The Curation Newsletter is available on the Internet through the WWW at http://muse.bio.cornell.edu/ and Gopher at gopher://muse.bio.cornell.edu:70/

SUBSCRIPTION RENEWAL

In the past, the Curation Newsletter has been distributed at ASIH meetings and sent by mail to approximately 400 addresses. In the interest of minimizing reproduction and mailing costs, we are asking interested recipients of the Newsletter to access it electronically rather than requesting a printed copy. This issue of the Newsletter will be mailed to all on the current mailing list but we are requesting a subscription renewal ONLY by those who do not have access (or do not expect it within the next year) to Gopher or WWW, or can not otherwise access the Newsletter electronically. Libraries and those wishing to renew should send their name, title, institution name, department and address to Susan Jewett (see address listing at the end of newsletter for Susan's address as well as complete physical and email addresses of all contributors).

Future issues of the curation newsletter will be mailed only to those who renew at this time.

Articles

BIS-CARBONYL FIXATIVES FOR MUSEUM SPECIMENS?
Douglas W. Nelson, Philip W. Willink, Barbara A. Shields

The effectiveness of a bis-carbonyl "fixative" for preservation of fish specimens for archival storage was tested at the Division of Fishes, Museum of Zoology, University of Michigan (UMMZ). These observations and tests were undertaken as part of an ongoing program of the Ichthyological and Herpetological Collections Committee (IHCC) of the ASIH to test and report on products, procedures, and policies relevant to acquisition, maintenance, and uses of ichthyological and herpetological collections.

Materials and Methods.

NoTox Histological Fixative (Earth Safe Industries, Inc., Belle Mead, NJ 08502), a bis-carbonyl based "fixative", which also contains ethanol, propylene glycol, and "antiseptic and antifungal agents", was used to fix a collection of Michigan freshwater fishes taken by R. M. Bailey and W. C. Latta in July, 1994. Following the manufacturer's directions, the fish were placed into a 100% solution of NoTox and were left in this solution for a period of one week. After one week approximately one-half of the fish in the collection were transferred (after a brief rinse in cold water) into successive baths of 50% and 70% ETOH and were then placed into a final 70% ETOH solution for archival storage. A second collection, made by Bailey and Latta in August, 1994, was also fixed in NoTox Histological Fixative. Approximately one-half of the fish in the second collection were transferred into NoTox Biological Preservative, a preservation solution containing essentially the same ingredients. This product is also stated by the manufacturer to be capable of "fixing" animals and cadavers; however, in these tests this product is treated as a "preservative" and only its effectiveness as a solution for long-term specimen storage is being evaluated. Results of these longer-term tests will be reported later. The remainder of both collections were left in the original "fixative". Control samples of fish, taken by Bailey and Latta during these same surveys, were fixed in a 10% formalin solution and transferred to ETOH using these same procedures. All specimens have been placed into permanent archival storage in an unsorted condition for further observation and testing.
Loans and gifts from these collections may be made to persons interested in conducting further investigations.

Results.

Observations made on the fish "fixed" in NoTox Histological Fixative approximately 24 hours after initial fixation revealed the following morphological features. The eyes of the specimens were white and cloudy -- similar to the eyes of fish that have been initially preserved in ethanol. The coloration of the fish had begun to fade badly in comparison with the formalin-fixed samples. Although not quantified, the fish had apparently begun to shrink: this was particularly evident in the abdominal region. Some specimens exhibited considerable discoloration and clearing of the abdominal region, similar in appearance to autolysis of tissue resulting from incomplete fixation of digestive enzymes immediately after death. In addition, the NoTox fluid had acquired an oily appearance.

Upon transfer into 50% ETOH the specimens initially floated: some specimens continued to float after 20+ hours in this solution. After transfer to 70% ETOH, almost all specimens had sunk after 6 hours.

During sorting and identification of these specimens (both the fish left in NoTox and those that had been transferred into ETOH) by Bailey and Latta, the following observation were made by these investigators. The coloration of the fish had faded badly. The fin membranes had become fragile and tended to tear badly when elevated to count fin rays or to examine the fins for color patterns. The fish had an unpleasant "coating" on the body. In addition, the fish appeared to have undergone some differential shrinkage, especially noticeable in the abdominal region and in the lateral components (i.e., the girth dimensions of the fish).

Subsamples (from both the NoTox and formalin-fixed lots) of three taxa *Cyprinella spiloptera*, *Fundulus diaphanus*, and *Etheostoma nigrum* were cleared and stained for bone and cartilage following the procedures of Taylor and Van Dyke (1985). The osteological preparations from the formalin-fixed specimens stained well and maintained their body integrity. The specimens fixed in NoTox stained well for cartilage, but disintegrated after a short period of time in trypsin. The bones of the disarticulated specimens later stained well with alizarin red S. All osteological preparations were then placed into archival storage in 100% glycerin.

An independent test, using muscle tissue from a pink salmon (*Oncorhynchus gorbuscha*), was conducted to evaluate the suitability of this preservative for DNA studies. Specifically, the manufacturer claims NoTox to be polymerase chain reaction (PCR) compatible. Neither extraction of high molecular weight DNA nor PCR amplification was successful using this tissue, although these procedures worked well for tissue that had been frozen or preserved in other media (Queen's buffer, several alcohols). Detailed results of these tests will be published later.

Discussion.

In the discussion below the authors are not seeking to attribute any of the observations or results of these tests to any particular chemical constituent in NoTox Histological Fixative. We consider that these conclusions are preliminary and are representative of the effectiveness of the solution AS A WHOLE (authors' emphasis).

The observations suggest that NoTox Histological Fixative does not behave as a true fixative for whole specimens as traditionally understood, for example, in the cases of formaldehyde or glutaraldehyde solutions. The morphological appearance of the specimens (white eyes, loss of pigment, body shrinkage) tends to indicate that this solution relies on a high percentage of an alcohol to preserve rather than fix the tissues. The low specific gravity of the NoTox-fixed material (which floated in 50% ETOH) further tends to corroborate this. The disarticulation of the specimens during the trypsin digestion portion of the clearing and staining procedure also is similar to that experienced with non-fixed, alcohol-preserved material. We also suggest that the shrinkage of the specimens, presumably due to the loss of lipid material (fats and oils) and water (possibly due to large amounts of propylene glycol and alcohols in the solution), may make accurate morphometric comparisons with specimens fixed using more traditional methods difficult.
The lack of success with molecular testing of the tissue, however, indicates that some denaturation or destruction of the DNA had occurred during preservation in NoTox Histological Fixative. Further histological and biochemical examinations of the tissues is required to clarify the precise nature of the behavior of this product on both tissues and whole specimens.

Based on these preliminary observations and tests, we do not think that this product is an adequate substitute for formaldehyde as a fixative for specimens destined primarily for morphological examination. However, the specimens appear to be adequately preserved for purposes of identification and may be suitable for archival storage. The UMMZ presently will accept fish "fixed" in this fluid as identification voucher specimens, recognizing the apparent limited scope of ichthyological value of these materials.

**Acknowledgments.**

The authors thank R. M. Bailey and W. C. Latta for the collection and field preservation of the specimens used in these experiments. We also thank John Simmons (Univ. of Kansas) and Susan Jewett (National Mus. Nat. History) for their critical reviews of the manuscript.

**Literature Cited**


**ISOPROPANOL REVISITED**


In recent years the preferability of using ethanol or isopropanol in ichthyological collections has been the subject of much discussion. Establishing cause for the occasional, poorly-preserved specimen stored in either isopropanol (ISO) or ethanol (ETH) collections is difficult since the precise curatorial history of many specimens is sketchy at best. Deteriorated specimen condition may reflect inadequate original fixation procedures, poor post-fixation curatorial attention, or the type of alcohol utilized. Fixation and preservation methods have changed little since the advent of collections and are in use because, by trial and error or pure chance, they were found to work (Simmons 1992). It is clear that there is a pressing need for well-documented studies addressing the comparative utilities of these alcohols and also other preservatives. Such studies must be made prior to making recommendations for collection-wide uniformity in choice of an ichthyological preservative. This is not an attempt to promote one preservative over another, but simply an article reporting our observations.

Our combined experience in ichthyological collections represents over 80 years of working with isopropanol on a daily basis. We have found virtually none of the problems that have been anecdotally associated with ISO fish collections. The group discussion following the recent Curation Workshop (see Laframboise, et al. 1992) raised some issues which should be addressed. The two major concerns associated with preservatives are specimen condition and safety. Generally, one of the desired qualities for a preserved specimen is to be lifelike (Simmons 1992). During the typical fixation (formalin)/preservation (ETH or ISO) process all colors except brown or black are lost, regardless of the choice of ISO or ETH. However, bony fishes in ISO are more flexible, seem to shrink less (Laframboise, et al 1992, found noticeable dehydration when transferring specimens from 45% ISO to 70% ETH and a graded series of solutions was needed to minimize this shrinkage; see also Figures in Lai 1963) and fin rays are often less brittle than those stored in ETH. One criticism of ISO is that this more lifelike condition "may mask" specimen deterioration, but after decades of observations on millions of specimens, we find virtually no deterioration (clearing, fragmentation, swelling, discoloration of specimen or cloudiness of fluid, dehydration beyond the initial processing, flotation, shape distortion, fungal growth, etc.). Clearing and staining specimens presents no problem; we simply soak out the ISO in water and put the specimens in ETH. The specimens come through the process just fine.

The reported immiscibility of ISO and water is
easily overcome by thoroughly mixing the stock solution initially and exchanging the liquid for newly preserved specimens two or three times depending on the volume of fish in the container. The percentage of ISO in solution can quickly and effectively be obtained using a calibrated hydrometer. Although digital density meters are handy and quite accurate, they may not be cost effective with negligible levels of contaminants in jars and even large specimen tanks. In reference to other contaminants, we represent different parts of the U.S. and Canada and we have always been able to locate reliable sources of pure, technical grade (inexpensive) ISO. (Using ISO also saves money since ETH collections (at 75%) are using approximately 50% more alcohol than we use.)

Recent conversations/inspections with safety personnel at SIO, CU and ARC raised other issues. Although ISO is technically more toxic than ETH, all our labs have no safety problems related to toxicity. However, the Uniform Fire Code exempts 50% ISO (or ETH) solutions from those requirements for storing 70-75% ETH (or ISO) solutions (Canada recognizes all solutions as 100%). Thus a 50% ISO collection requires a less costly system and/or equipment to meet the Fire Code.

Based on these observations we conclude that ISO ichthyological collections are not substandard facilities for scientific specimen storage. There is, as yet, no quantifiable data on the two alcohols which suggests whether or not a choice should be made (Fink, et al 1979). Although we prefer the smell of ETH, ETH has not been shown clearly superior or the obvious choice as the best preservative. We believe that valuable curatorial dollars should not be spent financing a conversion of a collection from one preservative to another until documented studies have been performed and we call upon the collections community to initiate such studies.

Literature Cited


Laframboise, S., R. M. Rankin and M. M. L.


MUSE

Julian Humphries, Jr.

The MUSE project has recently been funded by the National Science Foundation for another three years. The purpose of the MUSE Project is to design, program, distribute and support software for the management and curation of natural history collections. The new round of funding will allow the MUSE staff to continue support for existing and new MUSE users. Effort will also be continued in the improvement of MUSE software, geographic authority files, user security, as well as other improvements. We will also be working towards adding new taxonomic disciplines to the project and creating a version of MUSE for Windows users.

Currently we have approximately eighty sites supported by the MUSE Project, spanning a broad array of taxonomic disciplines. Ichthyology was our first specialization and because of the large number of MUSE sites in Ichthyology we have dealt with most issues that fish collections face.

To date we have produced fifty-two Ichthyology versions of MUSE, evenly split between national and international institutions including collections with extensive freshwater and marine holdings. Five (soon six) of the eight sites rated International Centers (Poss and Collette, Copeia, 1995) use MUSE to manage their collections. Collections in this discipline range in size from small university collections (<20,000 lots) to large international centers (>200,000 lots). Complete taxonomic authority files down to
the level of genus are available with species level data available for limited geographic areas (primarily North America).

Our second area of strength is Herpetology, with fourteen institutions using MUSE. Again, the breadth of collections is extensive including eight USA and six international collections. Taxonomic authority files are incomplete, consisting of currently accepted names down to the level of genus.

MUSE has also moved to the Internet. The latest version of our newsletter "MUSENEWS" was dedicated to our use of the Internet. We have a home page on the World Wide Web; The Biodiversity and Biological Collections WWW Server (http://muse.bio.cornell.edu/), which includes up to date information on the MUSE Project. The WWW Server also contains valuable information about specimens in major biological collections and allows users access to directories of biologists including ASIH, and an archive of listservs including Taxacom, Cichlid-L, and our own listserv - MUSE-L. Numerous documents including ASIH curatorial reports and workshop proceedings are available on the server. The WWW server allows real time query access to several hundred thousand records of fish data in MUSE databases as well as the ability to view those query results as distribution maps.

Another recent addition to the MUSE project Internet resources is the MUSE Server. This is a Windows based add-on to MUSE databases that allows WWW and Gopher based queries of collection data. The MUSE Server requires an Internet connection, Winsock software and a spare 386 or faster computer. Currently, Cornell, Michigan, Harvard and Sweden fish collections are running MUSE Servers.

The FishGopher located at URL gopher://muse.bio.edu:70/11/fishgopher is a collaboration between free-standing museums and university collections in the development of open-access biological collection community databases. It was funded by a grant from the NSF program on Research Collection in Systematics and Ecology and facilitated by support from the MUSE Project. Currently seven large fish collections are searchable through FishGopher.

More information on the MUSE Project can be obtained by subscribing to the MUSE-L listserv at cmusa.berkeley.edu or by sending an e-mail message to MUSE@cornell.edu.

**PRESERVATION OF COLOR IN LARVAL FISHES**

David G. Smith

It is not generally appreciated that larval fishes have colors. Reds and yellows are most common and are distributed in characteristic patterns. Freshly caught larvae observed under a microscope can be as aesthetically striking as any adult fish, and their color patterns often form important taxonomic characters.

The problem with color in fishes is that it does not persist in preservative. In a matter of days or weeks after death, the brilliant colors fade and leave only patterns of light and dark pigmentation which may also disappear in time. With adult fishes, this problem is usually solved by photographing freshly caught specimens. Photography is not as practical with larvae, as it requires elaborate photomicrographic apparatus. During the 1960's, the antioxidant BHT (butylated hydroxytoluene, also known by the trade name "ionol") was introduced and enjoyed a brief period of popularity among ichthyologists (although, to my knowledge, it was not used on larvae). For some reason, BHT lost its appeal and was largely abandoned. In recent years, it has begun a modest comeback among certain larval-fish workers.

I have experimented extensively with BHT during the past three field seasons in Belize. Many of the larvae collected here display striking and distinctive colors. Using standard preservation techniques, these taxonomically important colors rapidly fade and are gone by the time the specimens are returned to the museum.

BHT in its pure form is an odorless, white, crystalline substance. It is nearly insoluble in water, and thus in formalin. In its earlier incarnation, it was used mainly in an emulsified form, placed directly in the formalin in which the fish were fixed. My current use of BHT involves some differences from earlier procedures.
These differences are due principally to the following: 1) although BHT is essentially insoluble in water, it is readily soluble in ethyl alcohol; 2) colors do not fade instantly (except for certain structural colors that are not important in larvae) but gradually over a period of days or weeks; 3) larvae are small and require only a short time to be adequately fixed in formalin. My standard procedure is to fix the larvae as usual in ca. 10% formalin, without BHT, and let them stand overnight. The following day I transfer them to 75% ethanol (usually going through at least one intermediate stage) with some BHT added (see below). BHT dissolves very slowly in 95% ethanol; it requires a lot of stirring. As the concentration of ethanol is reduced, the solubility of BHT declines sharply. I can dissolve at least 10 teaspoons of BHT in a quart of 95% ethanol, but barely a third of a teaspoon will dissolve in a quart of 75% ethanol. After preparing the solution in 95% ethanol, I diluted it to 75% with rain water. The excess BHT precipitates out, leaving a saturated solution of BHT in 75% ethanol. To test the effect of different concentrations, I did three 1:1 dilutions of the BHT saturated 75% ethanol solution with plain 75% ethanol and ended up with four samples: saturated, one-half, one-quarter, and one-eighth saturated. I also included one sample of 95% ethanol with about 10 teaspoons of BHT per quart. For controls, I used 75% ethanol without BHT and 5% formalin in seawater, also without BHT. I then filled each of the samples with representatives of the various larvae collected that night.

The sample with the worst color preservation was that in 5% formalin; the colors faded completely after a few weeks. The second-worst color preservation was the sample in 75% ethanol without BHT. The colors were badly faded, but traces were still visible after the formalin-preserved specimens had lost all their color. At the other extreme, the specimens in 95%, BHT-enriched ethanol faded rather quickly. The other four samples of 75% ethanol/BHT produced excellent results and seemed to result in about the same degree of color preservation. I noticed little obvious difference between the specimens preserved in saturated BHT and those in one-eighth saturated BHT. A year and a half later, these samples still retain color.

BHT is by no means a panacea. It is impractical to use in standard sampling programs because it requires specimens to be transferred to ethanol a day after collection. The long-term effects on specimens are still unknown. After two and a half years, all of the specimens I preserved in BHT are still in good condition, and I have cleared and stained many of them with excellent results. Whether colors will last permanently or whether they will eventually fade is still to be determined. Although many questions remain and much work must be done, BHT has proven itself a useful tool. The delicate red and yellow pigments of larval fishes can be captured and preserved, at least long enough to return the specimens to the laboratory and examine them at leisure.

LASER PRINTER LABELS: POTENTIAL DISASTER WHILE SPECIMENS ARE ON LOAN
Susan L. Jewett

Many fish collections are now using laser printer labels for their wet specimen catalog labels. It has been known for some time that labels generated by the usual office variety laser printer (unlike the high temperature/pressure laser printers) are not very durable and that the letters are susceptible to rubbing off if the label is abraded. Most fish collections have made a practice of including a catalog number tag in each jar in addition to the wet label, and this serves as a backup system should the label get destroyed or otherwise become illegible.

The purpose of this note is to forewarn all managers of wet specimen collections using laser printer labels to provide a backup system when sending specimens on loan, as well. We have received fish on loan from a large U.S. ichthyological collection, accompanied by labels that were illegible on arrival, presumably due to the abrasive forces of wrapping and packing. Fortunately we discovered this when we were unpacking and prepared, by hand, a backup label with pertinent data (copied from the loan invoice). I wish to make the following suggestion to all those who send laser printed labels with fish loans: send a copy of the label data on the shipping invoice AND send a
catalog number tag to keep in the jar with the specimen, while on loan.

ANNOUNCEMENTS


In January 1995, the ASIH Supplies and Resources Subcommittee began its survey of products, manufacturers and vendors used by collections of fishes and herps. We started with 22 collections and expand the survey list to include more collections in South America. The Subcommittee hopes that those of you asked to participate in the survey this year will do so and return the forms in a timely manner. We know that surveys are time consuming and tedious. However, once we have a products and supplies database established and available on the Internet Gopher, future surveys and access to the information will be much easier. Our Subcommittee would like to thank (ahead of time) all the participating collections for their patience and effort in responding to this survey. We will be sending these survey forms out over the next year (or two?) so "do not despair" if you don't receive a survey at the beginning of the year; we will not forget you!

ASIH SUBCOMMITTEE ON SUPPLIES AND RESOURCES, Lex Snyder, Chair Museum of Southwestern Biology, Univ. of New Mexico, Albuquerque, NM 87131 [amsnyder@bootes.unm.edu].

SPNHC

The 1995 Annual Meeting of the Society for the Preservation of Natural History Collections will take place 2-6 June at the Royal Ontario Museum in Toronto, Canada. A training workshop, "Managing the Modern Herbarium" is planned for 5-6 June. For more information about this meeting, please contact Janet Waddington, SPNHC'95 Organizing Committee, Royal Ontario Museum, 100 Queen's Park, Toronto, Ontario, CANADA M5S 2C6, Telephone 416-586-5593 or FAX 416-586-5863.

DIGITAL DENSITY MEASUREMENT OF PRESERVATIVES.

The most accurate way to determine the strength of alcoholic preservative solutions is to use a digital density meter. A hand-held model made by PAAR company is available from Fisher Scientific for US $2024. This unit measures density accurately to within 0.001. Density in g/cm3 and temperature in degrees C are shown in an LED digital readout. The telephone for Fisher Scientific is 1-800-766-7000. The meter is item number 10-820-10 in their catalog.

The temperature and density readings obtained with this meter can be quickly and accurately converted to percent alcohol with a "shareware" program available from the Canadian Conservation Institute. The program is available in two versions, for both ethyl alcohol and isopropyl alcohol concentrations. For further information, contact Thomas Strang, Canadian Conservation Institute, 1030 Innes Road, Ottawa K1A 0C8, CANADA, telephone 613-998-3721.

SUPPLIER FOR SMALL ORDERS OF JARS AND LIDS.

A company which will accept small orders (minimum US $20) for glass jars with polypropylene lids and polyethylene liners is Scientific Specialties Service, Inc., telephone 1-800-648-7800.

INSERTS FOR SCREW-TOP JARS.

Clear, flexible polyethylene inserts that fit down into the necks of screw-top jars provide a much more effective seal than do flat lid liners. Previously, these have only been available for jars made by the Abico Company in Japan. However, there is now a United States manufacturer for inserts in four standard sizes. For information on prices, contact Dr. Robert Timm, Division of Mammalogy, Natural History Museum, University of Kansas, Lawrence, Kansas 66045-2454. The inserts were developed under an IMS grant to the Natural History Museum of the University of Kansas.

The previous four items were submitted by John E. Simmons.
RECENT LITERATURE OF INTEREST


Literature compiled by John E. Simmons and Susan Jewett

Except where noted, this Newsletter is written and compiled by the Newsletter Subcommittee of the Ichthyological and Herpetological Collections Committee, American Society of Ichthyologists and Herpetologists, and is intended for use by its membership. Comments are not to be construed as an endorsement of practices or products by ASIH. Members of the Subcommittee are:

H.J. Walker, Jr. (chair), Scripps Institution of Oceanography, U.C.S.D. 0208, La Jolla, CA 92093-0208 [hjwalker@ucsd.edu] phone: 619-534-2199 FAX: 619-534-5306

George H. Burgess, Florida Museum of Natural History, University of Florida, Gainesville, FL 32611 [gburgess@flmnh.ufl.edu]

Julian M. Humphries, Jr., Cornell Vertebrate Collections, 83 Brown Road, Bldg 3, Cornell University, Ithaca, NY 14850-1247 [jmh3@cornell.edu]

Susan L. Jewett, Division of Fishes, National Museum of Natural History, MRC 159, Smithsonian Institution, Washington, DC 20560 phone: 202-357-3300 [mnhvz020@sivm.si.edu]

Cynthia I. Klepadlo, Scripps Institution of Oceanography, U.C.S.D. 0208, La Jolla, CA 92093-0208 [klepadlo@ucsd.edu]

Lou VanGuelpen, Atlantic Reference Centre, Huntsman Marine Science Centre, St. Andrews, New Brunswick, Canada E0G 2X0 [arc@bionet.bio.dfo.ca]

Other Contributors:

Douglas W. Nelson; Museum of Zoology, University of Michigan, Ann Arbor, MI, 48109-1079 [dwnelson@umich.edu]

Arnold Y. Suzumoto; Bishop Museum, 1525 Bernice St., P.O. Box 19000-A, Honolulu, HI 96817.

Barbara A. Shields; Department of Biology, 316 Mark Jefferson, Eastern Michigan University, Ypsilanti, MI 48197.

John Simmons; Natural History Museum, University of Kansas, Lawrence, Kansas 66045-2454 [jsimmons@kuhub.cc.ukans.edu].

David G. Smith; Division of Fishes, MRC-159; National Museum of Natural History; Washington, DC 20560 [mnhvz077@sivm.si.edu].

Philip W. Willink; Museum of Zoology, University of Michigan, Ann Arbor, MI, 48109-1079