



California Academy of Sciences
Department of Invertebrate Zoology and Geology
55 Concourse Drive
Golden Gate Park
San Francisco, CA 94118

August 2024

Protocols and standards for preservation and archival of vernal pool crustaceans.

Specimens of vernal pool crustaceans listed as endangered or threatened by the U.S. Fish and Wildlife Service (USFWS) and collected during surveys permitted by the USFWS may be deposited as voucher specimens at the California Academy of Sciences, Department of Invertebrate Zoology and Geology (CASIZ). At the discretion of the Collection Manager, CASIZ will accept a small number of voucher specimens documented with both USFWS and California Department of Fish and Wildlife (CDFW) collection permits valid during the dates of collection. Only those specimens that have been properly fixed, preserved and documented in accordance with these protocols will be accepted for archive and archive is at the Academy's discretion. The vendors listed below can supply necessary materials for specimen storage, however these vendors are only suggestions and materials from other sources will be acceptable **provided they meet the standard requirements of CASIZ**. Any questions regarding these standards and protocols should be directed to Christina Piotrowski, Collection Manager of Invertebrate Zoology at CASIZ, preferably by email to cpiotrowski@calacademy.org. **Visits to the collection to deposit potential voucher specimens must be scheduled at least 7 days in advance. Due to experiences with past shipments, specimens shipped to CASIZ will not be accepted and must be hand-deposited.** Each deposit must be accompanied by a packing list of specimens and a completed Excel data sheet. CAS staff will assign CASIZ catalog numbers and notification will be sent to you by email. This will be done in an expeditious manner, however staffing limitations may cause delays. Please allow two weeks for notification of CASIZ catalog numbers.

Alternatively, wet-preserved specimens and dried cysts may be submitted for archive without museum standard containers but with the same quality of data and permits. CASIZ staff will re-house the samples into standard archival containers for \$20 per specimen lot. [A specimen lot contains a single species with identical data (collector, locality, time, date, depth, etc.). Each specimen lot must be accompanied by all relevant data (see details below) and a check for \$20 per specimen lot payable to: "California Academy of Sciences". You must receive approval directly from the Collection Manager of Invertebrates at CASIZ prior to deposit using this method. Again, all specimens must be properly labeled as described below and delivered in person, not shipped, for acceptance as vouchers. Otherwise deposited materials will be returned to sender.

Please see attached Protocols for preparing a specimen deposit.

Standards and Protocols

Wet-preserved specimens:

Materials required:

- 100% or 95% non-denatured ethanol
- 2 dram, 4 dram, 6 dram glass shell vials (screw top vials are not acceptable)
- Clean cotton
- 8 oz. tall flint glass bottles, 58 mm aperture (outer diameter) or 32 oz. glass bottles, 89 mm aperture (outer diameter)
- White polypropylene screw-top closures with solid (no holes) smooth surface fitting 58 mm diameter (8oz. bottles) or 89 mm diameter (32 oz. bottles), with foam F217 liners
- Byron Weston Resistall Paper (must use ethanol resistant paper)
- Technical pen with alcohol-proof ink, or #2 pencil

Procedures:

- Vernal pool crustaceans must be fixed and preserved in 95% non-denatured ethanol. A minimum ratio of 10 parts 95% ethanol to 1 part tissue is required for preservation.
- All specimens must be sorted in a "lot" by collecting event (one lot holds specimens with identical locality/date/time of collection/collector and all other data). Specimens must be identified to species level, each species lot from each collecting event placed into a vial or vials in its own 8 or 32 oz. bottle (use 8 oz if possible).
- Specimens are placed into 2 dram, 4 dram, or 6 dram glass shell vials with **straight sides (no shoulders)** filled with 95% ethanol. Do not submit screwtop vials. The vial or vials are filled with 95% ethanol, then tightly plugged with clean cotton in such a manner that no air bubbles are trapped inside and placed inverted into an 8 or 32 oz. glass bottle filled with 95% ethanol. If open vials with specimens are inserted upright into the larger container, then plugged with ethanol-damp cotton, air bubbles will not be trapped in the vial. The vial may then be removed and placed back into the bottle with cotton plug oriented down for archive. It is important to remember that the specimens should not be jammed into the vials. The purpose of placing specimens into vials is to protect them from potential damage that could be caused by contact with labels placed into the bottle and/or during removal from the 8 or 32 oz. container. However, putting too many specimens into a vial or putting specimens into a vial that is too small, will damage them. The required ratio of preservative to tissue inside the vial is at least 10 parts 95% ethanol to 1 part tissue. This may require splitting a species sample from a single collecting event into two or more vials within a bottle or even into two bottles.
- The 8 or 32 oz. glass bottle is securely capped with the chemical resistant foam lined screw top polypropylene closure.
- Each 8 or 32 oz. bottle must contain a label with collecting event data on Nalgene polypaper in alcohol-proof ink or #2 pencil. Labels must be placed into the specimen bottle, which contains the specimen vial(s), and not directly inside the vials and not attached to the outside of the bottle. The data may be hand printed with technical pen using alcohol-proof ink or a #2 pencil. **Laser printed or photocopied labels are not acceptable.** All labels must be easily readable by CAS staff. If labels are not legible, specimens will not be accepted or cataloged into the CAS collection. Labels should be no larger than 3 x 5 inches and no smaller than 2 x 3 inches.

Dry preserved specimens (cysts):

Materials required:

- 4 dram, 7 dram glass shell vials (screw top vials are not acceptable)
- Gelatin capsules
- Clean cotton
- Permalife 20 lb Bond Paper
- Technical pen with archival ink (permanent, fade resistant, chemically stable, water-proof) [eg. Pigma® Micron®] or #2 pencil

Procedures:

- Specimens are placed into gelatin capsules (available in many pharmacies) within 4 dram, or 7 dram glass S-930 shell vials with **straight sides (no shoulders)** and plugged with cotton.
- Specimens must be sorted and labeled properly, as noted above in wet-preserved specimen section. However, labels should be placed into the vial in this case, as there is no larger container.
- Each vial must contain an archival quality label that includes collecting event data handwritten in archival ink or #2 pencil. Labels must be placed into the specimen vial, which contains the cysts in a gelatin capsule. All labels must be easily readable by CAS staff. If labels are not legible, specimens will not be accepted or cataloged into the CAS collection. Labels are to be no larger than 2.25" wide x 3 inches long and no smaller than 1 1/2 x 2 inches. Data for each specimen lot is entered into an Excel sheet that is emailed to CAS in advance of deposit. A hard copy accompanies the specimens.

Data required for specimen labels:

- Species name
- County, city/town, and other clearly worded description of collection locality so as to enable another scientist to find the collection locality
- Latitude and longitude in decimal degrees (UTM is not acceptable)
- Environmental data regarding habitat (temperature, turbidity, depth and size of pool)
- Full names of collector(s) and identifier
- Dates of collection and identification, dates should clearly indicate day, month and year (e.g. 10 Jan 1995)
- The phrase "Voucher specimen: Vernal Pool Crustacean Survey"

Note that valid USFWS and CDFW permits must accompany all deposits.

Supply Vendors:

The vendors listed below can supply the necessary materials for specimen storage, however these vendors are not specifically required and materials from other sources will be acceptable provided they meet the standard requirements of CASIZ. CASIZ will not act as a supplier of materials.

Glass vials: (S-930)	Acme Vial and Glass 1601 Commerce Way Paso Robles, CA 93446 (805) 239-2666 http://www.acmevial.com
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Glass bottles and polypropylene lids:	General Bottle Supply 1845 Daly Street Los Angeles, CA 90031
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(323) 581-2001

<http://www.bottlesetc.com/>

<https://www.generalbottle.com/glass-containers/glass-jars/paragon-jars-ct.html>

<https://www.generalbottle.com/plastic-ribbed-caps-with-foam-liner-f217-6498.html>

Paragon Jar, continuous thread, flint glass with 58/400 neck

White plastic ribbed caps with F217 foam liners, 58/400

Cotton:
(nonsterile)

PBS Animalhealth

2780 Richville Drive, S.E.

Massillon, OH 44646

Tel. 800 321-0235

<http://www.pbsanimalhealth.com/>

Gelatin capsules: available at most pharmacies

Permalife 20 lb Bond Paper [dry cysts]:

Archival Methods

230-2 Middle Road

Henrietta, NY 14467

Toll-Free Order Line (US): 866-877-7050 (M-F, 9am-5pm EST)

Item No. 132-8511

University Products Inc

517 Main Street

Holyoke, MA 01040

(800) 628.1912

<http://www.universityproducts.com>

Item No. 680-1211

Gaylord Archival

PO Box 4901

Syracuse, NY 13221-4901

1.800.448.6160

[http://www.gaylord.com/Preservation/Conservation-Supplies/Boards-%26-Paper/Permalife-20-lb-Bond-Paper-\(500-Sheets\)/p/HYB00968](http://www.gaylord.com/Preservation/Conservation-Supplies/Boards-%26-Paper/Permalife-20-lb-Bond-Paper-(500-Sheets)/p/HYB00968)

Part. No. PB811

Resistall paper [wet specimens]:

University Products Inc

517 Main Street

Holyoke, MA 01040

(800) 628.1912

<http://www.universityproducts.com>

(item 219-288511)

Non-denatured
ethanol
190 or 200 proof
(95% or 100%):

Gold Shield Chemical
3111 Depot Rd.
Hayward, CA 94545
(510) 782-2040
<http://www.goldsd.com/>

Example of select supply items for fluid preserved vouchers:



4 dram vial with cotton



8 oz bottle and lid