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### **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. 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 [cpiotrowski@calacademy.org](mailto:cpiotrowski@calacademy.org) or 415-379-5256. **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 archival 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 specimen shipment 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, 48 mm aperture (inside diameter) or 32 oz. glass bottles, 74 mm aperture (inside diameter)
- White polypropylene screw-top closures with solid (no holes) smooth surface, 58 mm diameter (8oz. bottles) or 85 mm diameter (32 oz. bottles), with foam or plastic liners
- Nalgene polypaper
- Technical pen with alcohol-proof ink, or #2 pencil

#### Procedures:

- Vernal pool crustaceans must be fixed and preserved in 95% non-denatured ethanol. A ratio of at least 3 parts 95% ethanol to 1 part tissue is required for preservation.
- All specimens must be sorted in a “lot” by collecting event (identical locality/date/time of collection). They 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 the smaller size 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. The vial or vials are 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 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 down for archival. 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 jar or during removal from the 8 or 32 oz. container. However, putting too many specimens into a vial or putting specimens into a vial, which is too small, will damage them. The required ratio of preservative to tissue inside the vial is at least 3 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 capped with the foam or plastic 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), 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
- Nalgene polypaper
- Technical pen, 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 a label with collecting event data on Nalgene polypaper in water-proof ink or #2 pencil. Labels must be placed into the specimen vial, which contains the cysts in a gelatin capsule. The data may be hand printed with technical pen using water-proof ink or a #2 pencil. 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 2 x 3 inches and no smaller than 1 1/2 x 2 inches. Accompanying data should also be deposited by Excel sheet, either by email or hard copy.

**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 documentation of 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>

Glass bottles  
and polypropylene  
lids:                              General Bottle Supply\* *NEW VENDOR, 2015*  
   8350 Pardee Dr #300  
   Oakland, CA 94621  
   (510) 300-2600  
   <http://www.bottlesetc.com/>  
   <http://www.bottlesetc.com/glass-containers/glass-jars/paragon-jars-ct.html>

Cotton:  
(nonsterile)                      PBS Animalhealth  
2780 Richville Drive, S.E.  
Massillon, OH 44646  
Tel. 800 321-0235  
<http://www.pbsanimalhealth.com/>

Gelatin capsules                      Reliable Drug Co.  
801 Irving St.  
San Francisco, CA 94122  
(415) 664-8800  
(or other pharmacy)

Resistall paper:                      University Products Inc  
517 Main Street  
Holyoke, MA 01040  
(800) 628.1912  
<http://www.universityproducts.com>  
(item 219-288511)

Bioquip  
2321 Gladwick Street  
Rancho Dominguez, CA 90220  
(310) 667-8800  
<http://www.bioquip.com>  
(item 1223RA)

Non-denatured                      Gold Shield Chemical  
ethanol                              3111 Depot Rd.  
190 or 200 proof                      Hayward, CA 94545  
(95% or 100%)                      (510) 782-2040  
<http://www.goldsd.com/>

**Example of select supply items for fluid preserved vouchers:**



4 dram vial with cotton



8 oz jar and lid