 440) and include:

- Leg Curl: This represents the degree of leg curling past the carapace. The more dehydrated the specimen, the more fluid is pulled out of the legs and the more the leg will curl.
- Leg Joint Swelling: The swelling of the joint membrane between the sections of the legs. This is a sign of over-hydration of the leg and will often cause the leg to become straightened.
- Leg Brittle: The brittleness of the femur when pinched with forceps. If the leg is dehydrated, it will become brittle and break.
- Leg Pull: If the specimen is picked up and shaken by the third leg, does the leg pull away from the specimen? If the spider is over-hydrated the tissue will become soft and the leg will easily tear away from the body. Spiders are often removed from vials by their legs so this also measures the ability of the researcher to remove a specimen from a vial without damage.
- Abdomen Pull-Away: Has the internal abdominal tissue pulled away from the exoskeleton? This also indicates dehydration.
- Spinneret Swelling: Is the membrane around the spinnerets swollen? This indicates over-hydration of the specimen.
- Abdomen Pattern Degradation: Has the abdominal pattern become faded? A degradation of the color patterns may indicate over-hydration or a degradation of the pigment due to overall specimen deterioration.
- Leg Flex: The time it takes for a leg that is pulled straight to rebound. If the leg rebounds, this time is recorded in seconds. A one second rebound indicates that the leg is dehydrated and may break off. A non-rebounding leg indicates that the leg is over-hydrated and may be torn off.

The data for Leg Curl, Leg Joint Swelling, Leg Brittle, Leg Pull, Abdomen Pull-Away, Spinneret Swelling and Abdomen Pattern Degradation were recorded as no change (0) or degradation (1) for each of the specimens between initial assessment and final assessment. These data were analyzed with a *G*-test comparing all three treatments and with Chi-Square tests comparing the Control with each of the experimental treatments individually (Sokal and Rohlf 1981).

The Leg Flex data were a measure of the amount of time it took a leg to rebound when pulled. We first determined how long it took a leg to rebound in the initial conditions (75% EtOH for C or 45% EtOH for G and Q) and then determined whether rebound time increased or decreased with the specimen in the final 75% solution.

Table 1. Families and numbers of vials used in value assessment.

Family	1940s	1950s	1960s	1970s	1980s	1990s
Araneidae	0	1	6	6	6	0
Dictynidae	0	0	6	6	6	0
Gnaphosidae	0	2	6	6	2	0
Lycosidae	2	5	6	6	6	0
Oxyopidae	0	0	6	6	5	0
Salticidae	0	4	6	5	6	0
Tetragnathidae	0	0	6	6	5	0
Theridiidae	0	2	6	6	6	2
Thomisidae	0	0	6	6	6	0

Measure of Alcohol Concentration

Because the alcohol concentrations in the CSU vials were unknown, an attempt to determine the percentage alcohol concentration was made. Commercial hydrometers were prohibitively expensive so we used a Beaume bulb hydrometer instead and calibrated the markings on the hydrometer for alcohol concentrations using known concentrations of EtOH. This hydrometer could not be used to measure the ethanol concentration for individual vials since the volume per vial was too low. Thus, we combined vials with visually similar liquid levels and preservative conditions (e.g., the color of the preservative and the level of preservative was approximately the same for combined vials).

We determined average alcohol concentration for four different families: Tetragnathidae, Agelenidae, Dictynidae, and Araneidae. These four families were chosen due to their very different morphology and, thus, potentially different levels of alcohol absorption into the tissues. For example, tetragnathids tend to have long thin bodies so alcohol may absorb into the tissues fairly rapidly; agelenids have more robust bodies and their abdomens tend to be subjected to higher degrees of damage and degradation, suggesting that the cuticle covering the abdomens may be thinner or less sclerotized; dictynids tend to be smaller than average, thus exchange between fluid preservative and body tissues may be rapid; and araneids tend to have short robust bodies with relatively large abdomens.

Assessment of Specimen Value

The CSU specimens were surveyed using a non-invasive method to get an idea of the intrinsic value of the specimens for a research collection. A mathematical equation was developed to allow for a rapid determination of the value of each specimen. For the assessment, nine families were chosen. Within each family, vials were separated by decade and then up to six vials per decade were chosen when available (Table 1). The donation had a mixture of family-sorted, species-sorted, and unsorted vials. In sum 173 vials were surveyed out of a total 2,965 vials, constituting 5.8% of the collection.

To assess the value of the specimens in these vials, we used morphological features diagnostic for specimen identification as long as those characteristics could be seen without opening the vials or handling the specimens. We also took into account the kind and completeness of data associated with each vial (data written on labels) and recorded criteria that reflected the degree of preservation of specimens. We used a total of eight value assessment criteria:

- Date: The older the specimen, the more likely the habitat in which it was collected has been altered. Thus, older specimens have a higher potential value. The year the specimen was collected is subtracted from the year the assessment is carried out.
- Data: A good data label for biological specimens has, minimally, date collected, collection locale and collector's name. If all three pieces of information were on the data label, that label received a score of 1. A score of 0.25 was subtracted from one for each missing piece of information. Even if a specimen lacked all three pieces of information, it potentially could be used as a teaching specimen. Thus, its value was not zero. However, all vials used for this assessment had at least the collection year affiliated with the label.
- Maturity: Only adult spiders can be accurately identified to species. Thus adult specimens received a score of 1 and juveniles received a score of 0 for this criterion.
- Genitalia Present: It is usually necessary to examine a spider's genitalia in order to identify it. Typically, the genitalia of adult specimens remain with the bodies; however, occasionally dissection of the genitalia is required for identification and the genitalia are separated from the remaining parts of the specimen. If these genitalia are subsequently lost, it is nearly impossible to identify the specimen. Therefore, a score of 1 was assigned to specimens with intact genitalia or with genitalia clearly present in the vial and a score of 0 was assigned specimens with missing genitalia.
- Abdomen Pull-Away: When a specimen has become dehydrated, the internal structures of the abdomen can pull away from the abdominal exoskeleton. In some families, e.g., Araneidae, abdominal patterns are important in identifying specimens and it is difficult to see these patterns in degraded specimens. However because other diagnostic features may still be observable, such as genitalia, a specimen with the abdomen pulled away from the exoskeleton did not receive a zero for this criterion. Instead, 1 was assigned to specimens with no abdomen degradation and 0.75 if there was some evidence of tissue separation from the exoskeleton.
- Abdomen Attached: Because of the difficulty in removing a separated abdomen from a vial without damaging it and because female genitalia are located on the abdomen, damage to the abdomen may also damage the genitalia or obscure other characteristics. A score of 1 was assigned specimens with abdomens still attached to the cephalothorax; a score of 0.5 was given if the abdomen was not attached.
- Body Damage: Any physical damage, besides those accounted for in the other criteria. Such damage, such as an abdomen that is crushed or a broken cephalothorax, may hinder identification. However, it is often possible to remove, manipulate and identify a damaged specimen. Therefore, an intact, undamaged specimen received an assessment score of 1.0 and a damaged specimen received a value assessment of 0.75.
- Number of Detached Legs: The loose legs in each vial were counted and recorded. In some families (e.g., Salticidae), leg characteristics are important. Presence of loose legs in the vials may reflect specimen degradation and/or past mis-handling of the specimen. However, even if all the specimen's legs were loose in the vial, the specimen, as long as it was an adult and had intact genitalia, was of value. Thus, we used the following calculation for value assessment for this characteristic: $1/[(\# \text{ loose legs} \times 0.3) + 1]$. Using this formula, a specimen with all legs loose in the vial still received a value score of approximately 0.3.

Table 2. Numbers of specimens (out of 50) showing changes in condition parameters among treatments ($n = 50$ for all three treatment groups).

Condition parameter	Control	Gradual	Direct	G Value	P Value
Leg Curl	0	2	1	2.813	$0.1 < P < 0.5$
Leg Joint Swelling	1	0	3	4.386	$0.1 < P < 0.5$
Abdomen Pull-Away	0	2	4	5.712	$0.05 < P < 0.1$
Spinneret Swelling	0	1	2	2.813	$0.1 < P < 0.5$
Abdomen Degradation	0	2	3	4.352	$0.1 < P < 0.5$
Leg Brittle	0	0	0	0	1
Leg Pull Off	0	0	0	0	1

RESULTS

Comparison of Re-Curation Techniques

The number of spiders from each treatment showing degradation for each variable is shown in Table 2. For example, no Control spiders showed a curling of the legs between the initial assessment and the final assessment. Two Gradual treatment spiders showed an increase in Leg Curl, and one Direct treatment spider showed an increase in Leg Curl. No spiders in any of the treatments showed a change in Leg Brittle or in the Leg Pull Off condition parameters. None of the treatments negatively affected the condition of the specimens using these seven condition parameters (G-values between 2.8–5.7, P-values > 0.05; Table 2). Chi-square tests comparing the Control spiders with either the Gradual or the Direct treatment specimens also showed no significant differences ($P > 0.05$).

We decided that Leg Flex was not an accurate or meaningful measure of specimen condition because 28 of the 50 Control spiders showed a change in leg rebound time between the initial assessment and the final assessment (Table 3). Therefore, these data were not analyzed and this condition parameter will not be considered further.

Measure of Alcohol Concentration

In order to measure the average alcohol concentration for each of the four families, we combined the alcohol from 19 vials of Tetragnathidae to get an alcohol concentration measurement, combined 44 vials of Agelenidae, 33 vials of Dictynidae, and 71 vials of Araneidae. The alcohol concentrations were: 73% for the Tetragnathidae; 73% for the Agelenidae; 70% for the Dictynidae and 70% for the Araneidae with mean = 71.5 ± 1.7% ($n = 4$).

Table 3. Change in rebound time for the Leg Flex condition parameter for spiders in the three treatments ($n = 50$ for all treatment groups).

Rebound time	Control	Gradual	Direct
More time	13	12	23
No change	22	21	16
Less time	15	17	11

Assessment of Specimen Value

The equation we derived for assessing specimen value was:

Value = (Date[yr assessment carried out − yr collected]) × (Data)

$\times$ (Maturity) × (Genitalia Present) × (Abdomen Pull Away)

$\times$ (AbdomenAttached) × (Body Damage) × (1/[(# of loose legs × 0.3) + 1])

$\times$ conversion factor

The conversion factor was calculated in order that the most valuable specimen in the donation would receive a score of 100. Thus, all other specimens were compared against the most valuable specimen in the donation. For our calculations and for this donation, the conversion factor was 1.5625. For any such value assessment and any new donation, the conversion factor would have to be calculated. Such a per-donation calculation of a conversion factor is useful if it is known a priori that at least some specimens from the donation will be of value for a collection. Alternately, a calculation could be done without a conversion factor and the top-ranked specimens evaluated for inclusion in a collection.

For example, an adult, intact 1940 specimen whose data label contained all three minimal pieces of information with evidence of abdomen pulled away from the exoskeleton and two loose legs in the vial but with the genitalia intact would receive a score of:

$$(2004 - 1940) \times 1 \times 1 \times 1 \times 0.75 \times 1 \times 1 \times 0.625 \times 1.5625 = 46.87$$

A summary of our value assessments for the 173 specimens surveyed is presented in Table 4. Specimens with scores of 0 (13.9% of the specimens) in this assessment were all juveniles. Juvenile spiders cannot be identified to species and, therefore, are of little value for research collections unless they are definitively associated with adults (e.g., both adults and juveniles are in the same vial and were collected together). Furthermore, 21.3% of the specimens had scores between 10.0–30.0. These were generally more recently collected specimens with relatively extensive damage. Almost half of the specimens (47.9%) had scores between 30.1–60.0. These specimens were in relatively good condition. Specimens with scores greater than 60 (16.7% of the specimens) were in excellent condition.

DISCUSSION

Transferring specimens from low EtOH concentrations directly into higher ethanol concentration solutions did not detrimentally affect them. Therefore, the more time-consuming method of transferring specimens into graded series of increasing alcohol concentrations as was done by Pickering (1997) and recommended by Moore (1989) is not necessary for this group of arthropods. Since spiders have thinner cuticles than other groups of arthropods, it is unlikely that any other group of arthropods normally preserved in alcohol would be detrimentally affected by direct transfer from degraded alcohol into new, higher concentration solutions. However, preservatives also function, to some extent, as solvents (Simmons 1995). Components of specimens, such as lipids, are extracted by the preservative. Although, over time, specimens reach an equilibrium with the fluid preservative, if specimens are transferred into new solutions of differing

Table 4. Summary of value assessment for 173 specimens from CSU donation. Damage in last column includes assessment categories: Abdomen Pull-Away, Body Damage, and/or # Detached Legs (see text for explanation of these categories).

Value score	# Specimens	% Specimens	Year collected	Comments
0	24	13.9	1960s–1980s	Juveniles
10.0–20.0	11	6.3	1970s–1990s	Extensive damage
20.1–30.0	26	15.0	Most 1980s	Some damage
30.1–40.0	31	17.9	Most 1970s–1980s	Overall good condition
40.1–50.0	43	24.8	Most 1960s–1970s	Overall good condition
50.1–60.0	9	5.2	Most 1950s–1960s	Overall good condition
60.1–70.0	22	12.7	1960s	Excellent condition
70.1–80.0	4	2.3	1950s	Excellent condition
80.1–90.0	1	0.6	1950	Excellent condition
90.1–100	2	1.1	1940s	Excellent condition

concentrations, a new equilibrium must be reached and further extraction of lipids and other body constituents could occur (Simmons 1995). Our study focused on overall changes in specimen condition rather than changes in body constituent extraction.

Pickering (1997) found that jars containing invertebrate specimens in the collections at the Oxford University Museum of Natural History had an average alcohol concentration of 63.1%. However, 0.75 of the specimens were in higher concentrations between 60–75%. Thus, even older specimens that have not been curated for a long period of time still seem, on average, to maintain a relatively high alcohol concentration. This was also found in the present study.

In arachnid research collections, any adult spider with data and with intact genitalia can be identified and is of value. Therefore, any specimens with value assessment scores greater than 0 should be kept and re-curated. However, the value assessment methodology we present provides a means by which re-curation can be prioritized. It also provides a means by which museums with few resources can quickly determine whether a donation should be accepted. The formula we developed is specific to spider specimens. Nevertheless, a similar, simple formula could easily be developed for any museum collection based upon the criteria deemed important for those particular specimens or objects.

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ANOXIC TREATMENT OF INSECT COLLECTIONS AND IMPLICATIONS OF DRAWER DESIGN

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Abstract.—The practical effectiveness of anoxic treatment in entomological collections was tested in three models of wooden insect drawers. We conducted four experiments to examine mortality rates of five species of dermestid beetles while varying oxygen levels, number of steps to reduce oxygen levels, and exposure time. A VELOXY® nitrogen generator and the oxygen scavenger Ageless® Z200 were used for oxygen reduction. We found that treatment with a nitrogen generator in combination with an oxygen scavenger is a useful, though somewhat laborious, method for pest eradication. Its great advantage is its lack of toxicity to people and collections. Reducing the oxygen level in three steps is more effective than two. Double-grooved drawers require a longer exposure time than single-grooved drawers due to their tight construction but because of this they are more effective in preventing larvae from entering. Four sachets of Ageless® Z200 seemed to be optimal and resulted in 100% mortality of larvae of *Attagenus woodroffei*, the most resistant species, after 14 days of anoxic treatment. *Trogoderma angustum* and *Reesa vespulae* needed 10 days in the tightest type of drawer. For *Anthrenus verbasci* and *Attagenus smirnovi*, the least resistant species, a 7-day anoxic treatment was sufficient.

INTRODUCTION

Natural history collections are susceptible to attack by pest insects. Dead plants and animals, components of natural history collections, constitute the natural food for pest species like dermestid beetles and moths. For small items such as pinned insects, a pest infestation could have a devastating effect on the collection in a very short time and rigorous pest control is therefore essential.

Pesticides have been used and are still in use for insect control but many of them, like paradichlorobenzene, DDT and dichlorvos, are now banned in many countries (Pinniger 2001). Freezing is a common alternative method for pest control yet it has disadvantages. Some materials, such as composite materials, may be vulnerable to freezing. In entomological collections pinned insects usually are kept in special wooden storage drawers with glass lids. The freezing treatment may cause the glass to break, old pins to corrode, and items glued to paper to come loose. Therefore another method developed for museum use in the last two decades, anoxic treatment (replacement of oxygen with an inert gas like nitrogen), is of increasing value for pest control in natural history collections. Anoxic treatment methods have been described by Daniel et al. (1993), Gilberg (1989, 1990), Gilberg and Roach (1992), Grattan and Gilberg (1994), Hanlon et al. (1993), Koestler and Mathews (1994), and Reichmuth et al. (1993). A variety of pest species have been tested for estimating time limits for mortality (Bergh and Hallström 2000, Bergh et al. 2003, Kigawa et al. 2001, Rust et al. 1996, Valentin 1993, Valentin et al. 2002). The methods have been reviewed by Åkerlund et al. (1998), Linnie (2000), Pinniger (2001), and Maekawa (1999) and are already in use in several museums. These papers refer to studies done under strict laboratory conditions as well as to case studies of museum objects, but there are no data available for the type of treatment described here.

Insect drawers are often constructed with tight seals to prevent pest insects from entering. This could be an obstacle, however, to effective anoxic treatment, especially where widespread problems exist, as opening each drawer will be time-consuming and

also involve an enhanced handling risk for the collections. The aim of the present study was to find out whether the oxygen concentration inside the closed drawer could be decreased to a level low enough to kill pest insects and, if so, whether it could be done in a reasonable time.

MATERIALS AND METHODS

The VELOXY® System

VELOXY®, developed within the European project SAVE ART, is a nitrogen generator system (compressor, nitrogen generator, humidifier and plastic barrier film) used for anoxic treatment of museum objects to control insect pests. It produces an almost oxygen-free (<0.1% O_2) flow of 200 l/h that is used to modify the atmospheric composition inside enclosures, individually made from the gas-proof plastic barrier film. The nitrogen generator is based on the use of tiny semipermeable hollow fibres that are connected to a manifold to which pressurised air is applied. Oxygen permeates through the walls of the fibres leaving a stream of nitrogen and the other minor constituents of air. The permeation system is 37 cm wide, 40 cm long and 94 cm high and is equipped with wheels for easy transportation. The VELOXY® method uses a compressor and in the experiments described here a 1.1 kW single-phase unit was used.

Pest Species

Larvae of five species of dermestid beetles were used in this study, all of which can cause damage to objects of plant and animal origin including entomological collections:

- *Anthrenus verbasci* (L.), the varied carpet beetle, is cosmopolitan in distribution (Åkerlund 1991, Griswold 1941, Mroczkowski 1968).
- *Attagenus woodroffei* (Halsted and Green), Woodroff's fur beetle, originates from tropical countries and is one of the most common indoor pest insects in Scandinavian countries (Åkerlund 1991, Mathlein 1971, Palm 1987).
- *Attagenus smirnovi* (Zhantiev), the brown fur beetle or popularly named the vodka beetle, probably originates from Kenya. It is a common pest in Eastern Europe and is spreading in many European countries (Åkerlund 1991, Arevad 1975, Mroczkowski 1968, Zhantiev 1973).
- *Reesa vespulae* (Milliron), the American wasp beetle, is a parthenogenetic species, originating from North America. It was reported in Europe for the first time in 1963 from Norway (Strand 1970, Mehl 1975) but had already been found in an herbarium in Moscow in 1959 (Zhantiev 1973). The species is spreading in Europe (Adams 1978, Åkerlund 1991, Ólafsson 1979).
- *Trogoderma angustum* (Solier), a cabinet beetle, has spread from Chile to Northern America (Beal 1954) and to Europe. The first identified specimen in Europe was found in Poland 1921 (Mroczkowski 1960). The species continues to spread (Åkerlund 1991, Philipp 1968, Shaw 1999).

All test insects were reared at the Danish Pest Infestation Laboratory (DPIL), except one strain of *Attagenus woodroffei* that came from Central Science Laboratory (CSL), UK (Experiment 4). The larvae were sent to Stockholm, Sweden, by mail.

Experimental Design

Three types of wooden drawers with glass lids were used: old type of drawers with lids with one groove from the 1930s or older and new drawers with lids with one groove from
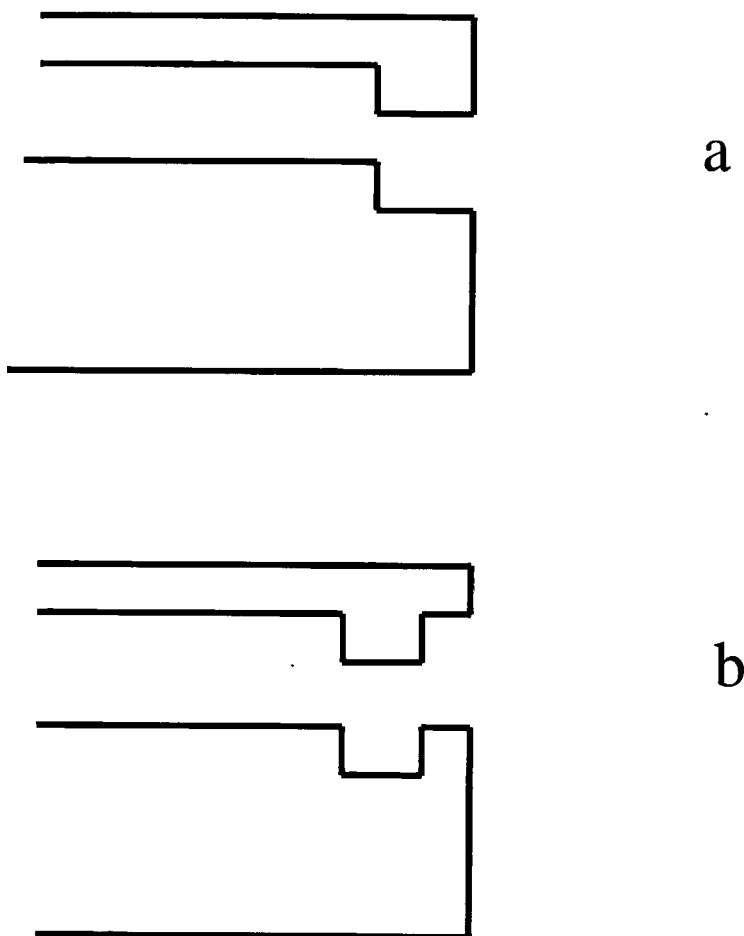
Figure 1.　Comparison of insect drawers with glass lids that have a single groove (a) or double groove (b).

the middle of the 1990s (Fig. 1a), as well as new drawers with lids with two grooves from the year 2000 (Fig. 1b). Old drawers, which still are used in many collections, might be less tight than the new constructions due to for example shrinking of wood and exposure to wear. Because of improved construction, double-grooved drawers could be expected to be tighter than single-grooved ones. All drawers measured 60 mm height × 400 mm length × 430 mm width.

Four experiments were performed and varied with regard to the type of drawer, amount of the oxygen scavenger Ageless®, exposure time, and test species (Table 1). In all experiments twenty larvae of the tested species were placed within each drawer in separate vials that were sealed with cotton gauze. To simulate an authentic situation a paper box with pinned insects was also placed in the drawer. Each drawer was enclosed in 0.09 mm thick RGI® low diffusion plastic film, consisting of nylon (15 μm) and polyethylene (75 μm) (oxygen permeability 0.45 cm^3/m^2 × day × atm; Fig. 2). The enclosures were sealed with a Hawo® heat sealer type hpl WSZ-300 with a seal width of 11 mm. Two plastic valves were attached in diagonal position. For anoxic treatment a VELOXY® nitrogen generator was used. The gas was humidified by a Rentokil® humidifier. The devise attached to the enclosure by means of a plastic tube (7 mm in diameter) and was
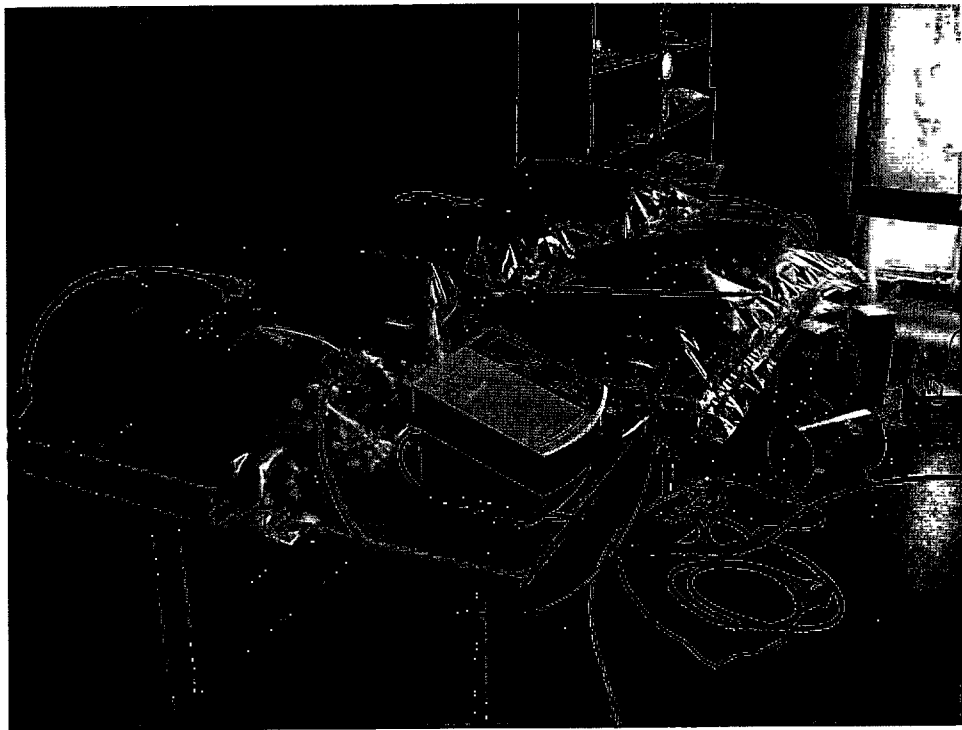
Figure 2.   Anoxic treatment setup showing enclosures sealed in plastic that contain insect drawers and the valves through which nitrogen gas enters and exits. The Dansensor oxygen analyser is seen in the foreground.

connected by one of the valves. The nitrogen gas entered as a gentle flow through one valve and left the enclosure through a non-return valve. The reduction of oxygen inside the drawers was due only to the diffusion of the nitrogen into, and the oxygen out of, the drawer. In the beginning of the treatment all enclosures were connected to each other through tubes and the non-return valve was placed at the end of the series.

After the treatment the condition of the larvae was checked three times during one wk. This entailed touching the larvae, gently exhaling towards them and observing them under a stereo microscope. Larvae not moving were regarded as dead.

Experiment 1.—We tested two species of dermestids, *T. angustum* and *A. verbasci.* The vials with larvae were placed horizontally in one corner of the drawer. The oxygen level was measured by an Analox Oxygen Analyser® 101D2 with a sensor Type 9212-5A (accuracy ± 1% of readout). When the oxygen level had decreased to about 5%, the drawers were treated one at a time. When Ageless® was used (Table 1), the enclosures were first cut open and then two sachets of Ageless® Z200 were added. The opening was then immediately heat-sealed and the oxygen level reduced to 0.3% by the VELOXY®. The drawers treated with VELOXY® only were separated from the nitrogen flow during nights and weekends. The oxygen level which then increased to between 0.4 and 3.4% was actively reduced to 0.2% during daytime. The enclosure for one of the single-grooved new drawers had a leak during the first night. This enclosure was resealed and the treatment was restarted at noon on day 1.

The ambient temperature was approximately 22°C but had a peak of 24°C on day 2. The RH level in the room was 15–30% and the nitrogen gas was conditioned to 50% ±

Table 1. Experimental design of tests to examine dermestid mortality in closed insect drawers with different anoxic treatments and exposure times. For drawer types, A = single-grooved old, B = single-grooved new, and C = double-grooved new. * = leakage. Dermestid species refer to Ta = *Trogoderma angustum*, Av = *Anthrenus verbasci*, Rv = *Reesa vespulae*, Atw = *Attagenus woodroffei*, Ats = *Attagenus smirnovi*. VELOXY® + number = VELOXY® + number of sachets of Ageless® Z 200.

Experiment	Drawer type	Species	Treatment	Exposure (days)
1	A, B, C	Ta, Av	VELOXY®	7
	A, B, C*	Ta, Av	VELOXY® + 2	7
	A, B, C	Ta, Av	VELOXY®	14
	A, B, C	Ta, Av	VELOXY® + 2	14
	A, B, C	Ta, Av	Control	7, 14
2	A, B, C	Ta, Av	VELOXY® + 4	7
	A, B, C	Ta, Av	VELOXY® + 8	7
	A, B, C	Ta, Av	Control	7
3	B, C	Ta, Rv, Atw	VELOXY® + 4	4
	B, C	Ta, Rv, Atw	VELOXY® + 4	7
	B, C	Ta, Rv, Atw	VELOXY® + 4	10
	C	Ta, Rv, Atw	VELOXY® + 4	14
	B, C	Ta, Rv, Atw	Control	4, 7, 10, 14
4	B, C	Ta, Av, Atw, Ats	VELOXY® + 4	7
	B, C	Ta, Av, Atw, Ats	VELOXY® + 4	10
	B, C	Ta, Av, Atw, Ats	VELOXY® + 4	14
	B, C	Ta, Av, Atw, Ats	Control	4, 7, 10, 14
	B, C	Ta, Av, Atw, Ats	VELOXY® + 4	7
	B, C	Ta, Av, Atw, Ats	VELOXY® + 4	10
	B, C	Ta, Av, Atw, Ats	VELOXY® + 4	14
	B, C	Ta, Av, Atw, Ats	Control	4, 7, 10, 14

5% RH. Controls were set up in an identical way, but without the nitrogen treatment. The experiment was carried out for 7 and 14 days, at which time larval mortality was checked.

Experiment 2.—We tested the same larval species but added 4 or 8 sachets of Ageless® Z200 (Table 1). In addition we placed a humidity logger (Mätman® datalogger) inside two of the drawers. The vial with the test insects was placed in an upright position in one corner of the drawer. The oxygen level, measured as above, was reduced to 0.2% and exposure time was 7 days. The ambient temperature was 22–24°C and the RH level in the drawers was approximately 28%. Control experiments and larval checks were performed as above.

Experiment 3.—The third experiment was performed with only new drawers that had either one or two grooves in the lids (Table 1). We tested three species of larvae, *T. angustum*, *R. vespulae* and *At. woodroffei,* which appeared to be more resistant to low oxygen than the two other species in laboratory tests (Bergh et al. 2003). For the control test insects, we added some breeding food material from DPIL to the vials to avoid cannibalism observed in the earlier experiments.

Four packets of Ageless® Z200 were used and RH of the nitrogen flow was 50% ± 5%. Oxygen levels were measured with a PBI-Dansensor Checkmate, a device that has a needle-like cannula that can penetrate the barrier film through a 15 mm septum of nitrile rubber and take a sample of the gas inside the enclosure. The oxygen level is directly analysed by the instrument which allows for taking multiple measurements during the treatment. Measuring range is 1 ppm–100% and accuracy is ±1% of the readout.

To test if oxygen reduction is more effective if performed in two or three steps, we conducted two additional tests. In test 1 oxygen level was reduced in three steps, first to 6%, then to 1.5%, and finally to 0.3%, in all enclosures before the oxygen scavengers were added. In test 2 the oxygen reduction was performed in only two steps, first to 3% and then to 0.3%, before the scavengers were added. The oxygen levels were checked in the enclosures after 4, 7, and 10 days (and after 14 days in one doubled-grooved drawer, test 2 only). In test 1 the temperature and RH in the enclosure were 20–23.5°C and 21.5–24%, respectively, and in test 2, they were 20.5–23.5°C and 24.5–32%, respectively.

Experiment 4.—In order to evaluate the results of the previous experiments, tests with nitrogen and 4 sachets of Ageless® Z200 were repeated in two tests. We used *T. angustum, A. verbasci,* and *At. smirnovi,* (20 larvae per vial of each species) and 13 larvae of *At. woodroffei,* and placed the vials in each drawer. In addition a vial with 20 larvae of *At. woodroffei* from Central Science Laboratory, England (CSL) was also placed in each drawer as there was a discrepancy in survival in low oxygen atmosphere between our experiments and earlier tests at CSL (see below). Some breeding food was added to the boxes with *At. woodroffei* and, for the other species, one dead house fly from DPIL was added. In the controls, consumed flies were replaced by new ones during the larval checks.

The nitrogen was conditioned to 50% ± 5%. The oxygen level was reduced in 2 steps, first to 3%, and then to 0.3% as in Experiment 3, test 2. The oxygen level in the enclosures was checked after 7, 10, and 14 days of treatment. This time a new kind of septum of nitrile rubber for the oxygen level measurements was delivered by the manufacturer. The new septum had the same diameter as the old one but was 3 mm thick instead of 2 mm and the quality was less dense.

As there seemed to be a leak through the septa, the oxygen level was reduced to 0.3% on days 3 and 4 in test 1. In test 2, the oxygen level was not further reduced. The oxygen analyser described in Experiment 3 was used.

RESULTS

Experiment 1

When using VELOXY® alone a 7-day treatment was sufficient to kill all larvae of both species in all drawer types (Table 2). Oxygen levels remained higher in the new double-grooved drawers over most of the 14-day interval (Fig. 3). Regardless of the drawer model, a 7-day treatment with nitrogen and 2 sachets of Ageless® Z200 did not result in 100% mortality of either species except for *A. verbasci* in the old type of drawer with a single groove (Table 2). In the double-grooved drawer a leakage resulted in an oxygen level of 19.3% after a wk. The survival rate of *A. verbasci* in this drawer was 53%, which is about the same as for this species in the new type with a single groove (60% survival, 0.6% O_2). The treatment killed both species in all types of drawers after 14 days. The control survival in Experiment 1 was 94% or more for both species after 7 days, and after 14 days it was 79% or more for *T. angustum* and 50–94% for *A. verbasci.*

Experiment 2

For single-grooved drawers, nitrogen treatment and 4 sachets of Ageless® were sufficient to kill both species in 7 days (Table 2). For double-grooved drawers, however, the treatment was sufficient to kill only the larvae of *A. verbasci.* 100% mortality of both species was achieved after 7 days using 8 sachets of Ageless® Z200 in combination with

Table 2. Number of days to reach 100% mortality of *Trogoderma angustum* and *Anthrenus verbasci* larvae inside three different models of closed insect drawers using Veloxy® and different numbers of sachets of Ageless® Z200 (Experiments 1 and 2). * = Leakage after the 7-day exposure.

Drawer type	Experiment number	Treatment	*Trogoderma angustum* (Ta)	*Anthrenus verbasci* (Av)
Single-grooved old	1	Veloxy	7	7
	1	Veloxy + 2	14	7
	2	Veloxy + 4	7	7
	2	Veloxy + 8	7	7
Single-grooved new	1	Veloxy	7	7
	1	Veloxy + 2	14	14
	2	Veloxy + 4	7	7
	2	Veloxy + 8	7	7
Double-grooved new	1	Veloxy	7	7
	1	Veloxy + 2*	14	14
	2	Veloxy + 4	10	7
	2	Veloxy + 8	7	7

nitrogen treatment (Table 2). In Experiment 2 the survival of the controls was 100% for both species.

Experiment 3

Test 1.—100% mortality of *T. angustum* and *R. vespulae* larvae was achieved in single-grooved drawers after seven days, however, 10 days were required for 100% mortality of *At. woodroffei*. In double-grooved drawers all three species were killed after 10 days (Table 3).

Test 2.—*T. angustum* and *R. vespulae* in single-grooved drawers did not survive a 7-day exposure. *At. woodroffei* in the same type of drawer survived a 10 day treatment but we have no data after 14 days. In double-grooved drawers *T. angustum* was killed after 7 days, *R. vespulae* after 10 days, and *At. woodroffei* after 14 days (Table 3).
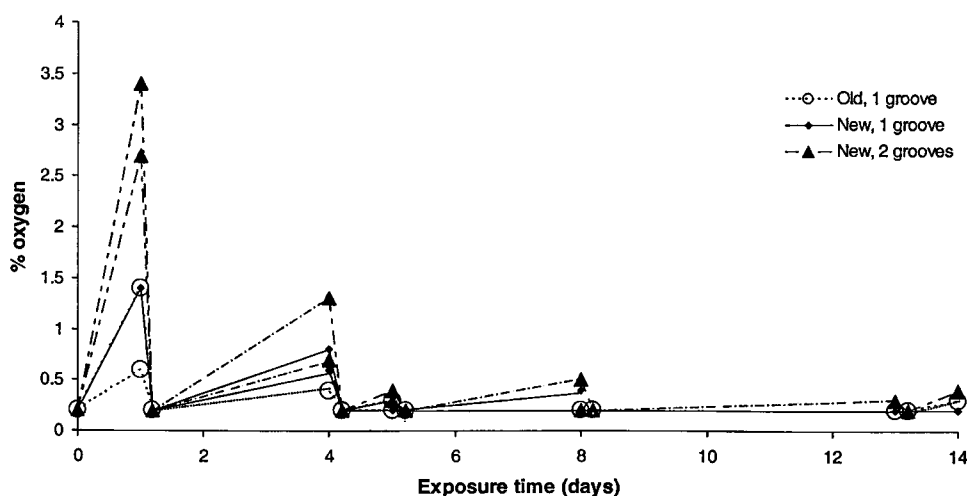
Figure 3. Variation in rate of oxygen reduction within enclosures containing insect drawers of different models (single-grooved of old and new type, double-grooved of new type) in Experiment 1 (VELOXY® only).

Table 3. Number of days to reach 100% mortality of three species of dermestid larvae inside two different types of closed insect drawers using Veloxy® + 4 sachets of Ageless® Z200 (Experiment 3).

Test 1 (oxygen reduction occurred in 3 steps)

Drawer type	*Trogoderma angustum* (Ta)	*Reesa vespulae* (Rv)	*Attagenus woodroffei* (Atw)
Single-grooved new	7	7	10
Double-grooved new	10	10	10

Test 2 (oxygen reduction occurred in 2 steps)

Drawer type	*Trogoderma angustum* (Ta)	*Reesa vespulae* (Rv)	*Attagenus woodroffei* (Atw)
Single-grooved new	7	7	—
Double-grooved new	7	10	14

The oxygen levels in drawers with one groove decreased faster than those with two grooves (Fig. 4). The exception in the figure was the result of a leak in the enclosure. The reduction of oxygen levels was more effective when done in 3 steps (Figs. 5, 6).

Experiment 4

Test 1.—In single-grooved drawers 100% mortality of the four species was reached after 7 days but *T. angustum* and *At. woodroffei* showed a 5% survival after 14 days that was due to a leak detected in this drawer (Table 4; the result due to leak is not indicated in the Table). In the double-grooved drawers all *A. verbasci* and *At. smirnovi* died after all exposures while 5–15% survival was found for *T. angustum* and *At. woodroffei* after 7 and 10 days exposure. No species survived after 14 days of exposure. Survival of the controls was 95–100% for all species.

Test 2.—100% mortality of *A. verbasci* and *At. smirnovi* was achieved in all drawers by day 7, while the mortality of *T. angustum* and *At. woodroffei* was 70–80% in both types of drawers (Table 4). After 10 days the survival of *At. woodroffei* from CSL was 5% in both types of drawers. After 14 days we found 100% mortality of all species in all drawers. In all single-grooved drawers and in the double-grooved drawer exposed for 14 days the final oxygen level was higher than 0.4% indicating small leaks. Survival of the controls
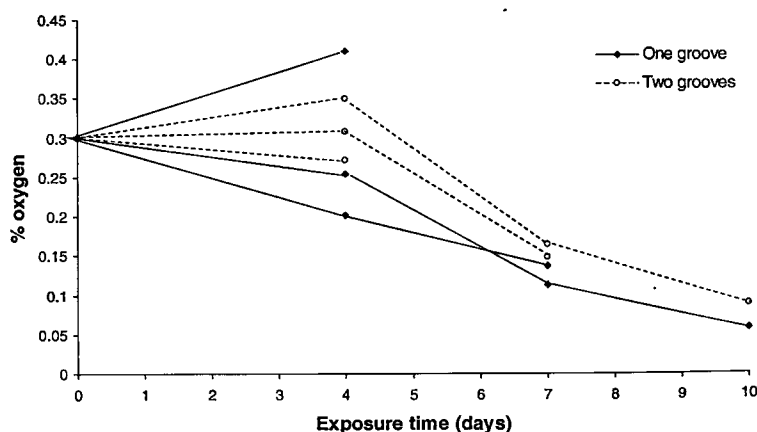
Figure 4. Variation in rate of oxygen reduction between single-grooved and double-grooved insect drawers in Experiment 3. The high value for one of the single-grooved drawers is the result of an enclosure leak.

Figure 5. Variation in rate of oxygen reduction in two tests using single-grooved drawers, in Experiment 3. In test 1 the oxygen was reduced in three steps, in test 2 in two steps.

was 85–100%, except for *A. verbasci* which had a lower survival rate (80, 75, 65%) after 7, 10, and 14 days, respectively, in the double-grooved drawer.

DISCUSSION

The treatment of insect drawers with VELOXY® alone was efficient but laborious as the oxygen level in each enclosure had to be checked and adjusted regularly to keep the level low. It is possible to maintain a constant flow of nitrogen during the entire treatment and thus keep the oxygen at a constant low level, however, this would occupy the equipment and prevent treatment of other objects during that time. Using both the nitrogen generator and an oxygen scavenger is a more efficient method.

The oxygen analyser used in Experiments 3 and 4 works by extracting a small amount of gas and is much more accurate in low values of $\leq 0.1\%$. This equipment was not available to us during the first two experiments. The septa used in Experiment 4 caused leak problems and therefore is not recommended by us.

In the 7-day treatment using VELOXY® and 2 sachets of Ageless® 200 the survival rate of the test insects was high except for *A. verbasci* in the old single-grooved drawer, where the mortality was 100%. The survival of the test insects in the new drawer with two
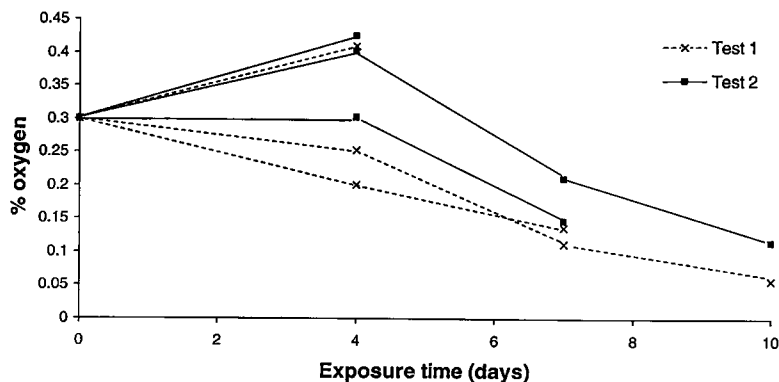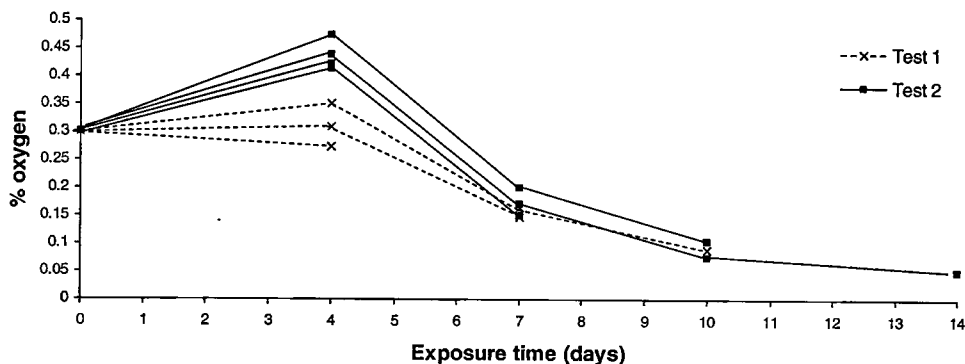

Figure 6. Variation in rate of oxygen reduction in two tests using double-grooved drawers, in Experiment 3. In test 1 the oxygen was reduced in three steps, in test 2 in two steps.

Table 4. Number of days to reach 100% mortality of four species of dermestid larvae inside two different types of closed insect drawers using Veloxy® + 4 sachets of Ageless® Z200 (Experiment 4). ** = only larvae from CSL (5% survival in 10 days exposure).

Test 1: oxygen reduction occurred in 3 steps

Drawer type	Trogoderma angustum (Ta)	Anthrenus verbasci (Av)	Attagenus woodroffei (Atw)	Attagenus smirnovi (Ats)
Single-grooved new	7	7	7	7
Double-grooved new	14	7	14	7

Test 2: oxygen reduction occurred in 2 steps

Drawer type	Trogoderma angustum (Ta)	Anthrenus verbasci (Av)	Attagenus woodroffei (Atw)	Attagenus smirnovi (Ats)
Single-grooved new	10	7	10 (14**)	7
Double-grooved new	10	7	10 (14**)	7

grooves, the most airtight drawer tested, is explained by the fact that the oxygen level was 19.3% due to a leak. The survival of the insects however, was less than in the new single-grooved drawer. This may indicate that the oxygen level might have been sufficiently low during the first part of the treatment period. The survival after 7 days in the control experiment was high.

After 14 days the survival of the *A. verbasci* controls in the double-grooved drawer was exceptionally low, 50%, compared to 100% survival after 7 days. The mortality rate found for this species in drawers with two grooves may thus be uncertain. The low humidity of the air may have been a stress factor for the larvae. The test insects in the controls in Experiments 1 and 2 had no access to food during the second wk and they had obviously consumed some of their companions. In Experiments 3 and 4, the test insects in the controls did have access to food.

At the first survival check some individuals initially showed slow movements but were found to be dead during the following check. On the other hand the opposite situation sometimes occurred. By observing the survival over several days, a good estimate of the survival rate could be done. Figures 5, 6 clearly show that reducing the oxygen level in three steps is more effective than doing it in two steps. The mortality rates do not always reflect this, but small leaks in some drawers may have influenced the result.

Bergh et al. (2003) found that larvae of *A. verbasci* were killed in 48 hr and larvae of *T. angustum* in 72 hr, when exposed to 0.3% oxygen in 25°C and 55% RH. *At. smirnovi* and *At. woodroffei* were even more resistant, however, to a low oxygen atmosphere. In Experiment 4 of this study *At. smirnovi* proved to be as susceptible to anoxic treatment as *A. verbasci*. A possible explanation could be that *At. smirnovi* might be more susceptible to the dry atmosphere inside the insect drawers than *T. angustum, R. vespulae,* and *At. woodroffei.*

According to the results of tests at CSL the mortality for larvae of *At. woodroffei* at 50% RH and 25°C and 0.3% oxygen was 100% after 72 hr. At 70% RH the mortality was about 98% (S. Conyers personal communication). Bergh et al. (2003) found that for the same species, however, the mortality was only 50% after 72 hr at 55% RH and 25°C and 0.3% oxygen. In the present study laboratory strains of *At. woodroffei* from both CSL and DPIL were tested. Differences in susceptibility to anoxic treatment between the populations from the two laboratories could be traced but may have been a random effect.

Due to the fact that the vials in Experiments 1 and 2 were placed horizontally, some of the first test insects escaped and had to be recollected. The fugitives were found inside the pinned insects, in the unit box in the drawer, and for both types of single-grooved drawers, at the groove between the drawer and the lid, and outside the drawer in the enclosure. Only double-grooved drawers were tight enough to prevent the larvae from leaving. This shows that the double-grooved new drawers also would prevent insects from entering them.

The results of the tests can only be referred to the particular conditions that existed during the tests. Other developmental stages, especially eggs, may be more resistant than larvae to anoxic treatment and would therefore require a different treatment period.

CONCLUSION

This study was designed to simulate an authentic treatment situation. This gives some limitations for the possibility of performing identical replications but offers more information for museological applications.

It is possible in practice to use the VELOXY® method for pest control of closed wooden insect drawers with glass lids. Its great advantage, besides being non-toxic to people, is that it is not harmful to the treated object. Adding enough of the oxygen scavenger to the enclosures instead of only using a nitrogen generator reduces the work input.

For the tested volumes, 4 sachets of Ageless® 200 seemed to be optimal and during these conditions larvae of *At. woodroffei* and *T. angustum*, being the most resistant species, should be treated for a minimum time of 14 days. *R. vespulae* will need 10 days of treatment in the tightest type of drawer. Recommended minimum time of treatment for larvae of *A. verbasci* and *At. smirnovi*, the most susceptible species, is 7 days.

Care must be taken to examine the construction of the drawer prior to beginning anoxic treatment as a tighter drawer e.g., double groove construction, needs a longer exposure time. The number of steps involved in oxygen reduction is also an important consideration in anoxic treatment, with 3 steps considered to be more effective than two.

ACKNOWLEDGMENTS

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RISK ZONES FOR IPM: FROM CONCEPT TO IMPLEMENTATION

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Abstract.—The Natural History Museum in London is the first national museum to introduce a museum-wide integrated pest management (IPM) strategy based on a concept of risk zones. This system is based on the principle of pest prevention within a museum building, which will always have a resident population of pest insects. The loss of dichlorvos [DDVP] resulted in an urgent need to implement a museum-wide IPM program to protect vulnerable collections in both storage areas and displays. With such a large, diverse collection in a complex series of interconnecting buildings, it was necessary to break the program down into sections. A key to this was the decision to define and adopt the concept of "Risk Zones" from high risk A, to low risk D, for all areas of the museum. The paper describes the development of ideas and subsequent implementation of the "Risk Zone" concept in the Natural History Museum, including the outstations, and makes observations on the need to identify priorities and the importance of training staff at all levels. Risk concepts and staff awareness are an effective method, which can be adopted by other institutions as part of an IPM program.

INTRODUCTION

The Natural History Museum in London England is the first national museum to introduce a fully integrated pest management (IPM) strategy using a concept of risk zones. This provides a framework for preventive and remedial conservation strategies to help prevent and mitigate infestations and react appropriately in the event of an infestation. This concept has proven that whilst there will always be a resident population of insects and rodents within the building of a large national museum, control methods and protocols linked to risk zone concepts are an effective method of tackling pests.

BEFORE IPM

The Natural History Museum houses collections comprising of approximately 67 million specimens, many of which are vulnerable to attack by insect pests, particularly those belonging to the life sciences departments of Zoology, Entomology and Botany.

As was common practice in other institutions, including many galleries and historic houses, the Museum historically relied upon the use of vapor phase chemicals, such as camphor, naphthalene and para dichlorobenzene to discourage or control resident insect populations. These chemicals are solids and work by vapors off-gassing into closed environments such as display cases and collection storage areas (Pinniger 2001).

Following a serious infestation of varied carpet beetle (*Anthrenus verbasci*) in the collections housed at The Walter Rothschild Zoological Museum at Tring in the early 1970s, the Museum introduced a program based on the use of dichlorvos or DDVP (2,2-dichlorovinyl dimethyl phosphate, $C_4H_7Cl_2O_4P$) slow-release strips. This proved to be very successful in protecting vertebrate collections from insect attack both in storage cupboards and in display cases in the main museum and the outstations.

Large mounted mammal specimens, found to be infested with *Anthrenus*, were successfully treated by enclosing them in large plastic bags with DDVP strips. However,

this technique of treating individual specimens was soon replaced by bagging and freezing, which is deemed safer, and with less risk to objects. (Strang 1996).

Strips were not suitable for use in open displays or in very large exhibition galleries as the dichlorvos vapor needs to build up to a sufficient concentration in an enclosed space to produce 100% mortality of the pests (Pinniger and Child 1996). A further problem was that dichlorvos strips had an effective life of no more than six to nine months in cases or bags. Strips therefore needed to be regularly removed and replaced to remain effective. This required complex and labor-intensive opening of exhibition display cases and collections storage cupboards and as a preventive pest control strategy was costly in both materials and staff time and as consequence occasionally ignored or delayed.

When the registration and safety of dichlorvos was reviewed in 2002 by the UK Health and Safety Executive, it was clear that the timescale for continued use of this insecticide was limited and that a total ban of this product was likely to be introduced. The use of dichlorvos was suspended in 2002 [HSE E076:02 2002] and eventually prohibited from 15 April 2004.

It was also clear that there was unlikely to be any replacement chemical insecticide with a vapor effect, which could be used to substitute or replace dichlorvos, and that alternative appropriate measures had to be introduced well in advance of the total ban.

Introducing IPM

In the early 1990s, to combat increasing problems with pests, the Entomology and Botany Departments started to adopt the newly-termed Integrated Pest Management (IPM) concept (Rossol and Jessup 1996). Due to the increased concerns about health and safety, emphasis was moved away from reliance on chemicals and priority was placed on pest prevention. This included the introduction of trapping and monitoring protocols (Child and Pinniger 1994). It also required the adoption of new working procedures such as quarantine, inspection, and cleaning regimes. IPM was also adopted in a number of other collections areas, particularly Zoology, but it was not yet Museum-wide.

The trapping data showed that the major pest in the museum was now the Guernsey carpet beetle *Anthrenus sarnicus*, which had replaced the varied carpet beetle *Anthrenus verbasci*. There were also problems with biscuit beetle *Stegobium paniceum* and a new and relatively unknown Dermestid pest, *Attagenus smirnovi*. All of these species were increasing in areas which were not protected by DDVP vapor.

A proposal that the Museum needed to be prepared for the loss of dichlorvos and should investigate the feasibility of implementing IPM across the whole Museum was prepared by the staff involved with IPM and presented to the Directorate. The failure of introducing integrated pest management would rate the impact on the Museum as a corporate risk #1, the highest rating based on the corporate risk register. This was accepted by the Directorate, the success of IPM procedures already in use being a key factor in the adoption of the proposal.

Although the emphasis had previously been on insect pests, there had been serious and increasing problems in the Museum with house mouse *Mus domesticus*. Therefore the IPM remit was expanded to include prevention and control of mice (and rats), which had formally been under the exclusive control of the estates management department.

The proposed IPM strategy was museum-wide in scope and needed representation and commitment to the project from all departments across the whole museum. Therefore, in order to achieve a museum-wide status and effective impact, an IPM champion at senior manager (directorate) level and an IPM coordinator from the science group were

identified and given the task of working with the external pest management consultant to evaluate options and devise a workable IPM strategy for the whole Museum.

IMPLEMENTING IPM

The information on pest species presence and distribution in the Museum was provided by earlier trapping data from the Botany, Entomology and Zoology departments. This had shown that the main pest, Guernsey carpet beetle *Anthrenus sarnicus*, was now spread throughout the museum. It was also apparent that the Brown carpet beetle *Attagenus smirnovi*, also known as the Vodka beetle, was also spreading through the main South Kensington Waterhouse building (Ackery and Pinniger 1999).

Other pests which were present in the building included the biscuit beetle *Stegobium paniceum*, and cigarette beetle *Lasioderma serricorne*, mainly in Botany collections areas. The American wasp beetle *Reesa vespulae*, was however, confined to one particular building, the Entomology department.

A key factor identified as being essential to the successful implementation of the Museum-wide IPM strategy was the recognition that many of the pests were present throughout the museum building living on organic material in uncleaned or inaccessible dead spaces, including those in non-collection areas. The IPM strategy would therefore need to include all areas of the Museum, not just vulnerable collections in storage or on display.

Given the reality of finite resources, there would also be a need to prioritize any strategy to minimize the damage to high-risk collections in the most cost-effective way.

RISK ZONES

Collections risk management (Waller 1994, Waller 1995, Waller 2003) is a well-established tool for decision-making and implementation of collections care. This approach can be equally effective when applied to pest prevention and control (Egunnike 2001, Xavier-Rowe and Pinniger 2001, Strang and Kigawa 2006). To effectively manage and prioritize the museum-wide IPM program, we decided to divide the museum up into areas of "high risk" to "low risk." Specific protocols could then be devised and applied at the level appropriate to the vulnerability of the collection. This includes galleries, stores, offices and all other areas in the museum.

The Natural History Museum, with its large and diverse collection housed in a complex series of buildings, required this risk zone concept to encompass all the collections and non-collection areas in a simple, workable system which would be easily understood by all in-house staff as well as visitors.

Floor plans of the museum, provided by the estates management department, were color coded. Specifically; red for high-risk collections in stores, orange for high-risk collections on display, yellow for low risk collections and green for non-collection areas using risk zone definitions as shown in List 1. Staff from collections management teams, together with IPM representatives and researchers walked through the museum identifying collections and color-coding floor plans accordingly.

LIST 1: RISK ZONE DEFINITIONS

- Collection storage area (with or without workstations) holding material very vulnerable to insect damage: Insect (A) and Rodent (D).
- Collection storage areas (with or without workstations), offices and other areas holding collections less vulnerable to insect damage and/or very vulnerable material in
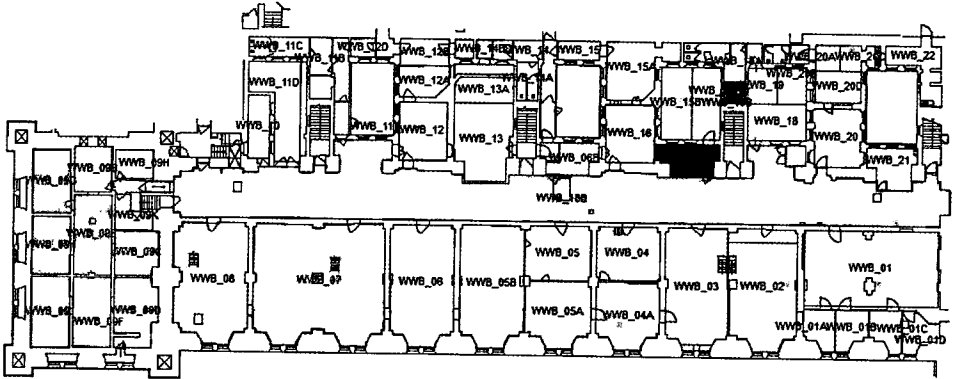
Figure 1. Color-coded Insect risk zone floor plan.

transit, where food is consumed, prepared or temporarily stored: Insect (C) and Rodent (A).

- Offices, labs & other areas temporarily holding collections very vulnerable to pest damage: Insect (C) and Rodent (C).
- Designated area for the preparation and/or consumption of food. Commercial catering outlets and kitchens, Staff Common Rooms, Meeting Rooms and Keeper's Suites etc: Insect (D) and Rodent (A).
- Public area: Exhibition display material vulnerable to pest damage: Insect (B) and Rodent (B).   ·
- Collection storage areas with Public Access holding collections less vulnerable to pest damage and/or very vulnerable material in transit: Insect (C) and Rodent (B).
- Collection storage areas (with or without workstations), holding collections less vulnerable to pest damage: Insect (C) and Rodent (D).
- Non-collections areas. Offices, labs and other staff areas (excluding designated eating areas): Insect (D) and Rodent (C).
- SPECIAL RISK ZONE: Dermestarium or specimen preparation areas: Insect (E).

The terms of reference for the floor plans also included mapping the following:

- Contents of cupboards and storage areas
- Doors into unmarked rooms and their use
- Corridor and access routes
- Potential zone boundary areas
- Areas of ownership (i.e., rooms) and responsibility (collection areas)
- Areas requiring further investigation including areas of concern (i.e., where previous, current or potential pest problems were apparent)
- Current and potential insect and rodent trap locations.

An example of color-coded area is shown in Figure 1. The mapping project required extensive revisions and other staff were consulted in the case of uncertainty and confusion. At a later stage, this information will be transferred to electronic mapping software known as Geographic Information Systems (G.I.S) into which other IPM data such as trapping and environmental monitoring will eventually be fully integrated.

Rodent Risk - Palaeontology, Ground Floor
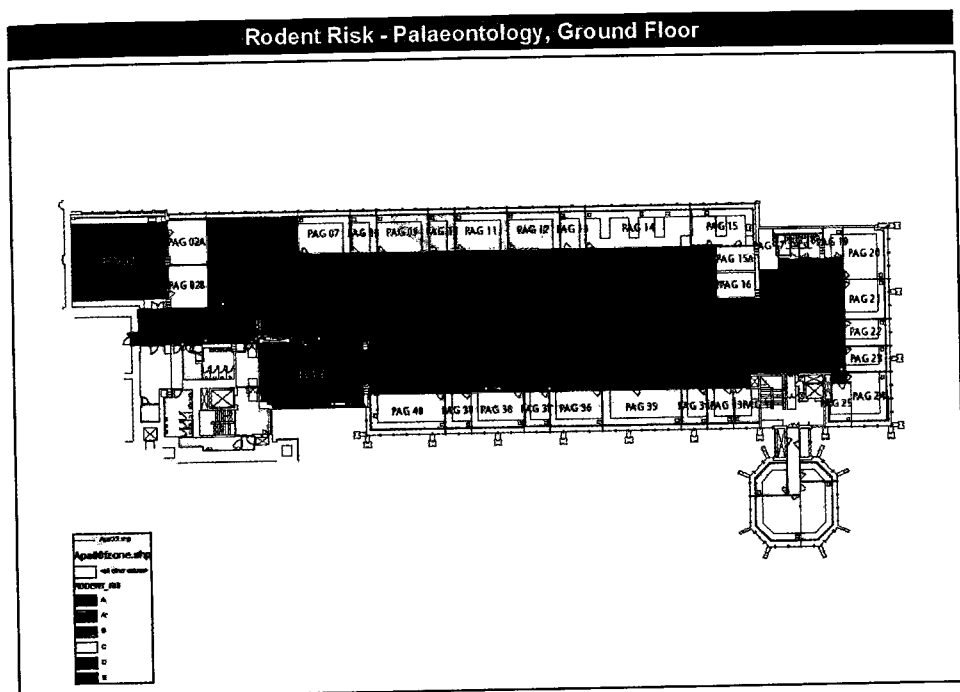
Figure 2.   Color-coded Rodent risk zone floor plan.

This exercise also identified high-risk rodent risk zones where food was stored, prepared or consumed. Unlike the insect risk zones, those for rodents relate to infestation risk in the museum. They are not for identifying collections at risk from rodents as there is little evidence that collections are at risk when other more palatable food sources are present (Pinniger 2001). An example of a rodent risk zone plan is shown in Figure 2.

By combining both color codes, a table was drawn up showing the possible combinations of risk zones that existed in the museum (Table 1) and a series of protocols for each combination was drafted such as this example for the highest risk categories (List 2). This procedure was a time-consuming but necessary process to establish a basis for the IPM program which would be practicable, achievable, and be perceived as non-draconian in nature.

LIST 2: EXAMPLE OF PROTOCOLS FOR RISK ZONE COMBINATIONS INSECT A, RODENT A

- **Do not** bring material through this area without IPM treatment, sealed plastic bagging or first showing it to a curator.
- **Eat only** in designated areas.
- **Keep all** food in containers and transport bin covered trolleys. Material for permanent installation that may be sensitive to IPM treatment such as computers and plants should be inspected or quarantined first.
- **Do not** leave food unattended for any length of time in corridors or lift lobbies. **Do not** leave waste food out overnight.
- **Keep** area tidy.
- **If you see** any evidence of **insects** please notify the local curation team immediately.

Table 1. Table of Risk zone combinations.

Insect	Rodent
A	A
A	D
A	E
B	B
C	A
C	B
C	C
C	D
D	A
D	C
E	—

- **If you see** any evidence of **rodents** (droppings, holes, gnaw marks or sightings) please notify your IPM representative AND the Estates Help Desk (6000) immediately.

RISK ZONE IDENTIFICATION

As a means of identifying the zone that staff and visitors are working in, a method of signage was devised similar to fire zone concepts. An identification symbol had to be designed that was clear, accurate and easy to understand for people who do not necessarily have English as their first language. Additionally, any symbol would have to comply with access regulations as well as color perception difficulties (such as red/green color blindness).

A simple symbol showing a generic insect silhouette and an alphanumeric letter on a color background in a circle provided a simple clear concept. An example of the symbol for high-risk collections vulnerable to insect attack is shown (Fig. 3). A series of test designs were made for signs labels and symbols using various icons to identify insect and rodent zones as well as choice of color for the backgrounds. They would need to be easily duplicated and the color standard maintained by different manufacturing processes (for example paper labels, laminated soft plastic and rigid polycarbonate).

Signs

A4 (297 mm × 210 mm) size information panels with symbol and detailed English language information. These would be on the perimeter of the collections or other areas, similar to fire zone information panels.

Labels

A5 (148 mm × 210 mm) size information panels which would be used within the risk zone for highlighting the risk and as a further reminder.

Symbols

A circular color identification symbol, (40 mm diameter), which would be used as a very close proximity reminder of the risk zone (for example, affixed to drawers, cabinet doors or collections shelves).

Insect A

Figure 3.   Symbol for high risk zone (A) Red.

Testing the Concept

Although the risk zone concept had been recognized as an appropriate course of action, it was still necessary to test this on a high-risk collection area as part of an information gathering exercise and in particular to understand other issues associated with zoning.

The Zoology Department has a high-risk collection of mammal skins which, conveniently, were housed within one particular building known as the Zoology Department Mammal Tower. This was ideal for testing the concept and a team of IPM representatives inspected the collections and storage areas in this building to deal with the following issues:

- Labeling of collections and zones with temporary symbols.
- Safe removal and disposal of dichlorvos.
- Removal of unwanted clutter, litter bins, rubbish and storage and packaging materials.
- Improvement to external window and door fittings to minimize ingress of insects and rodents where possible.
- Identification of access routes and, if necessary, suggests new routes through low-risk zones to avoid the high-risk areas.
- Discussions with staff, visiting scientists and other personnel to identify problems associated with categorization of this collection as a high risk A and the implications to working practices.

The process took several days and in addition to confirming that the concept was viable, became a very successful team-building exercise for the IPM group.

Implementing Risk Zones

The pilot study exercise proved that the concept would work and from that beginning the museum undertook a full risk zone mapping exercise. The full implementation will include adopting agreed protocols for trapping, monitoring and cleaning in each zone. In addition, IPM, including risk zones, is being adapted and implemented at the other NHM sites at Wandsworth and Tring.

The key to the success of this is to involve everybody in IPM by targeted staff training and awareness. To this end, a series of training and discussion sessions have been devised. The training is at four levels:

- Level 1. A basic 45 minute presentation introducing IPM and risk zones for all staff, including cleaning and security.

- Level 2. A 2 hour presentation with a discussion session specifically targeted to each of the Science departments.
- Level 3. A one day IPM workshop including a practical survey exercise for all staff directly responsible for collections care.
- Level 4. A one day insect identification workshop for all staff that check insect traps.

Additionally, all new starters (including contract staff, staff on short-term appointments and those working for organizations within the museum) are provided with a 20-minute presentation on the basics of IPM as part of an induction welcome program as well as personal tuition from departmental representatives as required.

Since IPM training was initiated, over 470 staff members have been trained to the minimum basic level (i.e., Level 1) which represents a significant percentage of the staff complement of the Museum.

Further developments include the adoption of a standard trapping record form and the annual analysis of insect trapping data. Protocols and procedures for insect trap placement and application of control methods have also been agreed and adopted across the museum. Treatment methods include freezing of specimens and applying residual insecticides, such as permethrin micro-emulsion or desiccant dust, to collections areas. These procedures were adopted with due regard to health and safety and COSHH regulations.

Each department currently has its own systems for inspection and quarantine of collections. The need for a centralized inspection and quarantine treatment facility has been identified to prevent insects becoming introduced on any incoming collection and non-collection material is being introduced as part of the Museum-wide collections management policy.

The system must be flexible and be able to deal with new issues such as the construction of the new Darwin Centre 2. It must also be able respond rapidly to new threats to collections from pests. Moth pheromone lures are now used in traps in the museum as a response to the increase in pest problems caused by webbing clothes moths *Tineola bisselliella* in London museums over the last 5 year. Because of this, a potentially serious infestation of moths was detected in a gallery at an early stage and eliminated before serious damage had occurred.

CONCLUSIONS

The Natural History Museum now has a coordinated IPM program which is evolving to meet the needs of the museum. As a consequence of introducing the IPM strategy, the rating of the corporate risk has been reduced from the corporate risk #1 through to corporate risk #5, corporate risk #10 and more recently is no longer on the corporate risk top-10 register. This is a major achievement to reduce risk to collections in such a large museum within a three-year time frame and subsequently and as a consequence, a rolling 5-year IPM strategy is now agreed policy for the museum.

It has clearly demonstrated that it is more cost-effective to prevent pest outbreaks than to spend large amounts on remedial measures.

Risk zones have been a key to the success in identifying priorities and concentrating effort in the most effective way. The risk zone concept as developed by the Natural History Museum has now been recognized as an essential IPM tool and has been adopted by the Imperial War Museum on all of its sites; it is also currently being incorporated into the IPM strategy of the Victoria and Albert Museum and the British Museum.

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PRESENCE OF ELEMENTAL MERCURY IN FLUID PRESERVED SPECIMENS

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Abstract.—A small amount of elemental mercury was discovered in two fluid preserved specimens of an African lizard collected prior to 1901. The droplets found inside the specimens formed near perfect spheres, showed low adhesive properties, strong cohesive properties, had a metallic color, and were opaque to x-rays, confirming the identification of mercury. The specimens containing mercury were notably darker than the other specimens of the same species collected from similar localities and dates. Although toxic, mercuric compounds (particularly mercuric chloride, also known as "corrosive sublimate") have a long history of use as a preservative or as a component of preservative solutions. Although it is not known how and when the mercury was added to the specimens, it was probably in the form of mercuric chloride added to an alcoholic solution. Mercuric chloride may be reduced to elemental mercury by: (1) the action of mercuric chloride resistant heterotrophic bacteria; (2) interaction with proteins; (3) reduction and oxidation reactions; or (4) the action of an organic solvent. Because of the widespread use of mercury in preservative treatments prior to about 1900, appropriate precautions should be taken when handling older specimens and preservative fluids, and such materials should be treated as hazardous materials.

INTRODUCTION

This report concerns the presence of mercury in two lizard specimens of *Chalcides ocellatus* (Sauria: Scincidae), which were examined for a taxonomic revision (Greenbaum et al. 2006). The specimens were on loan from the Naturhistorisches Museum Wien (NHMW), Austria. The two specimens, NHMW 10438:1 (sex unknown; 123.0 mm snout-vent length [SVL]) and NHMW 10438:5 (adult female, 97.0 mm SVL), were part of a lot of five specimens (NHMW 10438:1–5) which were collected in "Kordofan region," Sudan. Although the exact date of collection for these specimens is unknown, both were from the NHMW "Alte Sammlung" or old collection, which consists of specimens collected between 1796 and 1900. A total of 34 specimens of *Chalcides ocellatus* were borrowed from the NHMW, for which the known dates of collection were 1893–1914 (Greenbaum et al. 2006).

MATERIALS AND METHODS

Metallic mercury (mercury 0) was discovered while measurements were being taken of specimens NHMW 10438:1 and 10438:5. The elemental mercury was first mistaken for steel shot inside the body cavity. An attempt to remove the metallic spherules by means of forceps revealed them to be liquid. Radiographs of the two specimens (Fig. 1A–D) revealed inclusions opaque to x-rays. Mercury is the most commonly encountered metal that is liquid at room temperature, but some other metals, notably gallium and some gallium alloys, are liquid at common environmental temperatures. The melting point of mercury is −38.83°C, and its boiling point is 356.73°C. Thus, mercury remains in a liquid state under standard laboratory conditions. Pure gallium (melting point 29.76°C) would have been solid at the room temperature (24°C). The specimen, while being handled for examination, would have had a lower temperature than the room given the evaporative
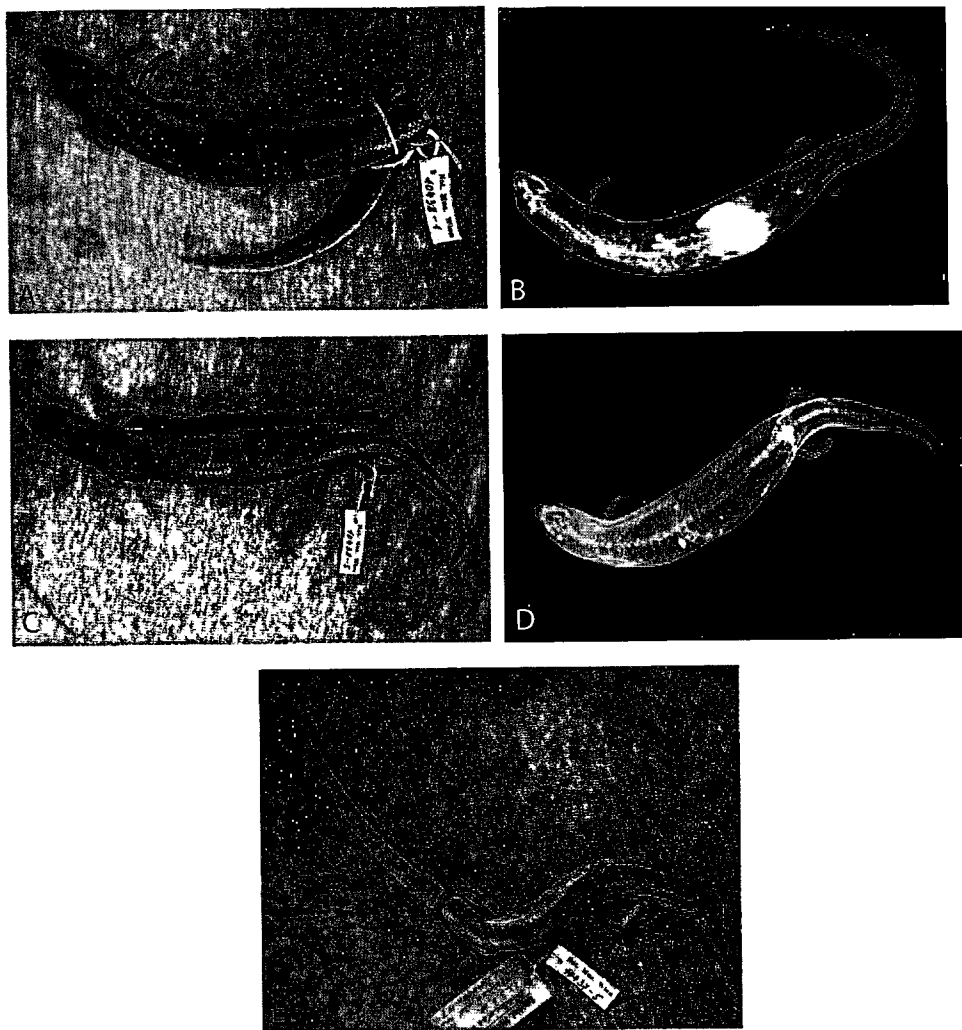
Figure 1. (A) Photograph and (B) x-ray of *Chalcides ocellatus* (NHMW 10438:1), SVL 123.0 mm, from "Kordofan region," Sudan, showing discoloration and mercury droplets *in situ*; (C) photograph and (D) x-ray of *Chalcides ocellatus* (NHMW 10438:5), SVL 97.0 mm, from "Kordofan region," Sudan, showing discoloration and mercury droplets; (E) photograph of *Chalcides ocellatus* (NHMW 10438:5) from Umm Rarnad, Kordofan, Sudan, illustrating typical color pattern of specimens collected from Sudan.

cooling effect of the ethanol preservative. The various gallium alloys which are liquid at room temperature are exotic and unlikely candidates for the liquid metal.

The droplets of the metal formed near-perfect spheres and showed low adhesive properties while exhibiting very strong cohesive properties. Gallium and its alloys are extremely adhesive to surfaces such as glass, while mercury has the highest coefficient of cohesion of any element. The metallic luster of the droplets, their opacity to x-rays, spheroid shape (indicative of very high surface tension), low adhesion to surfaces (slipperiness), very high cohesion (droplets introduced to one another very quickly formed a single large droplet), and the ready availability and historic use of mercury and mercury compounds complete the diagnosis of the liquid metal found in the two specimens as mercury.

Description of the Mercury as Found in the Specimens

The individuals found to have mercury in them were notably darker than conspecifics from the same museum that were collected at similar times and localities (Fig. 1E). Other specimens in the same lot (NHMW 104381:1–5) were discolored, but to a lesser extent.

The largest droplets found inside the specimens were less than 5 mm in diameter. A reticulated pattern of droplets was found along the folds of connective tissue. Most droplets observed were 0.5–1 mm in diameter, but microscopic examination revealed many droplets not visible to the unaided eye. Droplets were recovered with a concave probe. Approximately 1–2 mL of mercury were recovered from the two specimens. The mercury that was removed from the specimens was collected for safe disposal by the University of Kansas Department of Environmental Health and Safety.

DISCUSSION

Mercury has a long history of use as a preservative in the form of mercuric chloride ($HgCl_2$), a highly toxic white powder commonly called corrosive sublimate in the older literature (Farrar and Williams 1977). As a human toxin, mercury affects the tissues of the kidney, destroying its ability to remove waste products from the blood (Pauling 1988) and affects the brain and respiratory system (Turkington 1999).

Mercuric chloride was used both as a dry powder and as a component of fluid fixatives and preservatives (see reviews in Williams and Hawks 1987, Hawks and Von Endt 1990), but the authors are not aware of any use of pure mercury as a preservative. One of the earliest mentions of mercuric chloride as a preservative was its use in anatomical injections in 1678 (Cole 1921). Although mercuric chloride was most commonly used for the preservation of dry specimens, it was routinely added to fluid preservatives (e.g., Peck 1795), usually in concentrations of about 1 teaspoon per quart of preservative. For example, in 1825, Waterton recommended dipping bird specimens in a solution of corrosive sublimate (mercuric chloride) and alcohol before allowing them to air dry (Matthews 1973). A 1906 publication recommended that lizard skins be brushed with a solution of alum containing a few grains of mercuric chloride or arsenic (Anonymous 1906). Mercuric chloride was used as early as 1846 to fix flatworms (Jones 2001), and in 1854 was recommended as a fixative for liver cells in rabbit embryos (Galigher and Kozloff 1971). Mercuric chloride has been recommended for the preservation of *Ascaris* and *Planaria* (Anonymous 1944) and several other invertebrates (Lincoln and Sheals 1979).

For fluid preservation, mercuric chloride was sometimes mixed with a liquid to precipitate protein and harden tissue (Drury and Wallington 1980). When used in this way (or in combination with other fixing agents) mercuric chloride "almost invariably produces a brown to black granular deposit, distributed uniformly throughout the tissue" (Drury and Wallington 1980:45). Similar deposits were found in specimens NHMW 10438:1–5, which were stained dark gray, olive, and black.

Source of the Mercury

The introduction of mercury into the specimens is not recorded, and the curator of the NHMW was unaware of the presence of mercury in these specimens (Franz Tiedermann, pers. comm.). All five specimens in the series (NHMW 10438:1–5) were darkly stained, but mercury was recovered from only two of these specimens (NHMW 10438:1 and NHMW 10438:5). There was no elemental mercury found in the specimen jar in Vienna, but persons unknown may have removed the metal without making a note of their

actions. Metallic mercury may have been introduced into the specimens either intentionally or by accident. One scenario for intentional introduction of metallic mercury into the specimens would be a preparation for an x-ray of the alimentary canal (which would also explain the removal of the internal organs), however, this possibility was considered to be unlikely in this case as there are no records of such a procedure being carried out. Another possibility is that mercury may have accidentally been introduced into the specimens through a spill, such as the breaking of a thermometer or barometer.

Conversion of Mercuric Chloride to Elemental Mercury

Mercurous mercury (Hg_2^{2+}) or mercury (II), is the most common and easily obtained ion of mercury (Levason and McAuliffe 1977). Compounds with mercury (I) rapidly decompose into elemental mercury or mercury (II) compounds. Mercury (III) does not exist under standard laboratory conditions (Levason and McAuliffe 1977). Mercury (II) can be reduced to elemental mercury in a variety of ways, such as by replacement with other metals or hydrogen. These pathways of reducing mercury (II) to elemental mercury are dealt with only incidentally in the chemical literature as the desired product is usually the synthesized organic molecule rather than the mercury precipitate (Bloodworth 1977). The simplest of these processes is either acidolysis or reductive demercurization.

Mercuric chloride may be also reduced to elemental mercury in small amounts by the action of mercuric-chloride-resistant heterotrophic bacteria (Baldi et al. 1987), and mercury has a great affinity for amino acids and proteins as well (Falchuk et al. 1977). Another pathway is for the mercuric salts to be reduced to mercurous salts, which in turn are easily oxidized to the mercuric state (MacGregor and Clarkson 1974). Ethanol is known to react with aqueous solutions of mercuric salts to produce "various complicated mercury compounds" (Whitmore 1921:107), which may in turn be oxidized to elemental mercury, and the mercury-chloride bond "has appreciable solubility in organic solvents" such as ethyl alcohol (MacGregor and Clarkson 1974:465). A wide variety of chemicals have been added to fluid preservatives over the centuries in an attempt to improve their efficiency, or have leeched into the preservative from the container or closure components, including chromic acid, picric acid, glycerin, and arsenic. A survey of fluid preserved anatomy specimens in the Mütter Museum (Philadelphia) found that the preservative fluid in 21% of the containers tested positive for arsenic and 38% tested positive for lead (Thede 1996). Glycerin was often added to alcohol with the idea that if the solution evaporates, the glycerin would protect the specimens from dehydration (Beirne 1955). Thus, the necessary components for the activity of any of these pathways could be present in a fluid preserved specimen of this age.

CONCLUSIONS

The use of mercury in the form of mercuric chloride was fairly widespread in preservative treatments of both dry and fluid preserved specimens from the late 1500s to ca. 1900 when formaldehyde came into vogue as a fixative (Simmons 2002a, 2002b). Appropriate precautions should be taken when handling specimens and preservative fluids from this time period unless testing for mercury has shown it to be absent. Suspect or contaminated fluid preserved specimens should be handled only while wearing neoprene or nitrile gloves and working in a well-ventilated area. Avoid inhaling mercury vapors. Old preservative fluids and old specimens must be considered hazardous materials likely containing mercury or mercury compounds. In addition to the age of the

specimens, other indications that preservative treatments may have included mercury or mercuric chloride include darkening of specimens and unexplained spots in x-rays.

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FORMALDEHYDE HYPERBARIC FIXATION

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Abstract.—The technique described here relates to the fixation of fish in formaldehyde under hyperbaric conditions. Samples are placed in a chamber containing 5% formaldehyde. A film of oil is provided to create an interface between the liquid and the 2.5 bar air atmosphere. The minimum contact time at this pressure is 24 hr. The tissues are then desaturated in stages. At the end of the cycle the samples are rinsed and placed under ethanol. The formaldehyde is neutralized. All of the operations are performed with the principal concern being to expose the researcher to as little formaldehyde vapor as possible.

INTRODUCTION

Formaldehyde is a gas, sold in the form of a 40% aqueous solution, commonly referred to as formalin. It is generally used in a proportion of a few percent of stock solution in water. New regulations regarding carcinogenic, mutagenic and reprotoxic (CMR) products (INRS 2006) require that if a product cannot be replaced by a substitute, the quantities handled should be reduced and the workers maximally protected from exposure. We worked within this framework on the fixation of small-to-medium size fish. The basic principle is one of saturating tissue under pressure in order to ensure that samples are fixed to their core. Desaturation is carried out with a "decompression-stages" protocol modeled on those stages observed for resurfacing divers. The formaldehyde is neutralized during the final phase.

PRINCIPLE OF FIXING TISSUE IN FORMALDEHYDE UNDER HYPERBARIC CONDITIONS

The working principle is one of the directly saturating tissues with dissolved, pressurized formaldehyde gas. From a physiological point of view (Haldane model), various groups of tissues, the so-called "compartments" (Juvenspan and Thomas 1997), are thought to exist. This classification is based on the fact that tissues are more or less readily in contact with gases (in the lungs, for example) or circulating blood. Tissues saturated with the latter include bone and fat. According to American (US Navy 2005) and French (FFESSM 2000) diving table data, saturation of all tissue groups is completed after seven periods totaling 720 min (12 hr). It should be noted that the present study involves dead tissues, i.e., neither ventilated nor irrigated. Nevertheless, the values to be used are those of the two tissue groups mentioned above (bone and fat), even if the present paper relates more to muscle tissues.

After the period at 2 bar (roughly a 12-hr dive at −20 m), the tissues are thoroughly saturated with formaldehyde. The process of depressurization in stages begins next (as when resurfacing during a long-lasting conventional dive). Desaturation continues, in stages of several hours at various intermediate pressures, until all tissues are desaturated. The stage protocols are based on the above-mentioned French Navy tables, extended to diving at −60 m, and provide a factor-three safety margin (FFESSM 2000). These stage parameters, starting at −20 m, were modified and adapted to the fixation requirements.
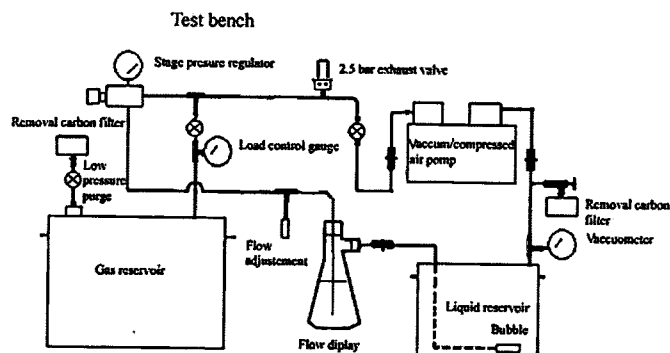
Figure 1. Component parts of the initial test bench.

EXPERIMENTAL PROCEDURES

Determination of Pressure and Time Constants for the Desaturation Stages

Initially, we sought to work directly in a phase of saturating, pressurized formaldehyde vapor. We were dissuaded, however, by the experiment's inherent danger, as well as the fact that formaldehyde breaks down in the presence of oxygen (Batista and Iwasita 2006). Nevertheless, we made use of the initial experimental set-up to develop the stage protocol.

A diagram of the test bench is presented in Figure 1. For reasons of asepsis (samples are tested over several days at room temperature), the following protocol was chosen:

1) The samples are placed in the "gas reservoir."
2) A small amount of 5% formaldehyde is placed in the "liquid reservoir." A Divac 1.2 (100 mm Hg) vacuum pump/compressor depressurizes the "liquid reservoir," thus turning the formaldehyde into vapor form and sending it to the "gas reservoir" after a few minutes.
3) A device for bubbling air in a sulfite bath is then put in place in order to eliminate oxygen in the air for the compression sequence.
4) The same pump, now switched to compression, pressurizes the samples at 2.0 bar for approximately 24 hr.
5) A micro-flow valve is then used to reduce the pressure in stages (residual vapors are trapped in a final outflow filter). The presence of a small amount of formaldehyde ·in vapor form is insufficient to fix the samples, but it ensures that the samples are partially sterilized, which helps to avoid decomposition at room temperature.

Various stage protocols were tested on various species. The following values were selected for the intermediate-stage pressures: 1.7 bar, 1.4 bar, 1.0 bar, 0.6 bar, 0.3 bar. The parameters for each stage are indicated in Table 1. For depressurization rates (Table 2), minimal values are expressed as the equivalent of a diver's surfacing rate in meters per minute (1 m/min is roughly 0.1 bar/min). The visual test selected to evaluate the proper execution of the desaturation procedure was the presence of bubbles in the vitreous humor of the eye, in accordance with hyperbaric ophthalmic medicine (http://www.snof.org/maladies/diving.html [in French]).

The fishes tested included breams, cods, mackerels and sardines. In fresh sardines (5 specimens), bubbles were not observed in the eye cavities using a binocular dissecting microscope (Fig. 2). On the other hand, two animals had damaged skin. It was not possible to know if this deterioration was due to the fact that the fish had spent a number

Table 1. Parameters for each depressurization stage.

Pressure (in bars)	Time under pressure
Initial = 2.0	24 hr
Stage 1 = 1.7	1 hr
Stage 2 = 1.4	2 hr
Stage 3 = 1.0	3 hr
Stage 4 = 0.6	4 hr
Stage 5 = 0.3	12 hr

of hours at room temperature or if this phenomenon was a problem of desaturation, given that sardines are by their nature fragile to handle. For mackerel, cod and bream we did not observe damage to the surface of or the presence of bubbles in the vitreous humor.

Fixation of Samples (Fish) under Hyperbaric Conditions

The test bench modified in July 2007 is presented in Figure 3 and the start-up procedure is presented in Figure 4.

1) The "transfer reservoir" is filled with 10 L of water; a film of oil (50 ml) is then deposited on the surface of the liquid. Formaldehyde QSP is added with a 50 ml syringe.
2) The samples are placed in the "experimental chamber."
3) The air circuit's release valve and the outlet gate are opened. The 'experimental chamber' fills by gravitational force. The outlet gate and the valve are closed.
4) Pressurization using a compressor (2 bar).
5) Fixation (24 or 48 hr) at 2.0 bar.
6) Stages: the micro-flow valve is opened to control the transition between pressure levels. Flow is bubble by bubble. Once the required pressure is reached, the valve is closed until the following stage.
7) Draining after return to atmospheric pressure: the outlet gate is opened and approximately 0.15 bar is injected into the chamber. The liquid and the oil are forced back into the transfer reservoir. The three-way control valve isolates the system.
8) The experimental chamber is opened. The samples, under a film of oil, are rinsed and placed under ethanol.
9) If no new samples are to be treated the remaining formaldehyde can be neutralized by emptying it into a neutralization tank containing an aqueous solution of ammonium bicarbonate or carbonate (Kawamata and Kodera 2004).

Table 2. Depressurization rates during each stage of the process.

Depressurization stages (in bars)	Rate during each stage (in m/min)
2.0→1.7	0.6
1.7→1.4	0.3
1.4→1.0	0.3
1.0→0.7	0.3
0.7→0.3	0.15
0.3→atm	0.06

Figure 2.   Absence of bubbles in the vitreous humor of sardines in comparison with a hyperbaric accident in the rat (bubbles in the eye of a rat subjected to high pressure followed by sudden decompression; the bubbles are visible evidence of a decompression accident). Radiograph provided by Dr. Lanphier at http://www.snof.org.

Several tests were carried out in July 2007 on batches of 4 or 5 frozen mackerels. No specific difficulties were encountered. The animals appeared properly fixed compared to controls treated conventionally (one week in a 5% bath).

Rinsing after Fixation

For rinsing the animals after fixation, and in order to save water and to shield the manipulator from residual formaldehyde vapor, we took advantage of an isolation spraying system which we developed and whose diagram is presented in Figure 5. This apparatus also makes it possible to treat small quantities of animals with formaldehyde at atmospheric pressure in a completely sealed manner.

Packaging under Alcohol

For samples of reasonable size (30 cm maximum) we also developed a novel technique for limiting the researcher's exposure to alcohol vapor. Heat-sealed pouches were produced and the alcohol was stored at –30°C. The fixed sample is placed in the pouch, –30°C alcohol is added and the entirety heat-sealed. The procedure is low-risk since the vapor pressure of alcohol is very low at this temperature (Figs. 6 and 7). Another advantage of the method is a substantial decrease in alcohol requirement, 30% less compared to conventional packaging. For larger animals, storage in drums remains useful.
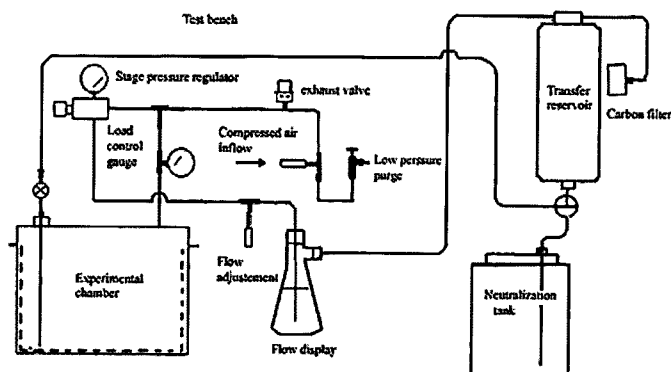
Figure 3.   Test bench modified for a liquid phase process.
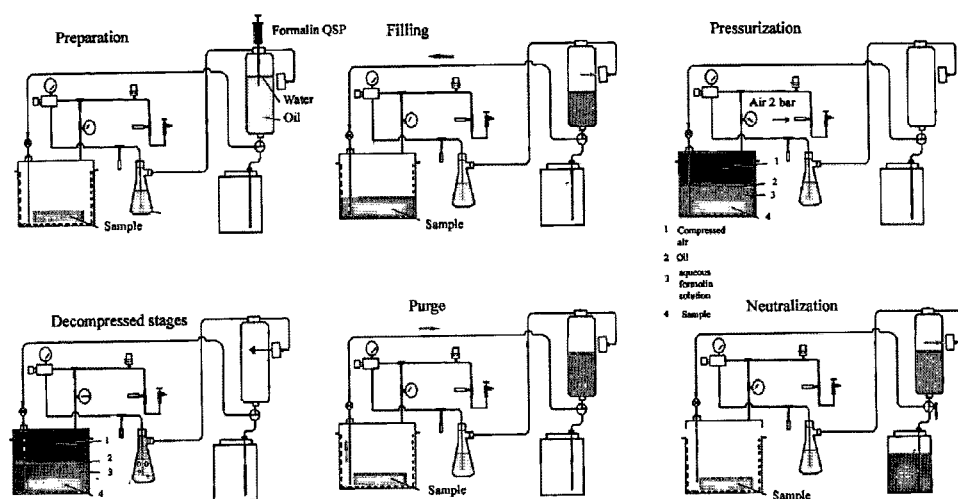
Figure 4.   Summary of the fixation process.

DISCUSSION

This novel method meets our three initial objectives: 1) To improve researcher' security by eliminating exposure to formaldehyde vapor, in accordance with new European Community's CMR regulations (INRS 2006); 2) To decrease the time lapse samples are in contact with formaldehyde; and 3) To produce a device that can be used as well in the laboratory as in the field.

The problem of vapor exposure is solved: the system is completely sealed in its hyperbaric phase. Workers are exposed a little during the rinsing process: the thin film of oil covering the samples upon exit from the chamber greatly reduces exposure by formaldehyde vapor. The film of oil is eliminated during the rinsing phase, which is carried out in a closed chamber and is autonomous. The formaldehyde is then neutralized by ammonium bicarbonate. The process (gravity) is such that the oil comes to the surface after neutralization. Any vapors are thus very few. In our view, ammonium bicarbonate is the most appropriate solution for neutralization: ammonia, which neutralizes formaldehyde by forming hexamine (Frosin et al. 1980), was also tested (hospital technique), as well as sodium hypochloride (bleach). The latter two products require the use of masks whereas ammonium bicarbonate, a skin irritant, requires only gloves. Moreover, bleach can, under certain conditions, react with formaldehyde with explosive results (Walker 1975) and form bis(chloromethyl)ether, a powerful carcinogen (http://www.ch-aix.fr/pro/theme/anapath/prevrisques.htm). We did not test the destruction of aldehydes by potassium permanganate (Picot and Grenouillet 1992).

Reducing the time necessary for the fixation of tissues was also an original objective. Saturation of tissues under hyperbaric conditions meets this aim. Maceration time under formaldehyde is clearly less compared to the conventional immersion method: for example, large samples typically requiring roughly one month of contact in a formaldehyde bath, not including rinsing time, can be treated in 48–72 hr.

Rinsing in an isolated system uses water economically: the typical rinsing process consumes roughly 7,200 liters of water (5 L/min) compared to 5×10 liters for our method. Note that in the chamber, the volume of compressed air is small compared to that of the water/formaldehyde mixture (this is also a pressurized-explosion safety

Sprayers

Acces
hatch

0   50   cm

At the bottom of the
chamber, residual lever
after emptying
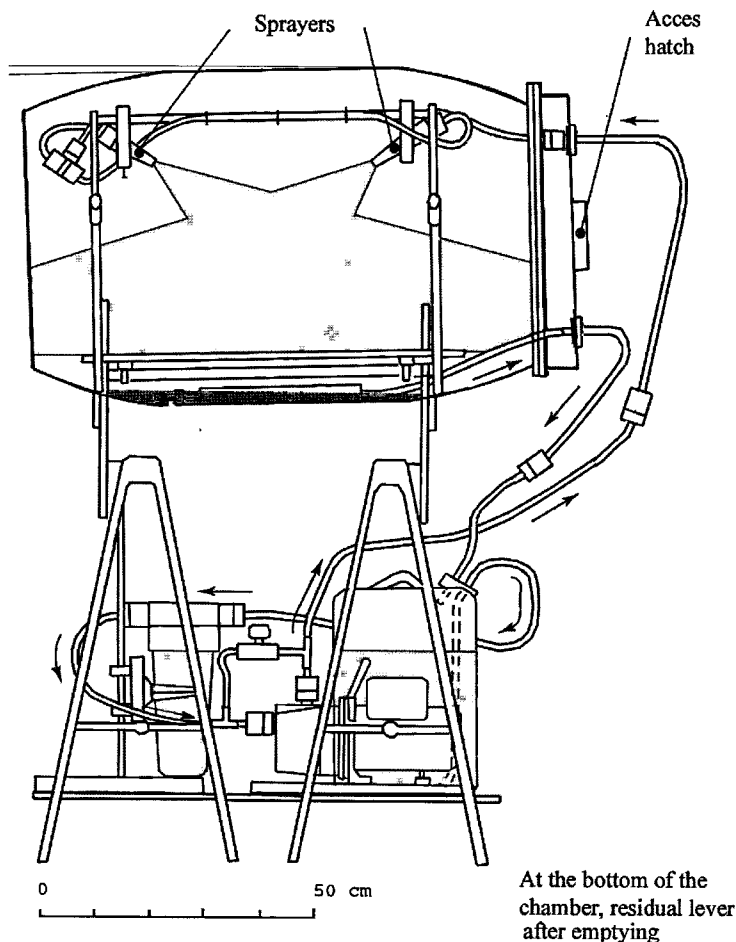
Fluid circulation diagram

Figure 5. Formaldehyde-treatment and rinsing apparatus (pressure of circulating liquid = 3 bars): in fixation mode, release via the spray-heads ensures the formaldehyde is vaporized throughout the chamber.

criterion). However, the presence of micro-leaks over long periods can jeopardize the experiment. As a safety measure, we coat our seals/joints with Loctite 5923®, a so-called "tacky film."

Determining decompression stages proved to be empirical in the absence of data on dead tissues. It should be noted, however, that the observation of bubbles in animal eyes (http://www.snof.org/maladies/diving.html) remains a rather reliable criterion for the success or failure of the decompression procedure.

All of our experiments were performed in the laboratory. This does not mean that development of a field method has been neglected. Most laboratories have basic equipment such as electric compressors, for example. Manual pressurization or a supply of pressurized gas from a scuba-diving tank, for example, could be considered for use in a field setting. All our decompression stages were carried out manually thus they are immediately applicable in the field. Nothing, however, prevents the use of an automated stage controller system fed by pressure sensors (either in the laboratory using its electrical
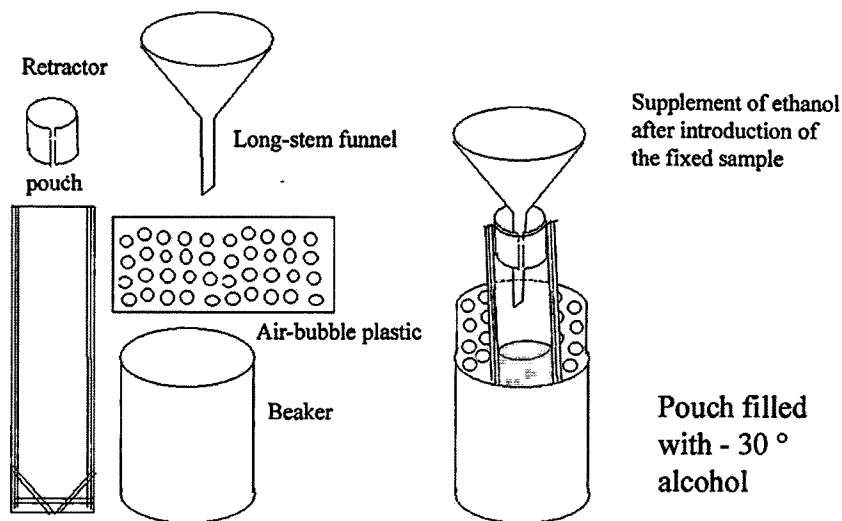
Figure 6.   Packaging under alcohol at low temperature.

supply or in the field using a battery source). There are two principal advantages of a computer-controlled system: on one hand, it would be possible to automatically compensate for possible micro-leaks, on the other, the researcher would no longer be restricted to a schedule governed by the requirements of the decompression schedule.

Figure 7.   Samples of mackerel packaged in plastic pouches.

The entirety of our process has been filed with France's *Institut National de la Propriété Intellectuelle* (INPI Soleau fund).

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POTENTIAL EFFECT OF RESIDUAL ANTI-PARASITIC COMPOUND IN MUSCLE TISSUE ON A MUSEUM DERMESTID BEETLE COLONY

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Abstract.—Many natural history museums with vertebrate collections process skeletal material using dermestid beetles (Dermestidae, *Dermestes* spp.). The present study experimentally tests the postmortem effects prophylactic drug treatment of vertebrates can have on dermestid beetles. A wolf specimen (*Canis lupus hudsonicus*) obtained from the Denver Zoo was processed in the Denver Museum of Nature & Science dermestid beetle colony. Osteological material with adhering muscle tissue was introduced into the beetle colony. Subsequently, a dramatic decline in the health of the colony was noted. A controlled experiment was conducted to determine if the wolf muscle tissue was, in fact, responsible for the decline. Dermestid beetles fed the wolf tissue showed much more pronounced mortality, particularly in the larval stages, than dermestids fed control beef. A review of the medical history of the wolf indicated that the most recent medicine the animal had been administered on a regular basis was a monthly dose of lufenuron, an anti-parasitic compound used to control fleas, and milbemycin oxime, used to control endoparasitic worms. According to the product information, lufenuron primarily affects the egg development of fleas and is also a chitin synthesis inhibitor. Lufenuron remaining in the muscle tissue of the wolf, approximately two months after its last treatment, appears to have had a profound effect on the development of the larval stages of the dermestid beetles and on the ability of final instars to successfully pupate.

INTRODUCTION

Many natural history museums maintain dermestid beetle colonies (*Dermestes* spp., Dermestidae) to process skeletal material before introducing the bones into collections. No work has been done to determine what effect prophylactic or medical treatments on living vertebrates may have on dermestid colonies after dermestids feed on potentially contaminated animal tissue. Several studies indicate that drugs or even insecticides ingested or injected into a human can be detected in the tissues of fly maggots feeding postmortem (Beyer et al. 1980, Goff and Lord 1994, reviewed in Gagliano-Candela and Aventaggiato 2001, Kintz et al. 1990, Lord 1990). Thus, it is possible that drugs administered to vertebrates while living can be metabolized by beetle larvae feeding on the vertebrates postmortem. We present evidence of just such an occurrence as well as the effect of this ingestion on the dermestid beetles.

At the Denver Museum of Nature & Science (DMNS), colonies of dermestid beetles have been maintained for over 20 yr. The DMNS has a cooperative affiliation with the Denver Zoo and periodically is asked to process Zoo specimens that have died. Previously, the DMNS has processed a rhino, an orangutan, and an elephant from the zoo in the dermestid colony with no ill effects observed among the dermestids. In August 2003, the DMNS received a dead male arctic wolf (*Canis lupus hudsonicus*) from the Denver Zoo. The Zoo acquired this wolf as a newborn May 1995 and it died 4 August 2003. The wolf was eviscerated and placed in a 6°C cooler for about 10 days at the Zoo. The wolf was then transferred to the DMNS and was frozen prior to removal of remaining tissues by trained volunteers. We introduced some bones with dried adhering muscle tissue into the dermestid colony on 20 August 2003. Wolf bones with adhering tissue were continually introduced into the beetle colony throughout the remainder of August and into October. In mid-October, a severe decline in the beetle colony was noted.

On 12 November 2003 new beetles were purchased from Carolina Biological Supply Company to augment the declining beetle population. Initially, the new beetles were maintained in a separate case (Case 2) from the original beetle colony and were fed non-wolf material. The original beetles were left in their own case (Case 1) and were also being fed non-wolf material. By January 2004, the new beetle colony in Case 2 was thriving while the population in Case 1 continued to decline. On 1 February 2004, we combined beetles from Case 2 with the remaining beetles from Case 1, placing all beetles in Case 1, and by 8 February we noted a decline in this combined colony. On 1 March 2004, it was observed that the colony contained mostly adult beetles but very few larvae. By 28 March, no larvae could be seen in the colony. No evidence of mite or other ectoparasite infestation in the colony was seen when nest material and dermestid life stages (eggs, larvae, pupae, and adults) were examined under a microscope, nor was there evidence of fungal or mold spores.

The decline in the beetle population was traced to the introduction of the wolf muscle tissue in Case 1 and it was suspected that the tissue may have been tainted with some chemical causing this decline, and that residue remaining in the case caused the continued decline even after no more wolf bones were introduced. On 18 April 2004, new beetles were procured from Carolina Biological Supply Company and quarantined from those remaining in the lab colony. They were placed in a sterilized aquarium and were fed non-wolf material. By 25 April the new beetles were thriving. On 5 May 2004, an experiment was initiated to determine if the wolf muscle tissue had been the cause of the beetle population decline.

METHODS

On 5 May 2004, two experimental beetle colonies and two control beetle colonies were established. The colonies were maintained in four sterilized plastic animal cages 33.5 cm long × 19.5 cm wide × 21 cm high. The cages were labeled Control Old, Control New, Experimental Old, and Experimental New. In the Control Old and in the Experimental Old cages, we introduced 12 adult beetles, 12 small larvae, and 12 large larvae from the original colony that had survived exposure to the wolf muscle tissue. In the Control New and Experimental New cages, we introduced 12 adult, 12 small larvae, and 12 large larvae from the newly established colony (beetles purchased from Carolina Biological Supply Company) that had never been exposed to the wolf tissue. In each of the four cages, a small covered jar of water was placed for moisture and a small piece of cotton batting to provide pupation and egg laying sites for the beetles (standard inclusions in beetle colonies; see Russell 1947, Wilkins 1981, and N. Pliler, pers. obs.). In the experimental cages, equal-sized pieces of the remaining wolf material were placed. In the control cages, pieces of beef bone with adhering muscle tissue were placed, approximately equal in size to the wolf bone with muscle tissue fed to the experimental beetles. Growth of each of the four colonies was monitored once a week for 14 wk. This interval is well within the 45-day life cycle of dermestid beetles, which spend 3 days as eggs, 30 days as larva (the first 20 of these are considered the rapid growth phase), 7 days as pupa, and 5 days as adults (Russell 1945). Thus, the interval should be of sufficient time to see changes in the various life stages of the control and experimental beetles. The number of dead adults, dead small larvae, and dead large larvae in each cage was recorded as well as the condition of the colonies. Dead larvae and adults were removed. Chi-square (χ^2) tests ($\alpha = 0.05$) were used to analyze differences in the number of dead beetles (adults and larvae) between treatments.

Table 1. Number of dermestid beetles of three developmental groups found dead each week in Control Old colony. *First row (Week 0) indicates number of beetles first introduced into the colony. Numbers in subsequent rows indicate the number found dead.

Week	#Adults	#Small larvae	#Large larvae	Notes
0*	12	12	12	
1	2	0	0	
2	1	0	0	
3	3	0	0	
4	0	0	0	Colony flourishing
5	0	0	0	Colony flourishing
6	1	1	2	Colony flourishing
7	0	0	0	Colony flourishing
8	0	0	0	Colony flourishing
9	2	0	2	Colony flourishing
10	0	0	0	Colony flourishing
11	0	0	0	Colony flourishing
12	0	0	0	Colony flourishing
13	0	0	0	Colony flourishing
14	0	0	0	Colony flourishing

RESULTS

Control Old and New.—By the end of the experiment (week 14) mortality in the Control Old (Table 1) and Control New colonies (Table 2) was very low. These colonies were thriving with numerous larvae of all sizes (too numerous to count) and adult beetles.

Experimental Old.—All the original small larvae were dead by the 3rd week although a few eggs hatched and developed subsequently (Table 3). No larvae of any size were present after the 8th week. After week 14, only two adult beetles were still alive in the cage. These two beetles were not from the original 12 adults first placed in the cage but developed from larvae that pupated into the adult stage over the course of the experiment.

Experimental New.—All the original small larvae were dead by the 6th week and none were seen by the 9th week (Table 4). No larvae of any size were present by the 10th week. After the 14th week, no adults or larvae were alive. In both Experimental colonies, there was evidence that many of the larvae died during molting. Many of the pupae also died.

A contingency table analysis indicated that the number of dead beetles of the various life stages is not independent of treatment (contingency $\chi^2 = 15.182$, df = 6, $\rho < 0.025$). There was no statistical difference in the number of dead adults among the four treatments (Table 5; $\chi^2 = 2.00$, df = 3, $0.5 < \rho < 0.9$). There was a statistical difference in the number of dead small larvae among the four treatments with far more found in the experimental treatments than in the controls (Table 5; $\chi^2 = 23.58$, df = 3, $\rho < 0.001$). Although more dead large larvae were found in the experimental cages, there was no significant difference in the number of dead large larvae among the treatments (Table 5; $\chi^2 = 7.52$, df = 3, $\rho \approx 0.05$).

DISCUSSION

The larval and adult stages of the dermestids in the Controls were healthy, growing colonies throughout the duration of the experiment. In contrast, both the New

Table 2. Number of dermestid beetles of three developmental groups found dead each week in Control New colony. *First row (Week 0) indicates number of beetles first introduced into the colony. Numbers in subsequent rows indicate the number found dead.

Week	#Adults	#Small larvae	#Large larvae	Notes
0*	12	12	12	
1	3	0	0	
2	2	0	0	
3	0	0	2	
4	0	0	0	Colony growing; one dead pupae
5	0	1	1	Colony flourishing
6	1	0	0	Colony flourishing
7	0	0	0	Colony flourishing
8	2	0	0	Colony flourishing
9	1	0	0	Colony flourishing
10	2	0	0	Colony flourishing
11	1	0	0	Colony flourishing
12	2	0	0	Colony flourishing
13	0	0	0	Colony flourishing
14	0	0	0	Beetles healthy; all food gone

Experimental beetles and Old Experimental beetles were detrimentally affected by exposure to the wolf tissue. The larval stages from the Old colony that had already been exposed in the past to the wolf muscle tissue were immediately affected by the exposure and a high mortality among the larval stages was noted after one week. The New beetles that had never before been exposed to the wolf material took only slightly longer to show a decline among the larval stages. Exposure to the tissue seemed to detrimentally affect larval development and pupation and may also have affected egg hatching. The

Table 3. Number of dermestid beetles of three developmental groups found dead each week in Exp. Old colony. *First row (Week 0) indicates number of beetles first introduced into the colony. Numbers in subsequent rows indicate the number found dead.

Week	#Adults	#Small larvae	#Large larvae	Notes
0*	12	12	12	
1	1	3	2	No pupae evident
2	0	6	2	
3	2	3	2	1 of large larvae almost dead; 3–4 tiny live larvae
4	3	0	1	1 of 3 adults barely alive; pupation problem (elytra missing, wings damaged)
5	1	0	0	No larvae seen; 5 live beetles
6	2	1	0	No larvae seen; 3 live beetles
7	0	0	1	3 live beetles left
8	2	2	0	3 live beetles left
9	1	0	0	3 live beetles left
10	0	0	0	3 live beetles left
11	0	0	0	3 live beetles left
12	1	0	0	2 live beetles
13	0	0	0	2 live beetles
14	0	0	0	2 live beetles

Table 4. Number of dermestid beetles of three developmental groups found dead each week in Experimental New colony. *First row (Week 0) indicates number of beetles first introduced into the colony. Numbers in subsequent rows indicate the number found dead.

Week	#Adults	#Small larvae	#Large larvae	Notes
0*	12	12	12	
1	0	2	1	No pupae evident
2	2	3	1	1 small and 1 large larvae did not look healthy
3	1	2	1	1 small larva died during molting
4	1	1	4	1 large larva died during molting; 1 adult seems to be unhealthy; 3 dead adults died during pupation
5	1	2	1	5 live beetles
6	3	3	0	
7	1	0	0	4 live beetles and 1 small live larva seen (nothing else seen alive)
8	3	0	0	1 live adult
9	2	1	2	1 live adult
10	1	0	2	1 live adult
11	0	0	0	1 live adult
12	0	0	0	1 live adult
13	0	0	0	1 live adult
14	1	0	0	No adults or larvae alive

experiment strongly suggested that the wolf tissue was responsible for the first decline in the beetle colony and that residual material from the wolf material left in Case 1 (the case originally housing the old beetles) caused the decline when the first batch of new beetles, originally housed in Case 2, was combined with the old beetles.

After conducting the experiment, the medical history of the wolf was investigated. The wolf was given a prophylactic containing 0.5 mg/kg body weight of milbemycin oxime and 10 mg/kg body weight of lufenuron against endo- and ectoparasites. The last tablet was administered on 1 July 2003, slightly over one month prior to death. According to the product information sheet, milbemycin oxime contains the oxime derivatives of 5-didehydromilbemycins in the ratio of about 80% A_4 ($C_{32}H_{45}NO_7$, MW 555.71) and 20% A3 ($C_{31}H_{43}NO_7$, MW 541.68) and is used to control the tissue stage of the heart worm larvae (*Dirofilaria immitis*), and the adult stages of hook worm (*Ancylostoma caninum*), round worm (*Toxocara canus* and *T. leonina*), and whip worm (*Trichuris volpis*). Lufenuron is a benzoylphenylurea derivative with the chemical composition N-[2.5-dichloro-4-(1,1,2,3,3,3,-hexafluoropoxy)-phenylaminocarbonyl]-2,6-difluro-benzamide ($C_{17}H_8Cl_2F_8N_2O_3$, MW 511.15) and is classified as an insect development inhibitor. Since the milbemycin oxime is used to treat endoparasitic worms, it is unlikely that this compound was responsible for the beetle decline. Lufenuron does not kill adult fleas but

Table 5. Summary of number of dead dermestids found in the four colonies between weeks 1–14.

	#Adults	#Small larvae	#Large larvae
Control Old	9	1	4
Control New	14	1	3
Experimental Old	13	15	8
Experimental New	16	14	12

disrupts the flea's life cycle at the egg stage (Dean et al. 1999, Rust and Dryden 1997) and also acts as a chitin synthesis inhibitor (Wilson and Cryan 1997). Several studies have demonstrated that lufenuron can affect growth and development of various insects including termites (Isoptera) (Su and Scheffrahn 1996), fruitflies (Diptera) (Wilson and Cryan 1997), caterpillars (Lepidoptera) (Da Silva et al. 2003, Edomwande et al. 2000, Gogi et al. 2006, Whiting et al. 2000), planthoppers (Homoptera) (Gogi et al. 2006), green lacewings (Neuroptera) (Bueno and Freitas 2004), and potato beetles (Coleoptera) (Zabel et al. 2002). Data from the present study suggest that lufenuron can also act to disrupt the larval development of dermestid beetles, although the adult stages of the beetles were not as detrimentally affected.

The Denver Zoo gives this prophylactic to wolves and other canids, and other zoos administer lufenuron to non-canids as well. Inoculations with lufenuron were given to the canids once a month via oral tablet, usually inserted into a meat ball and tossed into the enclosures of the canids. The treatments may be somewhat more frequent than once every 30 days depending on when the zoo keepers are doing their scheduled rounds. Thus, any given animal may have a 3–7 day period when a higher dosage of lufenuron is in their system. The substance begins to dissipate in the body around day 20.

The male wolf that was given to the museum was given its last lufenuron tablet on 1 July 2003, prior to its death. The animal was diagnosed with central neurological disorder resulting in an inability to walk and was examined on 3 August 2003. At that time, he was given salines, but no drugs. The wolf was anesthetized with isofluorine via a face mask, but this would not have entered the muscle tissue (the only tissue given to the beetles). The wolf was found dead on the morning of 4 August 2003 (slightly over one month after being treated with lufenuron). It appears that the lufenuron remained in the tissue of the wolf in a high enough concentration one month after treatment and nearly two months prior to being introduced into the dermestid colony to detrimentally affect the development of the beetles.

To test this hypothesis, a small amount of wolf tissue that was adhering to the pelt, as well as a section of the pelt, was sent to Pennsylvania Animal Diagnostic Laboratory System, New Bolton Center, (PADLS). Lufenuron was not detectable in the submitted samples at the method detection limit of 100 ppb, however, trace amounts of the compound were probably in the tissue samples but at levels too low to detect (Lisa Murphy of PADLS, pers. comm.). It also may be that lufenuron concentrates in muscle tissue rather than in dermal or subdermal tissue, however, by the time the material was sent to the PADLS, all that could be sent was a section of the pelt and a bit of subdermal tissue. Thus, despite the negative results from the PADLS lab, it is suspected that tiny amounts of lufenuron remaining in the muscle tissue fed to the beetles were responsible for the decline in the beetle colony. Other studies have shown that small amounts of lufenuron (<1 ppm) can affect insect development (Dean et al. 1999, Whiting et al. 2000, Wilson and Cryan 1997).

Because museums do, on occasion, process vertebrates obtained from zoos and since lufenuron is a common anti-parasitic compound administered to canids as well as other vertebrates, we recommend that museums with dermestid colonies explore the medical history and prophylactic treatment of vertebrates obtained from zoos prior to introducing any specimens to dermestid colonies. Lufenuron is also common in flea collars and in other products used to treat domestic pets and livestock so caution should be taken before introducing carcasses of such animals into dermestid colonies. In addition, it is recommended that museums avoid processing vertebrate material from

animals treated with lufenuron or provide the tissue treated with lufenuron to a small number of beetles to monitor its effect.

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PROFILING NATURAL HISTORY COLLECTIONS: A METHOD FOR QUANTITATIVE AND COMPARATIVE HEALTH ASSESSMENT

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Abstract.—Quantitative methods for assessing the health of a natural history collection are of paramount importance for prioritizing the investment of time and resources and ensuring the long-term stability and usability of a collection's invaluable specimens. Proposed profiling methods have provided institutions with important data on the condition of their collections, but to date, no method has been implemented to permit comparisons across multiple, unrelated collections at the same institution. Presented here is a profiling method developed to allow comparisons among the ten natural history collections at the Illinois Natural History Survey (INHS). The method employs eight profiling indicators, conservation status, processing state, container condition, label condition, identification level, arrangement level, data quality, and computerization level, each graded on a scale of 1 to 4 ("problematic" to "ideal"), with 3, across all collections and all indicators, being considered "acceptable." A database was developed for profiling data entry and analysis. Finally, in order to elucidate the value of collection profiling, the results of pilot studies in the insect and mollusk collections at the INHS are presented.

Introduction

In an era of declining funding for natural history collections, administrators need evaluative tools for prioritizing expenditures. Meanwhile, curators and collection managers need quantitative measures of the health of various parts of their collections in order to prioritize their efforts and make convincing arguments for their collections' financial support.

Although exhaustive evaluations have their place in collections management research (e.g., Cato 1990, Waller and Simmons 2003), collection profiling, as standardized collection health assessment is called, needs to be more efficient. Natural history collections are far too large to evaluate on a per-specimen basis, so profiling involves the assessment of a standard storage unit such as a drawer of pinned insects, an herbarium cabinet cubby, a shelved-box of fluid- preserved fish, a box of annelid slides, or a drawer of mammal or bird skins. The actual process involves the brief inspection of each storage unit in the collection and the evaluation of its condition in predefined categories on a predefined scale.

Various systems have been developed for profiling collections. One of the first such systems (McGinley 1989, 1993), developed in the Department of Entomology at the United States National Museum of Natural History (NMNH), was tailored for entomological collections. This system was used to compare different parts of the NMNH collection, and to compare it to entomological collections of other institutions. McGinley's (1993) profiling unit was a single drawer, vial rack, or slide box of insect specimens, graded on a single scale from 1 to 10 (Table 1). Williams et al. (1996) modified McGinley's (1993) method to assess vertebrate collections (Table 1).

The scales for both the McGinley (1993) and Williams et al. (1996) methods roughly followed the temporal process of specimen curation as performed by a typical taxonomist in his or her respective field, and were intuitive and easy to use. Also, because each system only had a single scale, collection profiling could be done relatively quickly. However,

Table 1. McGinley (1993) profiling system for insects and the Williams et al. (1996) modification for vertebrates.

Profile score	McGinley 1993 (insects)
1	Conservation problem (e.g., loose, unprepared specimens)
2	Specimens unidentified, inaccessible (e.g., pinned and labeled, but unsorted)
3	Specimens unidentified, accessible (e.g., rough-sorted)
4	Specimens identified but not integrated into collection
5	Specimens identified but curation incomplete (e.g., in substandard storage containers)
6	Specimens identified and properly curated in accordance with departmental collection standards
7	Data capture: species level inventory
8	Data capture: specimen label data capture
9	Data capture: research data capture
10	Scientific voucher material

Profile score	Williams et al. 1996 (vertebrates)
1	Acquisition: potential exists for loss of specimens, specimen parts, and/or associated data
2	Stabilization: basic preservation, processing, compilation and organization of records, and protection
3	Registration: cataloged and labeled (provisionally available for use)
4	Processing: supplementary processing and labelling completed
5	Curation: generally organized and retrievable
6	Storage: stored permanently with room for growth and associated materials
7	Maintenance: records quality-checked and cross-referenced and loan transactions updated

using a single profiling scale limits the assessment of particular problems. For example, in the McGinley (1993) method, a collection could be nearly perfectly curated, with full computerized data capture, yet still only rate a 5 out of 10 if the specimens were stored in substandard hard-bottom unit trays.

In order to address this weakness in assessing the true nature of a particular profile score, the NMNH developed an expanded profiling scheme. The method, as implemented by the Department of Invertebrate Zoology, included six dimensions, or profiling indicators, each scored on a scale of 1 to 5 (Table 2) (Bright et al. 2000, Moser et al. 2001). This new system provided for greater depth and usability of the profiling data. Scoring six profiling indicators takes longer than profiling on a single scale, however. Also, although the Moser et al. (2001) system allowed for comparisons among several natural history collections, it was not implemented beyond the NMNH Department of Invertebrate Zoology.

The mission of the Illinois Natural History Survey (INHS) is to "investigate and document the biological resources of Illinois and other areas, and to acquire and provide natural history information that can be used to promote the common understanding, conservation, and management of these resources" (www.inhs.uiuc.edu/welcome). Inherent to this mission is being a long-term repository of natural history specimens as

Table 2. Profiling system of the Department of Invertebrate Zoology at the NMNH (gray cells indicate unused profiling scores).

Profile score / indicator	1	2	3	4	5
Conservation Status	Unstable	Degraded	Stable*	Optimal	
Processing State	Unprocessed	Sorted	Fully Processed*		
Storage Containers	Substandard	Musem-quality containers*			
Arrangement	Not arranged	Needs improvement	Fully arranged*		
Identification	Not identified	Identified to gross level	Identified to useful level*	Identified to accepted standard	Identified by expert
Inventory	Not inventoried	Full inventory of some lots	Collection-level inventory	Lot-level inventory*	Full inventory

*These grades were considered "acceptable"

permanent records of the historic flora and fauna. To that end, the INHS maintains a diversity of natural history collections: fungi, plant, annelid, mollusk, crustacean, insect (and other arthropods), fish, reptile, bird, and mammal collections (geological and anthropological collections are the purview of the Illinois Geological Survey and Illinois State Museum, respectively).

Over the past several years, the Collections Resources Committee at the INHS developed a profiling system applicable and comparable across all of its collections. The assessments made possible by this profiling will permit 1) collection managers to quantify the relative health of various parts of their charges and develop informed priorities, 2) administrators to evaluate the relative needs and funding levels for the separate collections, and 3) a more persuasive argument to external funding agencies. The INHS collection profiling system is described herein, a FileMaker Pro® database for easy profiling data capture and analysis is described and offered to readers, and the usefulness of profiling is shown in comparing the INHS insect and mollusk collections.

PROFILING METHODOLOGY

Ideally, collection managers would have a health assessment of every specimen in their collections, but since acquiring those data is not practical, collection profiling is done on groups of specimens. Each storage method requires its own profiling unit. The various profiling units for each collection are simply the standard container storage units, and are presented in Table 3.

Eight profiling indicators were selected, each of which was scored on a scale of 1 to 4. A score of 1 is "problematic," and indicates that the immediate usability of the collection is in jeopardy. This would include fluid-preserved specimens that have desiccated, unsorted specimens, labels with nothing but a field notebook code, etc. A score of 2 is "substandard," but the immediate health of the material is not at risk, including specimens with improper seals on jars, hard-bottom pinning unit trays, specimen data not computerized, etc. A score of 3 is deemed "acceptable." These specimens are all curated to accepted standards, which may vary from collection to collection. There may be room for improvement, but all specimens are stable for the long term and readily accessible. A score of 4 is "ideal": all specimens in each profiling unit have been determined to the

Table 3.　Profiling units for different INHS collections.

Collection	Storage method	Profiling unit
Annelids	Fluid-preserved in vials or jars	Vial rack or jar rack
Annelids	Microscope slides	Slide box
Birds	Stuffed skins, skeletons, eggs	Cabinet shelf/drawer
Crustaceans	Fluid-preserved in vials or jars	Specimen jar tray or vial rack
Fish	Fluid-preserved in jars	Specimen jar tray
Fish	Fluid-preserved in tanks	Shelf of tanks or single tank
Fungi	Herbarium sheets or boxes	Cabinet cubby hole
Insects	Dry, pinned, in envelopes	Insect drawer
Insects	Fluid-preserved in vials or jars	Vial rack or jar rack
Insects	Microscope slides	Slide box, or row in slide tray
Mammals	Stuffed skins, skeletons	Cabinet shelf/drawer
Molluscs	Shells	Cabinet shelf/drawer
Plants	Herbarium sheets	Cabinet cubby hole
Reptiles	Fluid-preserved in jars	Specimen jar tray
Reptiles	Skeletons	Cabinet shelf/drawer

Table 4. Summary of INHS profiling method (gray cells indicate unused profiling scores).

Profiling indicator	Collection type	1 = problematic	2 = substandard	3 = acceptable	4 = ideal
Conservation status	wet	specimens damaged or desiccated	fluid level low or dark	fluid topped off and clear	▓▓▓
	dry	pest infestation or specimens unusable due to damage	specimens damaged	specimens intact and stable	▓▓▓
	slide	slide broken or mountant deteriorating	improper mounting medium, not ringed	slide ringed or in Canada balsam	▓▓▓
Processing state	all	bulk, unprocessed specimens	mixed field sample	properly sorted and labeled	▓▓▓
	dry	bulk, unprocessed specimens	specimens lacking labels, or not properly prepared	properly sorted and labeled	▓▓▓
	slide	▓▓▓	specimens not cleared and/or improperly oriented	specimens cleared and properly oriented	▓▓▓
Container condition	wet	bad stoppers/lids, loose vials/jars	degraded or poor stoppers/lids	neoprene stoppers, good containers	archival racks/trays
	dry	cigar boxes, pill boxes, paper bags	substandard containers	archival unit trays	herbarium sheets with archival glue and fragment folders
	slide	not stored in slide box or tray	slide boxes of non-standard size	good slide boxes or trays	slides stored flat
Condition of labels	all	faded to illegible, crumbling or missing	partially faded, on non-archival paper	labels on archival paper	▓▓▓
Identification level	all	all specimens not determined to any level	taxon dependent	taxon dependent	taxon dependent
Arrangement level	all	mixed taxa in same container	specimens crowded, arranged at higher taxonomic level	specimens arranged alphabetically, or with alphabetical list if arranged phylogenetically	specimens arranged geographically or numerically within a taxon
Data quality	all	data absent or in codes only	missing data can be inferred	all data fields intact	value-added data, including retrospective georeferencing
Computerization level	all	▓▓▓	no computerization	mollusk and vertebrates databased, others with taxonomic information computerized	all specimens databased and localities georeferenced

species (or subspecies level), they are stored in modern, archival containers, and taxonomic and collection locality data are fully computerized and value-added at the specimen level.

Profiling scores were selected relative to the collection being evaluated. For instance, having all bird specimens determined to the species level would be considered acceptable, whereas the genus level, or even the family level, would be acceptable, albeit not ideal, for most insect groups. The normative practices of the different disciplines dictated the profile scoring criteria.

The various collections were profiled by scoring the lowest possible value for the profiling unit. For example, if even a single insect specimen had fallen off of a pin, the entire profiling unit (specimen drawer), even if it contained several hundred intact specimens, was given a "1" for conservation status. Remaining conservative in the scoring helped standardize the profiling by minimizing the amount of subjective evaluation: e.g., how many specimens have to have fallen off of pins before the drawer is scored differently?

A summary of the profiling method, its indicators, scores, and a brief description of each scoring criterion is presented in Table 4.

Conservation Status

The conservation status of the specimens is perhaps the most critical dimension to evaluate as it assesses the long-term stability of the specimens. Mammal skins with damage from dermestid beetles or mollusk shells with Byne's disease need immediate attention lest the specimens be lost or damaged permanently. Because the long-term, stable, archival storage of specimens is the only acceptable practice, there is no Level 4 (ideal) conservation status.

Fluid-preserved specimens.—Level 1. Specimens are desiccated. Fluid does not completely cover specimen(s). Alcohol is opaque.

Level 2. Fluid level is low, but completely covers specimens. Alcohol is dark.

Level 3. Fluid is topped-off and relatively clear.

Dry specimens.—Level 1. Shells have Byne's disease. Specimens (of any kind) have signs of pest infestation. Insect specimens have fallen off of pin. Specimens are damaged to the point of being unusable.

Level 2. Specimens are damaged: broken into multiple pieces, with past pest damage, loose teeth or bones. Insect pins are broken or significantly bent.

Level 3. All specimens are intact and stable.

Slide-mounted specimens.—Level 1. Slide or cover slip is broken. Mounting medium is crystallized, running, or has receded up to specimen.

Level 2. Aqueous mounting medium is not sealed (ringed) under cover slip. Mounting medium has receded. Cover slip or slide is cracked.

Level 3. Slide in good condition. Mounted in Canada balsam or cover slip has been sealed (ringed).

Processing State

Specimens are often first brought to a collection as bulk samples. Unless they are processed immediately, they tend to end up in storage, often referred to as "backlog." As bulk samples are processed and incorporated into the collection, their profiling score improves. Processing is acceptable when it is complete, so there is no Level 4 (ideal) state.

Fluid-preserved specimens.—Level 1. Specimens stored in bulk and unprocessed. Unsorted samples stored in Whirlpac® bags, Nalgene® or other bottles, jars, or in the freezer.

Level 2. Mixed field sample, rinsed, stored in clean alcohol, in standard quality storage containers.

Level 3. Vertebrate samples sorted and tagged. Mollusk shells and soft body tissue separated. Insect specimens stored in proper vials with cotton and micro-vials, if necessary.

Dry specimens.—Level 1. Bulk insect specimens papered, or in jars, boxes, or cotton. Unsorted botanical specimens in newspaper or paper bags (backlog).

Level 2. Insect specimens pinned, but improperly mounted on pin or point. Mollusk and vertebrate samples not cleaned, cataloged, or numbered. Botanical material mounted to herbarium sheets with labels, but without accession numbers.

Level 3. Insects properly pinned, pointed, or enveloped. Vertebrate and mollusk specimens cleaned, cataloged and numbered. Herbarium sheets in folders with all labels and accession numbers.

Slide-mounted specimens.—Level 1. As soon as specimens are slide-mounted they are already semi-processed, so there is no Level 1.

Level 2. Specimens were not cleared prior to mounting, or were improperly oriented on slide.

Level 3. Specimens properly cleared and oriented on slide.

Container Condition

The condition of specimen containers predicts the longevity of the specimens themselves. The containers should be archival, easy to arrange, easy to retrieve, and easy to use (unlike hard-bottom insect trays, for example). The more degraded or complicated the storage system is, the more likely it is that specimens will get damaged.

Fluid-preserved specimens.—Level 1. Vial stoppers are cracked, broken, swollen, or disintegrating. Stoppers are made of cork. Vials are loose on shelf, or banded together, and not in vial rack. Jar lids are old and rusted (if metal), or are Bakelite® lids (which crack easily). Jar seals are missing, cracked, or shrinking. Five-gallon buckets have poor seals or loose lids.

Level 2. Hardened but intact vial stoppers. Vials aligned in wire-sided racks. Jar lids are metal or with non-polyethylene jar seals. Large specimens are stored in 5-gallon buckets.

Level 3. Vials have good quality stoppers. Vial racks are solid with no risk of vial loss. Jars are bail-topped with polyethylene gaskets, or have polypropylene lids. Large specimens are stored in archival barrels with clamping sealing mechanisms.

Level 4. Vials and jars in archival racks. Large specimens stored in stainless steel tanks.

Dry specimens.—Level 1. Specimens in old cardboard boxes, cigar boxes, pill boxes, or paper bags. Specimens not stored in unit trays. Plants mounted on cardboard with rubber cement.

Level 2. Specimens stored in new cardboard boxes or zip-lock bags. Vertebrate trays are unlined. Skulls or skeletal material are in substandard containers. Insects pinned in hard-bottom unit trays. Plants pressed in newspaper. Fungi kept in packets when they should be in boxes, or glued to paper in the packets.

Level 3. Unit trays are archival. Insects pinned in foam-bottom trays. Vertebrate trays are lined with acid-free paper. Plants and fungi are in/on acid paper/packet/box with Elmer's® or other non-archival glue, or lacking fragment folders.

Level 4. Plants and fungi in/on acid free paper/packet/box, fixed with acid free glue, and with fragment folders present.

Slide-mounted specimens.—Level 1. Slides not in slide box or tray. Slide box broken.

Level 2. Slide box not standard 100-slide box. Slides in trays are not protected by envelope or thick labels, which prevent the crushing of the cover slip on one slide by the adjoining slide.

Level 3. Good slide boxes or trays with rust-free hinges and substantial closure clasps.

Level 4. Tray slides stored flat.

Condition of Labels

As important as the specimen itself are the collection and determination data associated with it. For some taxonomic groups, an unlabelled specimen is not even worth keeping, so monitoring the health of the specimen labels is important. Similar to the specimen condition profiling indicator, impermanent labels of any kind are not acceptable, so there is no Level 4 (ideal) score.

Level 1. Labels are faded to illegible, crumbling, or missing. Labels have become detached from the specimen.

Level 2. Labels are partially faded, laser-printed in fluid or in pencil, or on non-archival paper.

Level 3. Labels are readily legible, printed with non-bleeding (if in fluid) archival paper and ink.

Identification Level

Specimens in a collection that have not been determined to any level are difficult to access and are not typically examined by taxonomists. Also, the more precise the determination is of a specimen, the more valuable it becomes to researchers. The level of determination useful for taxonomists will differ depending on the group.

Level 1. All specimens undetermined and major groups mixed.

Level 2. Insects determined to order or family (depending of the size of the group). Not all annelid slides in a slide box fully determined. All other groups determined to the family or genus level.

Level 3. Insects determined to the genus or family level. All other groups determined to species.

Level 4. Insects determined to the species level. All other groups determined to species or (often) subspecies and verified by a specialist.

Arrangement Level

Once specimens have been identified (to any level), they need to be put away. Different collections have different standards of arrangement. For instance, the INHS insect collection stresses an alphabetic arrangement, the herbarium arrangement is somewhat more phylogenetic, and the annelids are not stored taxonomically, but rather together with each collection event.

Level 1. Mixed taxa stored in the same vial, jar, unit tray, slide, etc. Annelid slides made from same collection are in different boxes.

Level 2. Specimens crowded. Species sharing trays, or taxa scattered in two or more places. Arrangement is only at a higher taxonomic level. More than one annelid sample or collection site is stored in the same box.

Level 3. Specimens arranged alphabetically by family, genus, and species, or, if arranged phylogenetically, with an alphabetical cross-referenced list. Annelid slides arranged in boxes according to collection event and/or locality.

Level 4. Specimens arranged geographically within a taxon, or arranged numerically by catalog number if specimens have been databased.

Data Quality

Even with intact specimen labels, the quality of the data can vary greatly, from simple codes referencing field notebooks or accession logs, to labels with full determination and locality data, including geo-reference coordinates (e.g., latitude and longitude or universal transverse mercator).

Level 1. Data are in codes or missing entirely.

Level 2. Some data are missing but can be inferred. Specimen containers (vials, jars, or slides) lack determination labels.

Level 3. All data fields are complete for all groups except pinned insects may have determination labels missing.

Level 4. Localities fully geo-referenced. All species-level insect pins have determination labels.

Computerization Level

Finally, most natural history collections have some level of computerization of specimen data. For some groups, such as vertebrates, it is standard practice that all specimens be computer-cataloged, whereas entomological collections remain largely undatabased. Because the lack of computerized data does not present a significant obstacle to the health and accessibility of the specimens themselves, there is no Level 1 (problematic) profile score for computerization.

Level 2. No computerization at all.

Level 3. All herbarium, mollusk, and vertebrate specimens databased. Taxonomic information of other groups electronically inventoried, but specimens themselves not yet databased and assigned catalog numbers.

Level 4. All localities geo-referenced and stored electronically. Invertebrates databased at the level of storage unit (pin, vial, jar, slide).

PILOT PROJECTS

Two of the INHS collections have been largely profiled, allowing for comparisons both within and between collections. The insect collection profiling represented a significant investment in time and resources (see results), but the resulting data have allowed evaluation of priorities, including establishing an NSF-funded project to database the Hymenoptera (ants, bees, and wasps), which constitute one of the insect collection's more significant and important holdings. The databasing and concomitant specimen curation is a long-term goal of the INHS insect collection (Favret and DeWalt 2002).

Comparisons were made among four broad taxonomic groups of Hymenoptera: Symphyta (primitive, broad-waist wasps, including sawflies), Apoidea (the superfamily comprising all of the bees), Parasitica (a paraphyletic grouping comprising most of the parasitoid wasp families), and "other Hymenoptera" (ants, non-parasitoid wasps, and relatives). As most Hymenoptera research is conducted with pinned material, alcohol-preserved collections tend to be neglected. The dry and wet Hymenoptera collections were profiled and compared in an effort to quantify the differences in condition of these two methods of preservation. The Hymenoptera slide collection is comparatively small and, although it was profiled, the results are not presented here.

Over the recent past, the INHS has been responsible for the care and management of both the INHS collections and some of the University of Illinois Museum of Natural History (UIMNH) collections, the latter scheduled for incorporation into the INHS in the near future. Evaluating the condition of the UIMNH collections will help assess their relative need for curatorial attention before incorporation. To this end, the UIMNH mollusk collection was profiled, also permitting an opportunity to compare the profiling results of two dissimilar collections: specifically, the UIMNH mollusk shell collection and the INHS fluid-preserved beetles.

Profiling data can be presented in any number of ways. A mean profiling score provides a general overview of the profiling and allows for simple comparisons (Table 5). Alternatively, the actual number of profiling units with each profile score, 1 through 4, can be tabulated and presented as a chart on a per-profile indicator basis. For example, Figure 1 presents the relative proportions of profiling units (pinning drawers) that scored a 1, 2, 3 or 4 for identification level.

In order to expedite both profile data entry and analysis, a relational FileMaker Pro® database was developed. Related tables include one for the profiling units themselves, one for the data entry personnel, and a look-up table that provides customizable descriptions of each of the profiling scores. Each database record represents a single profiling unit. It contains fields for the exact location of the unit (room, cabinet, shelf, position on shelf), the type of unit (dry, wet, slide), taxonomy, date of entry, and all eight profiling indicators. On each tenth record (customizable to any n^{th} record), the database prompts the user to enter the number of specimens in the profiling unit; this allows for a sub-sampling regime and an eventual collection size estimate (see Table 5). The data entry can be done either with a notebook computer in the collection proper or on paper spreadsheets to be entered into the computer later.

Table 5. Average profiling scores for various collections. 1 = problematic, 2 = substandard, 3 = acceptable, 4 = ideal.

| Insect groups/Profile indicators	All Dry Hymenoptera	All Wet Hymenoptera	Symphyta	Apoidea	Parasitica	Other Apocrita	UI Mussels (Unionidae)	INHS wet beetles
Estimated no. specimens	327,623	106,119	27,919	136,849	76,413	50,679	na	550,389
Number of profile units	979 drawers	893 vial racks	104 drawers	370 drawers	288 drawers	195 drawers	1,144 trays	4,569 vial racks
Conservation status	2.39	1.92	2.48	2.50	2.22	2.37	3.00	1.99
Processing state	3.00	2.90	3.00	3.00	3.00	2.99	2.85	2.85
Container condition	2.97	2.10	3.00	3.00	2.91	2.99	2.70	2.46
Label condition	1.60	1.60	1.81	1.65	1.38	1.41	2.73	1.56
Identification level	3.12	2.85	3.39	3.48	2.92	3.03	2.95	2.67
Arrangement level	2.09	2.00	2.11	2.12	2.23	2.03	2.23	2.11
Data quality	1.75	2.61	1.96	1.83	1.53	1.51	2.01	2.86
Computerization level	2.01	2.00	2.00	2.00	2.02	2.02	2.07	2.01
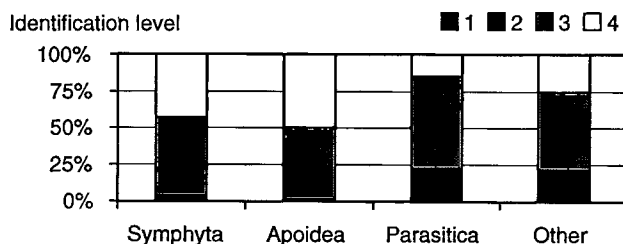
Figure 1. Proportional comparison of profiling scores for identification level of four taxonomic groups of Hymenoptera.

When profiling and computer data entry are complete, a simple query can be made of the database requesting a certain set of records, e.g., all the records of a particular collection, taxonomic group, preservation method, storage location. The user then proceeds to a summary layout and calculation fields return the average profiling score for each indicator in the found set, the number of records with each profile score, and the estimated number of specimens based on the sub-sampling. Interested readers are invited to contact the first author for a clone of the FileMaker Pro® database.

Results and Discussion

Three hourly workers profiled the insect collection over the course of three years. The profiling of the pinned collection averaged 28 drawers per hour, the wet collection 27 vial racks per hour, and the slide collection 18 slide boxes per hour. The pinned Hymenoptera, with 1,433 drawers, took 50 hr to profile, the alcohol-preserved Hymenoptera, with 893 vial racks, took 34 hr, and the alcohol-preserved beetles, with 4,569 vial racks, took approximately 172 hr. Results from the slide profiling are not presented here, but, the largest slide collection, the thrips, with 743 slide boxes, took 42 hr to profile. An estimate of the time required to profile the entire insect collection of approximately seven million specimens, preserved in 7,161 drawers, 23,132 vial racks, and 1,509 slide boxes/trays, is 1,200 hr, or approximately seven months of full time work.

Profiling within the pinned Hymenoptera showed that the groups that have received the most attention historically, the Symphyta and the Apoidea, scored higher than the others (Table 5, Fig. 1). The conservation status was lower than acceptable for all groups, probably the result of specimens falling off of pins, particularly within the frequently point-mounted (glued-on) Parasitica. Specimen label scores were low for all groups, a consequence of unlabeled material from the recently incorporated International Soybean Arthropod Collection.

The dry, pinned, Hymenoptera collection scored higher than the wet, ethanol-preserved, collection on several profile indicators, including: conservation status,
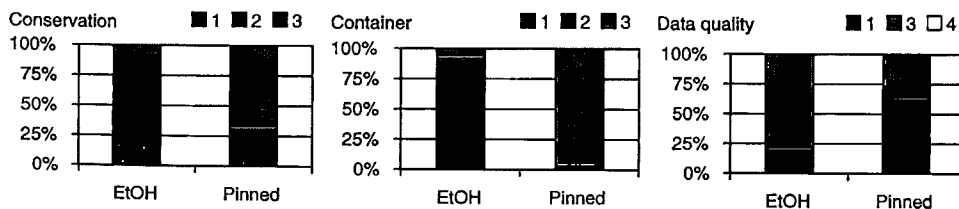
Figure 2. Proportional comparison of profiling scores for conservation status, container condition, and data quality in ethanol-preserved and pinned Hymenoptera collections.
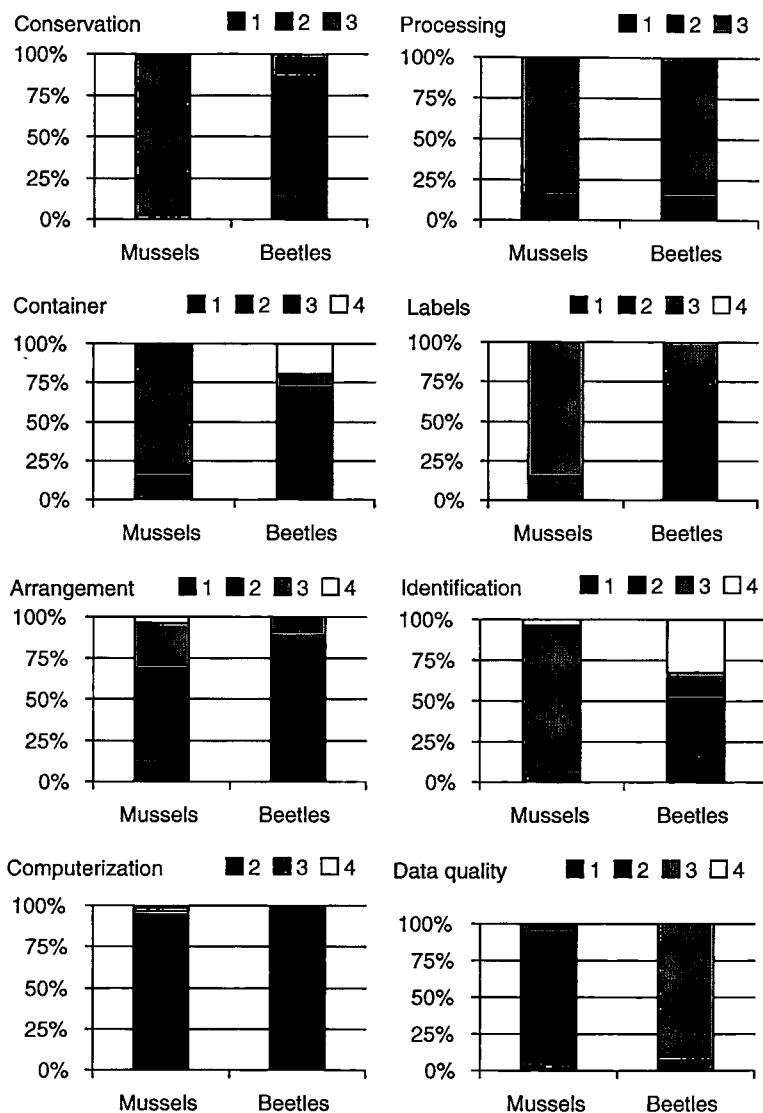
Figure 3. Proportional comparison of profiling scores for all eight profiling indicators in UI mussel and INHS ethanol-preserved beetle collections.

processing state, container condition, and identification level (Table 5, Fig. 2). For the most part, these disparities are attributable to the neglect the wet collection experiences in comparison with the more actively-used pinned collection. In an effort to address the poorer condition of the wet material, all the alcohol was replaced (improving the conservation score) and all of the old vial stoppers and wire-sided vial racks were replaced (improving the containers score). In contrast, the wet Hymenoptera scored better for data quality than the dry collection (Table 5, Fig. 2). This disparity is partly attributable to the Charles Robertson collection, a large and historically important collection of pinned bees (Marlin and LaBerge 2001), each specimen of which was assigned a single label with a number, referencing Robertson's collection logs.

In comparing the mussel and beetle collections, it is evident that the mollusks are in better overall condition than the beetles: the mollusk collection scores higher than the beetles in every category except processing state (where they are equal) and data quality (where the beetles outscore the mollusks) (Table 5). Parsing out the data more fully is especially instructive. All of the mussels are in good conservation state, whereas roughly 13% of the vial racks of beetles are problematic, and only 15% are in acceptable condition (Fig. 3). Surprisingly, the beetles and mollusks are almost identical with regard to processing state, but the beetle labels are in very poor condition, possibly due to the dark alcohol (low score on conservation status) discoloring the label paper. The specimen containers scores are a good example of the different messages received from arithmetic means as compared to profiling score distributions. Although the mean score for mollusks was slightly higher than the beetle score (Table 5), Figure 3 indicates that the mollusks have a higher proportion of containers rated as problematic, whereas the beetles have far more ideal containers (in this case, archival, plastic vial racks containing the well-curated aquatic beetles). Limited resources may best be allocated toward replacing the problematic mollusk containers first, and then working on the large number of substandard beetle containers.

Conclusion

Collection profiling has established itself as a useful tool for evaluating the health of any natural history collection. However, collection managers everywhere, for a variety of reasons, have been slow to initiate profiling of their respective collections. Perhaps the time commitment of profiling thousands of units is not seen as returning enough value. Perhaps the personal working knowledge of the collection is thought to be sufficient in making collection management decisions and prioritizing resources. With respect to the single collection manager who has relative autonomy in prioritizing projects, this hesitation towards profiling is understandable. However, with respect to museum directors, or other administrators who are called on to distribute funds or other resources to multiple collections, an honest and quantified assessment of the needs of the various collections under their directorship would be of great value. It is often easy to discount the hand-waving of collection managers who may complain of being under-funded, but it is much harder to ignore the hard data associated with collection profiling.

Likewise, without actual numbers, it is easy to ignore one collection to the benefit of another. General working knowledge of the collection indicated that the fluid-preserved Hymenoptera were in poorer condition than the pinned collection, but the stark reality of that disparity, presented quantitatively, is what spurred corrective action.

Profiling, albeit no panacea, is an important tool in reinvigorating collection management and in particular providing data to support funding requests. In today's political climate, unhealthy as it is for collections, the need for useful and direct diagnostic tools is greater than ever.

Acknowledgments

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SHIPPING AND HANDLING OF NATURAL HISTORY SPECIMENS IN DANGEROUS GOODS

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Abstract.—Most collection-holding natural history institutions, as part of their daily operating procedures, deal with the shipping of specimens, through loans and gifts of material to other institutions as well as the accepting of incoming material. A large number of these shipments contain flammable or hazardous solutions such as ethanol, isopropanol or formaldehyde in varying concentrations. Dangerous goods regulations, most of which were in place long before 11 September 2001, were brought sharply into focus after that tragic event. The shipping and handling of wet-preserved natural history specimens have been affected by the more rigorous enforcement of these regulations, which has impacted the methods and frequency with which museums and other collection-holding institutions can send loans and gifts of materials to others. There is a great deal of confusion concerning the application of these regulations which, along with a lack of knowledge, has resulted in serious misinterpretations of the regulations within the natural history community.

Most alcoholic specimen shipments are sent by airmail to minimize the length of time specimens are exposed to the hazards of transport, thereby reducing the chances of damage and dehydration. Shipping dangerous goods by air presents particular problems. International shipments must comply with both the International Civil Aviation Organization (ICAO) technical instructions as well as national regulations. In order to meet commercial standards, shippers are also required to meet the International Air Transport Association (IATA) Dangerous Goods Regulations. Furthermore, some countries have added variations to many of these requirements.

REGULATING AGENCIES

The ICAO governs the implementation and adoption of standard aircraft shipping and packaging regulations by both the Department of Transportation (DOT) in the United States and IATA internationally. DOT regulations are unique to the United States. Other countries have similar domestically enforced regulations while a large number rely on IATA regulations for both domestic and international regulations. Domestic shipments sent through the mail within the United States must also conform to United States Postal Service (USPS) regulations while courier shipments (FedEx, UPS and DHL) must conform to the individual company's specific regulations (which for the most part follow DOT or IATA regulations). USPS and private courier regulations must meet or exceed the DOT or IATA regulations respectively; in many instances they are more restrictive.

TRAINING

The first and most important requirement stipulated by all regulations is that people who pack, handle or ship dangerous goods must be properly trained and certified. Training can be obtained from any number of commercial companies that specialize in Dangerous Goods or Hazardous Materials Training, and may range in price from $300 to $500. Training programs can take from a couple of hours to two days, depending on the scope and complexity of training, and cover general shipper's compliance and responsibilities together with specific case scenarios. Participants should be provided with a copy of the relevant regulations, and the training should cover restricted quantity (small quantities for DOT and excepted quantities for IATA) dangerous goods packing and

shipping. For quantities above and beyond restricted quantities, more extensive training is required, which involves additional time and cost. The majority of museum shipments will fall within the restricted quantity regulations outlined below.

Every employee who handles, packs or ships dangerous goods is required to complete this training and maintain current certification. In addition, refresher training is required every 24 mo. Depending on the size of the institution, the training of a single person (or two) to handle, pack and ship all dangerous goods shipments may be sufficient. At some institutions, especially those affiliated to universities, there may already be trained individuals on staff (for example, in an environmental health and safety unit unit) who can ship and receive packages. University museums may also be able to make use of the institution's environmental health and safety unit for training of museum personnel, and for assistance with shipments larger than those covered by restricted quantities. There are also certified commercial re-packing companies that will handle packing and labeling requirements.

Dangerous Goods/Hazardous Materials

Dangerous goods/hazardous materials are classified according to Hazard Class and Packing Group. For example, most flammable liquids fall into Hazard Class 3 (flashpoint of less than 60.5°C or 141°F). Within Hazard Class 3, materials are classified into three Packing Groups. Materials in Packing Group I, considered the most dangerous, have a boiling point less than or equal to 35°C (95°F). Materials in Packing Group II, considered moderately dangerous, have a boiling point above 35°C (95°F) and a flashpoint less than 23°C (73°F). Materials in Packing Group III have a boiling point above 35°C (95°F) and a flashpoint between 23°C (73°F) and 60°C (140°F).

Of the four substances most commonly used in wet collections only ethanol, isopropanol and formaldehyde are covered under dangerous goods regulations. Glycerin (glycerol) used for cleared and stained specimens, is not regulated in any concentration.

Ethanol (ethyl alcohol), most commonly used in concentrations of 70% and above, is regulated for transport. Concentrations between 10% and 80% fall into Packing Group III while concentrations above this fall into Packing Group II.

Isopropanol (isopropyl alcohol), most commonly used at concentrations of 50% and above, falls into Packing Group III at concentrations of 10–30% while concentrations above this fall into Packing Group II.

Formaldehyde (formalin), usually sold as a saturated solution of formaldehyde gas in water and measured by weight or volume concentration, is most commonly used in concentrations of 3.7% or 4.0% (what is called 10% formalin in natural history collections) and is unregulated for transport. Above 10% is a Class 9, packing group III substance and is regulated for transport.

Other solutions used in tissue storage include dimethyl sulfoxide (DMSO), propylene glycol and proprietary solutions such as RNAlater. DMSO and propylene glycol are unregulated in any concentration. RNAlater is proprietary (of unknown composition, although thought to be made up primarily of propylene glycol), but is not listed as a dangerous good.

The shipment of infectious substances, natural history specimens not containing dangerous goods (pinned insects, skins, skeletons etc.), biological materials other than natural history specimens and any material on dry ice is covered by a separate set of regulations, and are outside the subject of this paper. There may also be ancillary permitting/documentation requirements for the domestic or international transfer of biological specimens (U.S. Fish and Wildlife import/export or CITES permits, APHIS,

etc.). APHIS in particular has specific approved treatment methods required for import of avian, ruminant, equine and swine specimens. These regulations (when relevant) should be adhered to during the transport of any natural history materials.

REGULATIONS

Domestic and international shipping and packing guidelines vary slightly in scope and limitations but both include special dispensations for smaller quantities of dangerous goods. The two sets of limited quantity regulations are very similar in scope and content but have a number of limitations that must be adhered to. It is important to consult the original texts of both the DOT and IATA regulations before shipping. USPS and DOT regulations are available online (see references cited 1 & 2) while IATA regulations must be purchased (reference 3).

DOMESTIC REGULATIONS

In the United States, the shipment of dangerous goods (referred to as hazardous materials) is covered in DOT Title 49 CFR[1] (Parts 100 to 185) and USPS Publication 52[2]. An exception to the regulations is made for dangerous goods in restricted quantities termed "small quantity regulations" outlined in DOT 173.4 and USPS Publication 52 (334). These small quantities are considered exempt from regular DOT and USPS hazardous goods requirements. Most fluid preserved natural history specimens can be packed and shipped utilizing these small quantity regulations. Institutions in other countries should consult their national dangerous goods regulations to ascertain if similar regulations exist and ensure compliance. In some instances this may mean using IATA regulations as outlined below in the international section of this document. Outlined below are the important relevant points from the DOT and USPS regulations:

1. Small quantities may be sent through the United States Postal Service via air transportation (Express, Priority and First-Class mail) or surface transportation as Standard or Parcel Post, or by any of the three major courier companies (FedEx, UPS and DHL) that follow DOT 49 CFR 173.4 small quantity regulations.
2. Class 3 dangerous goods (all packing groups) are acceptable (ethanol and isopropanol).
3. The maximum quantity of dangerous goods per inner receptacle cannot exceed 30 ml for acceptable liquids (as above). This inner receptacle cannot be liquid full at 55°C (131°F) and is to be constructed of plastic (having a minimum thickness of 0.2 mm) earthenware, glass, or metal. A removable closure on an inner receptacle must be held securely in place using wire, tape or other positive means.
4. Each inner receptacle must be placed within a securely sealed secondary package.
5. Sufficient cushioning and absorbent material (that will not react chemically with the dangerous goods) must surround each inner receptacle and be capable of absorbing the entire contents of the receptacle.
6. The secondary packages must be securely packed in a strong outer package (box) which complies with DOT mandated drop and compressive load tests without breakage or leakage from any internal receptacle:

    a. Drop tests—free drop on top, bottom, long and short side and the junction of three sides of the package from 1.8 m (5.9 ft) onto a solid unyielding surface.
    b. Compressive load test—stack packages of similar size and weight to a height of no less than 3 m (10 ft) for 24 hr.

7. The gross mass of the package must not exceed 29 kg (64 pounds).
8. Labeling—the address side of each package must be clearly marked with "This package conforms to 49 CFR 173.4" and complete return address and delivery address must be furnished. There are no other labeling requirements. Custom labels can be produced that follow these guidelines:

> The University of Kansas
> **Natural History Museum and Biodiversity Research Center**
> 1345 Jayhawk Blvd.
> Lawrence, KS 66045-7561 U.S.A. • (785) 864-4540
>
> # Scientific research specimens
>
> ## No endangered species
> ## No commercial value
> ### This package complies with 49 CFR 173.4
>
> 050805

INTERNATIONAL REGULATIONS

International shipments of dangerous goods are covered in Section 2.7[3] of the IATA regulations. As above, restricted quantity regulations exist for international shipping, contained in IATA Section 2.7.1 and referred to as "Dangerous Goods in Excepted Quantities." Dangerous goods in excepted quantities, in contrast to DOT and USPS regulations, are considered dangerous goods under IATA regulations but are exempt from large portions of the dangerous goods regulations applicable to larger quantities.

1. The United States Postal Service may not be used for international shipping of dangerous goods. All international shipments must be sent using a private courier service (FedEx, UPS or DHL) while adhering to IATA regulations.
2. Class 3 dangerous goods (all packing groups) are acceptable.
3. As above, each inner receptacle may not contain more than 30 ml while the same construction, liquid full and closure security regulations apply.
4. Each inner receptacle must be placed within a securely sealed secondary package.
5. Sufficient cushioning and absorbent material (that will not react chemically with the dangerous goods) must surround each inner receptacle and be capable of absorbing the entire contents of the receptacle.
6. The same package drop and compressive load test regulations as above apply.
7. IATA regulations state that each inner receptacle must be placed within a securely sealed secondary packaging the total contents of which may not exceed 500 ml for Packing Group II liquids and 1 liter for Packing Group III liquids.
8. Labeling—each package must be labeled with the label below (Fig. 1), having minimum dimensions of 100 mm × 100 mm (4 in. × 4 in.). This label must be completed and signed by the packer. The "Nature and Quantity of Goods" section of the air waybill must be completed with the words "Dangerous Goods in Excepted Quantities."

DANGEROUS GOODS IN EXCEPTED QUANTITIES

This package contains dangerous goods in excepted small quantities and is in all respects in compliance with the applicable international and national government regulations and the IATA Dangerous Goods Regulations

_____

Signature of Shipper

_____   _____

Title   Date

_____

Name and address of Shipper

This package contains substance(s) in Class(es)
(check applicable box(es))

Class: 2   3   4   5   6   8   9
           ☐   ☐   ☐   ☐   ☐   ☐   ☐

and the applicable UN Numbers are:

Figure 1. Dangerous goods in excepted quantities label for international shipments.

All three major courier services (FedEx, UPS and DHL) accept dangerous goods in excepted quantities for international delivery[4,5,6] and waive their normal dangerous goods surcharges for packages containing excepted quantities. All three couriers do, however, only accept dangerous goods on a contract or pre-approval basis and will only accept dangerous goods in boxes (no envelopes). FedEx has the added stipulation that the box must measure at least 7 in. × 4 in. × 4 in. All three companies will only ship dangerous goods to approved countries as there are various countries within which they are prohibited from shipping (due in part to these countries not adopting IATA dangerous goods regulations for domestic transport). This means that the courier could deliver a package to the designated international airport but no further. There are also various countries where shipment is allowed but only to certain regions or postal codes. The list of countries to which this applies changes constantly, so the carrier should be contacted for an up-to-date list[4,5,6]. It should also be noted that in some countries, additional customs, veterinary, or fish and wildlife fees may be incurred which will need to be paid by the recipient of the package. The list of these fees and to which countries they apply are not available or, in most cases, unknown to the courier.

It has recently been noted that FedEx has regulations in place against the carrying of "whole dead animals" and that museum specimens fall into this category and are therefore prohibited in FedEx mail. There are various groups working with FedEx to institute exempt status for museum specimens and resolve this impasse.

Transport in Personal Baggage as Carry-on or Checked Luggage

With so many variables and so many different people and organizations to deal with, there are inevitably differences in interpretation of regulations–for these reasons, I do not recommend attempting transporting specimens on board an airplane. In the majority of cases it is easier and safer to send the specimens by courier.

Due to the fact that DOT defines small quantities as non-hazardous, these quantities are allowed in hand and checked baggage on domestic flights but must be declared to the airline staff before boarding. The final decision as to whether or not to accept these packages is made by the pilot of the aircraft being boarded, thus you may be denied permission to carry the package on board at the last minute. Whether or not the package will be allowed on board varies from flight to flight and from airline to airline. Some individuals have suggested simply pouring off the excess liquid preservative before flying but there is no guarantee that this will be acceptable and it has yet to be determined whether removing liquid alcohol from specimens and carrying them "dry" would negate the need to declare these as dangerous.

With the present heightened security measures in force at airports and the policy of no liquids or gels (or limited to 3 oz bottles in a clear quart zip-lock bag depending on which airport you fly through) no specimens in fluid would be allowed as carry-on baggage at all.

Internationally, dangerous goods in any quantity are prohibited as carry-on or checked baggage and cannot be carried on your person or checked onto any international flight (IATA Section 2.7.3).

Natural History Specimens

In real world collection scenarios, the common practice of wrapping specimens in cheese cloth or gauze moistened with alcohol and sealed in plastic would keep the material from being a dangerous good as long as no more than 30 ml of 70% ethanol was used in each individual package and the heat sealed plastic bags are at least 0.2 mm thick. Each package would need to be placed in secondary packaging material (usually another bag) sealed in the same way and with sufficient absorbent material (vermiculite or 3M absorbent pad) and then placed in an approved box with cushioning material (packing peanuts). Purchasing boxes of various sizes that can be cut down to the appropriate size (available from ULINE) reduces the number of differently sized boxes kept on hand. It is recommended that old boxes not be re-used for shipping specimens, as they may have old labels that cause confusion at mailing facilities. All old labels must be defaced or removed before packing.

Specimens preserved in 3.7% formaldehyde can be shipped in regular mail both domestically and internationally without any dangerous goods requirements.

Tissues can be placed in cryo-vials or glass vials in less than 30 ml of 99% ethanol if the caps are secured with tape or Parafilm, and the vials placed in a secondary heat-sealed plastic bag with absorbent material and packed as described above.

Although it is widely believed that reducing the concentration of the alcohol below 24% renders specimens outside of the scope of dangerous goods regulations, from Tables 1 and 2 this is clearly not the case. Even at concentrations of 10%, both ethanol and isopropanol fall within the bounds of Packing Group III (flash points greater than 23°C and less than 60°C). The confusion stems from passages in the IATA regulations (Section 2.3) pertaining to beverage alcohol which state that "alcoholic beverages containing 24% or less alcohol by volume are not subject to any restrictions." This

Table 1. Flash points of ethanol based water solutions.

		Conc. (% by volume)										
		0	10	20	30	40	50	60	70	80	90	100
Temperature	(°F)	—	135	105	90	80	80	80	80	75	65	55
	(°C)	—	57	41	32	27	27	27	27	24	18	13
Packing Group						PG III					PG II	

regulation only pertains to beverage alcohol in retail packaging and cannot be used for natural history specimens.

It has also been suggested that fluid preserved specimens may be placed in water for shipment. Although this may put specimens outside of the scope of dangerous goods regulations, the possibility of damage to specimens from swelling and subsequent shrinkage upon reinsertion into alcohol, cell wall rupture, mold, and bacterial growth will severely endanger the specimens, particularly if the shipment is delayed.

PROBLEMS AND CONCERNS

These two scenarios cover the majority of all natural history dangerous goods shipments but there are still some areas of concern or where problems still exist:

1. Large specimens that require more than 30 ml to adequately moisten the specimen for transport must be packaged as regular dangerous goods (not excepted/small quantities) and are subject to the more restrictive regulations, labeling and paperwork of such shipments.

2. Specimens may not be sent to countries that do not accept dangerous goods. At present there is no solution to this problem.

3. Specimens may not be carried internationally as carry-on or checked baggage due to dangerous goods restrictions. As discussed above, it has yet to be determined whether specimens can be drained of alcohol thereby negating the need to declare them as dangerous goods.

4. Specimens sent on loan to researchers who have not had the necessary training to repack and return the material (or have no access to a certified packer) once they have completed their study can also pose a problem. This is especially pertinent for international shipments as regulations and training requirements differ between countries.

5. According to the regulations, packages entering the USPS postal system (whether sent as loan, gift or exchange) by international institutions and packed by untrained staff should be refused and returned to the sender. To do this would expose the specimens to the vagaries of the international postal system a second time, which would also

Table 2. Flash points of isopropanol based water solutions.

		Conc. (% by volume)										
		0	10	20	30	40	50	60	70	80	90	100
Temperature	(°F)	—	105	85	75	70	65	65	65	65	65	53
	(°C)	—	41	29	24	21	18	18	18	18	18	12
Packing Group			PG III					PG II				

expose the postal system to an illegal package a second time that may not have been packaged correctly or may be leaking fluid.

It is important to remember that dangerous goods regulations are not written to specifically address the shipment of natural history specimens. This is a shortcoming that would ideally be addressed through the planning of a meeting at which all of these issues will be discussed with representatives of all involved parties—ICAO, IATA, DOT, USPS, FedEx, UPS and DHL. This author has been working in conjunction with the Society for the Preservation of Natural History Collections (SPNHC) and the American Society of Ichthyologists and Herpetologists (ASIH) to put together such a meeting. However, even if legislation is written specifically for natural history specimens, it would take five to six years to take effect. Clearly, a short term solution to these problems is necessary and this author will be working to achieve this, keeping the museum community updated on any progress made.

<div align="center">LITERATURE CITED</div>

[1] US Department of Transportation. 2007. Title 49 CFR, Subtitle B, Chapter 1, Subchapter C: Hazardous materials regulations.
US Department of Transportation (DOT). http://www.access.gpo.gov/nara/cfr/waisidx_99/49cfrv2_99.html

[2] United States Postal Service. 1999. Publication 52: Hazardous, Restricted, and Perishable Mail. Part 3: Hazardous Materials. Pp. 15–60. http://www.usps.com/cpim/ftp/pubs/pub52.pdf

[3] International Air Transport Association. 2007. IATA Dangerous Goods Regulations, 48th ed. Section 2—Limitations Pp. 9–80.

[4] FedEx Dangerous goods shipping website: http://www.FedEx.com/us/services/options/express/dangerousgoods/hidden.html
FedEx Dangerous Goods/Hazardous Materials Hotline: 1-800-463-3339.

[5] UPS dangerous goods shipping website: http://www.ups.com/content/us/en/resources/prepare/idg/information/definition.html
UPS Hazardous Materials Support Center: 1-800-554-9964.

[6] DHL dangerous goods shipping website: http://www.dhl-usa.com/usgov/servopt
DHL Hazardous Materials Hotline: 1-866-588-2002.

BOOK REVIEWS

**HUMAN REMAINS: GUIDE FOR MUSEUMS AND ACADEMIC INSTITU-
TIONS, 2006, V. Cassman, N. Odegaard, and J. Powell, eds.** (Altamira Press,
Lanham, MD, 310 pp.) At once both clinical and empathetic, this remarkable guide
will be a benchmark for many yrs to come regarding the care of human remains.
Each of the book's seventeen chapters contribute to vigorously span a multidisci-
plinary arc from ethical to practical concerns. Equally important, the book is a
gentle disquisition about how ethical and practical concerns are, at the end of the
day, one and the same thing.

M. Alfonso's and J. Powell's chapter titled "Ethics of Flesh and Bone, or Ethics
in the Practice of Paleopathology, Osteology, and Bioarchaeology" begins with a
succinct history of the origins of ethical codes, proposes a "Code of Ethics for
Biological Anthropology," and concludes with a helpful list of standards and
guidelines adopted by an array of professional organizations and museum
associations. The following chapter, "Policy," was written by all three editors,
and begins where all such explication should begin, with a dictionary. (Perhaps
because it is irresistible, the authors use the Merriam-Webster definition of policy:
"prudence or wisdom in the management of affairs.") Policies are composed of
mission statements ("a short description of institutional goals that guides staff in
their work towards common goals"), vision statements ("the institutional desires
for the future, including plans for new infrastructure or new ways of serving
stakeholders and new audiences"), policies (which "are more in depth and further
define roles and action for staff"), and procedures (which are "specific guidelines
for specific actions"). It is always helpful to have examples; accordingly, an insert
lists the Internet-accessible policies of twelve museums, universities, professional
organizations, and governmental entities. A. Sadongei's and P. Cash's chapter
titled "Indigenous Value Orientations in the Care of Human Remains" briefly
and effectively outlines the need for institutional policies which "enable practices
that ensure greater cultural sensitivity to affected indigenous populations." A
suite of four chapters coauthored by V. Cassman and N. Odegaard form a
comprehensive practicum concerning skeletal materials and their disposition in
institutional settings. In the first, "Condition Assessment of Osteological
Collections," the basic morphology and composition of skeletal components is
described in preparation for a discussion about condition assessments. A glossary
differentiates hairline cracks from open cracks, and explains the etymological gulf
between split and splintered. A pop quiz might ask a student to distinguish
between fractures, breaks , splits, and cracks, and woe betide anyone who cannot
discern between consolidant, hardener, and preservative. But don't worry about
differentiating between soiled, dirty, dusty, grimy, smirched, and sullied, for
"these six terms are quite similar; however, soiled and dusty are the most
appropriate for condition assessments of bone." (One need never have met the
authors to like them a lot.)

The second, "Examination and Analysis," is illustrated with an economy of
drawings and photographs, the former to instruct in handling techniques, the
latter to identify measurement and documentation tools. Before the handling and
hardware, however, the authors address the personal and mental preparation

required when one endeavors to learn from human remains in a respectful manner.

The third, "Treatment and Invasive Actions" reviews the spectrum of cleaning methods from mechanical and aqueous to the use of solvents and soaps. Pesticides are invasive and problematic for objects and handlers alike, consequently integrated pest management (IPM) has become a defining mantra of the conservation and collections management set. Frequently, treatments involving coatings, consolidants, and adhesives can be side stepped by holding bones together in "passive cavity-cut support trays," an inspiring example of which appears in before-and-after photographs. (If this level of storage were required for many long bones, however, the unused corners of the tray would represent unrealized storage volume, a very real concern in many institutional settings.)

The longest discussion about DNA analysis appears as a two-page insert by A. Vuissoz and M. Gilbert, appropriately contextualized in this chapter as "The Impact of Preservation Treatments on DNA."

"Storage and Transport," the last of the four chapters written by Cassman and Odegaard, epitomizes the manner in which ethical and practical concerns merge to realize respectful and protective storage environments for human remains. The authors use the effective analogy of Russian nesting dolls, a ubiquitous art form with which almost everyone can identify. The outermost to innermost dolls, six of them, represent the building, the room, cabinets, boxes, trays, and. bags. The specification, design, construction, implementation and maintenance of these enveloping barriers contributes tremendously to preservation by mitigating against physical damage, pests, particulates, dodgy climate control systems and other annoyances. Sometimes human remains must travel, and an insert, "Moving Kennewick Man," relates the authors' harrowing tale of assessing and packing the fossils in under twelve hrs. The following insert, "Rehousing the Kennewick Remains," describes in detail the materials the authors used to construct passive cavity-cut supports for permanent housing, for which they had more time. Another of the book's qualities is this attention given to the unheralded work which attends public controversy.

A pair of chapters, "Documentation," by A. Morris, and "Associated Records: the Kennewick Project," by T. Militello, C. Pulliam, and N. Drew, identifies the sources of skeletons in collections, and itemizes associated records under the headings of "Activity" (e.g., carbon 14 dating analysis), "Record Format" (e.g., paper), and "Description of Records" (e.g., datalogger readings). Few collections have the level of documentation even approaching that of the Kennewick remains, but it is useful to have a systematic framework for organizing any records one is fortunate enough to have.

A good book about collections preservation thoroughly identifies problems and offers thoughtfully measured solutions. A great book about collections preservation does both, but also transcends its immediate subject in such a way as to become relevant to all collection types. This is a great book.

It is not within this book's purview to enumerate, photograph or illustrate human bones and teeth. Two more books are essential for this purpose, and complete the human osteology bookshelf.—*Paul Beelitz, American Museum of Natural History, Central Park West and 79th Street, New York, New York 10024-5192, USA*

HUMAN OSTEOLOGY, SECOND EDITION, 2000, T. White (Text) and P. Folkens (Images). (Academic Press, San Diego, CA, 563 pp.) The author and photographer of this indispensable volume share equal billing, as well they should, for the text and images are mutually dependent. With every bone meriting equal attention, this is the most detailed book about the human skeleton. In the chapter on the skull, exquisite 2.7 × 2.7 cm. illustrations complement the photographs by indicating the positions of bones in the skull. A chapter titled "Ethics in Osteology" addresses standards applicable to forensic osteology, archaeological osteology, and human paleontology.—*Paul Beelitz, American Museum of Natural History, Central Park West and 79th Street, New York, New York 10024-5192, USA*

HUMAN OSTEOLOGY: A LABORATORY AND FIELD MANUAL, FIFTH EDITION, 2005, W. Bass. (Missouri Archaeological Society, Columbia, MO, 365 pp.) This coil-bound manual has been an indispensable part of the human osteology bookshelf since it was first published in 1971 and relies primarily on drawings, not photographs. Throughout the manual, 5 cm high illustrations of the entire human skeleton from the front or the side indicates the relative positions of the bones.—*Paul Beelitz, American Museum of Natural History, Central Park West and 79th Street, New York, New York 10024-5192, USA*

IN PURSUIT OF PLANTS, 2004, Philip Short. (Timber Press, Portland, Cambridge, 351 pp.) This book describes interesting, extraordinary, dangerous and life-threatening aspects of the lives of 39 plant collectors who lived and worked in the 19th and early 20th centuries. The earliest expeditions are those of Drummond and Douglas in 1825 in North America and the latest is that of Forrest around 1931. At least 16 have connections to the Royal Botanic Gardens, Kew as staff or Director and some were funded by Kew so that Kew's collections could be developed. A few trained at Kew, some corresponded with Kew and some collected to supplement their income. Only one is a woman which presumably reflects social and family constraints of the time. As appears general for collectors, there was often no or very little correspondence relating to their activities.

The choice of collections included in the book has been to some extent related to the exposure they have already had. Some are famous, others less so and some you might expect to be in the book are missing altogether. The choice is also connected to the author who used part of his time as the Australian Botanical Liaison Officer (ABLO) to research the exploits of collectors using often unpublished documents in the Kew archive.

The book is divided into regional sections—Africa (6), Asia (9), Australia and New Zealand (11), Europe (2), North America (4), Central America and South America (4) and Oceans and Islands (3). The number in brackets refers to the number of collectors included. A chapter is devoted to each collector with the exception of Milne and Hooker who appear twice; Milne in Africa and Oceans and Islands, and Hooker in Asia and Oceans and Islands. The text gives a short summary of the life of the collector, mostly less than half a page, and then details extracts of collecting expeditions from their own notes and correspondence. Modern collectors will recognize some of the problems encountered. Few, hopefully, will have

experienced being shot, overhearing a plan to murder you, or catching an incurable disease and dying as a result. At that time, there was a relatively high mortality among Europeans. However, there is little about the bureaucracy that now surrounds collecting, the time constraints of being in the field and the significantly increasing and irreversible impacts of human populations on the biodiversity.

While the book is very readable and one that I can pick up and read from time to time, it is sometimes a little disjointed and some descriptions leave you wanting more information. Of course, that extra information more may not exist. There are appendices: discussions of plant names, herbaria, the Wardian case, references & notes, and an index follow. There are a few black and white and color photographs of plants and localities, and portraits of some of the collectors.

Philip Short should be pleased with his contribution, and I can recommend the volume to anyone interested in collectors, collecting and plants. I particularly recommend it to administrators who sometimes consider plant exploration in countries of the world to be akin to a holiday on the Riviera!—*Simon Owen, The Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey, UK*

POLLUTANTS IN THE MUSEUM ENVIRONMENT: PRACTICAL STRATE-GIES FOR PROBLEM SOLVING IN DESIGN, EXHIBITION AND STORAGE, 2002, Pamela B. Hatchfield. (Archetype Publications, London, England, 203 pp. softcover.) This book addresses an important area of concern for natural history collections which few texts have hitherto analyzed. By offering a comprehensive review of this complex subject in a clear and accessible format, it makes a signal contribution to our understanding of how pollutants in the museum environment can cause deterioration of collections. Although written explicitly for people who care for cultural artifacts, it is just as relevant for custodians of natural history collections, and also those who plan exhibition and storage spaces for them— architects, engineers and project managers.

Recognition of the importance of environmental pollutants to the preservation of museum collections has lagged behind considerations of relative humidity, temperature, and light exposure. This is primarily because it has been difficult to attribute deterioration to any specific factor, because deterioration is often slow and subtle, and because "aging" has been assumed, by many, to be inevitable. Now, with better detection methods, a better understanding of the effects of pollution on different materials, and a greater awareness of public health issues, there is a general appreciation of the harmful effects of many environmental pollutants.

This book thoughtfully explores the sources of damaging contaminants in the museum environment and the ways in which we may assert control to minimize damage and prolong the useful life of collections. SPNHC members will certainly wish to consult this book.

Chapter 1, *Sources of Pollutants in the Museum Environment*, identifies the primary environmental pollutants that are found in museum storage and exhibition areas and discusses their probable sources. These pollutants, which are of both man-made and natural origins, may be generated from sources external to the museum such as the combustion of fossil fuels, industrial processes, and biological decay, or by sources within the museum including paints and other coatings, adhesives, plastics, wall and floor coverings, cleaning agents, inks, and many other products.

Much of this information is summarized in table form. There are, in addition, interesting discussions of how factors like relative humidity and light can influence the rate or degree of damage, and how certain materials, including building materials and even artifacts themselves, can act as sinks, adsorbing or absorbing environmental pollutants and then, with changes of temperature, relative humidity, and pollutant concentration, release them, thus becoming new sources of harmful products.

Chapter 2, *Damage to Materials*, discusses in specific terms the deterioration that different materials will undergo in to the presence of environmental pollutants. A wide array of materials: a variety of metals, mollusk shells, mineral specimens, fossils, stone, ceramics and glass, cellulosic materials, photographic materials, plastics, colorants, adhesives, leather, wool, and more are covered.

Chapter 3, *Testing for Pollutants*, explains the various methods that can be used to identify pollutants in the environment. Clear, step-by-step procedures are given for conducting all the most current micro-chemical and incubator tests of suspect construction or storage materials and external pollutants. This information makes this chapter particularly useful, because this valuable information has often been scattered among a variety of sources.

Chapter 4, *Mitigation of Pollutants in the Museum Environment*, discusses the different strategies that can be employed to limit damage to collections by environmental pollutants. One approach that is explored is lowering pollutant levels in the museum by regulating ventilation and filtration of incoming and recirculating air. Another tactic is to choose only low emitting materials when planning construction materials for collections areas, an important consideration that has, until recently, received little attention. The chapter also alerts the reader to potentially harmful construction processes, and includes a useful chart of pollutants associated with building activities ranging from carpentry to plumbing to roofing.

Chapter 5, *Using Materials in the Museum Environment*, describes the materials commonly used in association with museum collections, either in storage or in display environments for collections objects. The text is usefully supplemented with lists and charts of wood species, wood products, plastics, gasketing, caulking, and many other materials. We still have much to learn in this area so the information presented is not always clear-cut, but will nonetheless help the reader to select woods, plastics, adhesives, floor coverings, and many other materials that will emit the least amount of harmful volatile substances for use in proximity to collections.

Chapter 6, *Protecting Objects in Enclosures*, discusses in detail a broad selection of scavengers, adsorbents, buffers and vapor phase inhibitors that may be used to remove environmental pollutants from the vicinity of museum objects. Many different products are explored, and the way in which they work is explained, although the information that is available at this time still appears to be somewhat uneven.

The book's appendices provide further information on commonly used units of measurement of airborne pollutants, technical information on plastics, and a handy evaluation of product stability. There is a list of manufacturers and sources for many of the products mentioned in the text, and straight-thinking instructions, reprinted from Arts, Crafts & Theater Safety, Inc. (ACTS), on how to use Material Safety Data Sheets.

Pamela Hatchfield has done a great service for those responsible for the preservation of natural history specimens, as well as cultural artifacts and works of art. This volume is sure to remain a primary source for all collections managers, conservators, preparators and others concerned with the care of collections for the foreseeable future.—*Barbara P. Moore, 2 Lacoma Lane, Pittsford, New York 14534, USA*

INSTRUCTIONS TO AUTHORS

Articles for Collection Forum are six to 30 pages double-spaced (approximately 7500 words including abstract and literature cited), plus figures and tables. They may include original contributions to the literature or significant review articles dealing with the development and preservation of natural history collections. Contributions may include, but are not restricted to, reports of research and methodologies for the collection, preparation, conservation, storage, and documentation of specimens, and discussion of some philosophical, theoretical, and historical aspects of natural history collection management. Case studies that serve to document or augment a philosophy, methodology, or research activity will be considered, but general descriptions of a specific collection or institution are not accepted.

Manuscripts should be submitted digitally in Microsoft Word or WordPerfect, IBM format. All parts of the manuscript must be double spaced to letter (8 ½ × 11 inch; 21.6 × 27.9 cm) or A4 paper size with at least one inch (2.5 cm) margins on all sides. Each page of the manuscript should be numbered. Do not hyphenate words at the right-hand margin. Each table and figure should be on a separate page. Each table and figure should be in a separate file unless they are .doc files. The ratio of tables plus figures to text pages should generally not exceed 1:2.

On the first page indicate only the name, email address, telephone, and mailing address for the author to whom correspondence and proofs should be addressed. The second page then includes only the title of the article, names of the authors, affiliations and addresses of authors, and the abstract. Begin the text on the third page.

The preferred language for manuscripts is English, but a summary in another language can precede the literature cited, if appropriate. Manuscripts written in other languages may be considered at the discretion of the Managing Editor if the language uses the Roman alphabet, an English summary is provided, and reviewers are available for the language in question.

Abstract. An abstract summarizing in concrete terms the methods, findings, and implications discussed in the paper must accompany each article. The abstract should be completely self-explanatory and should not exceed 200 words.

Style and Abbreviations. Symbols, units, and nomenclature should conform to international usage. Cite all references in the text by the author and date, in parentheses. For example, (Deer et al. 1992, Fraser and Freihofer 1971, Mahoney 1973, Taylor 1967) would be an acceptable citation. Footnotes are not accepted in the text.

Literature Cited. This section includes only references cited in the manuscript and should be double spaced. References are listed alphabetically by authors' names and take these forms:

Brokerhof, A.W., R. Morton, and H.J. Banks. 1993. Time-mortality relationships for different species and development stages of clothes moths (Lepidoptera: Tineidae), exposed to cold. Journal of Stored Products Research 29(3):277-282.

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

Thomson, G. 1986. *The Museum Environment*, 2nd ed. Butterworth's, London, England. 293 pp.

For references to Internet sites, the format is:

ICOM International Committee for the Training of Personnel. 2000. ICOM Curricula Guidelines for Museum Professional Training. http://museumstudies.si.edu/ICOM-ICTOP (15 July 2002).

[the closing date of the reference is the date information was retrieved]

Tables. Tables should not repeat data contained in the text. Each table should be numbered with Arabic numerals, include a short legend, and be referred to in the text. Column headings and descriptive matter in tables should be brief. Vertical rules should not be used. Tables should be placed, one to a page, after the references.

Figures. Figures should not repeat data contained in the text, and must be of professional quality, because they will not be redrawn by the editorial staff. They may include line drawings, graphs, or black and white photographs. Colour images will be printed as black and white. Photographs should be cropped to show only essential features. Each figure should be numbered with Arabic numerals and referred to in the text. Captions for figures should be provided on a separate page at the end of the manuscript. Magnification scale, if used, should be indicated in the figure by a scale bar, not in the caption alone. Figures must be produced at a scale that will reduce clearly to a maximum page width of five inches (12.5 cm) or 8 inches (20 cm) in landscape orientation (portrait orientation is preferred). Please consult the Allen Press specifications for digital art.

Please see www.spnhc.org or consult the Managing Editor for additional information.

Articles

Book Reviews