

## Appendix L

Interim Survey Guidelines for  
Permittees for Recovery Permits under  
Section 10(a)(1)(A) of the Endangered  
Species Act for Listed Vernal Pool  
Branchiopods, USFWS, April 19, 1996.



IN REPLY REFER TO:

# United States Department of the Interior

FISH AND WILDLIFE SERVICE

April 19, 1996

Interim Survey Guidelines to Permittees  
for Recovery Permits under Section 10(a)(1)(A)  
of the Endangered Species Act for the  
Listed Vernal Pool Branchiopods  
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## Interim Survey Guidelines to Permittees for Recovery Permits under Section 10(a)(1)(A) of the Endangered Species Act for the Listed Vernal Pool Branchiopods

The endangered Conservancy fairy shrimp (*Branchinecta conservatio*), longhorn fairy shrimp (*Branchinecta longiantenna*), vernal pool tadpole shrimp (*Lepidurus packardi*), and the threatened vernal pool fairy shrimp (*Branchinecta lynchi*) were listed on September 19, 1994, under the Endangered Species Act of 1973, as amended (Act) (59 Federal Register 48136). These species are endemic to vernal pools in the Central Valley, coast ranges, and a limited number of sites in the Transverse Range and Riverside County, California. The endangered Riverside fairy shrimp (*Streptocephalus woottoni*) was listed under the Act on August 3, 1993 (58 Federal Register 41391). This species inhabits Riverside, Orange and San Diego Counties, California, and northern Baja California, Mexico. These five species, hereafter referred to as vernal pool branchiopods, are fully protected under the Act. The San Diego fairy shrimp (*Branchinecta sandiegonensis*) is a proposed endangered species. Surveys for all these species should follow the methodologies described in these Interim Survey Guidelines (Guidelines). It is expected that the Guidelines will be revised in the future as additional information becomes available.

These Guidelines are issued as guidance to section 10(a)(1)(A) permittees. Because taking (killing, injuring, harming or harassing) endangered species is strictly prohibited under the Act, a section 10(a)(1)(A) recovery permit must be obtained prior to initiating any surveys or studies that might result in the take of endangered or threatened branchiopods. Failure to obtain this permit may result in violation(s) of section 9 of the Endangered Species Act. Additionally, violation(s) of a section 10(a)(1)(A) permit may result in its non-renewal, suspension or revocation.

For the purposes of these Guidelines, vernal pools and swales are defined as follows:

Vernal pools and swales are ephemeral wetlands that form in areas of California with Mediterranean climates that have shallow depressions underlain by a substrate of

hardpan, clay, or basalt near the surface that restricts the percolation of water. They may be characterized by a barrier to overland flow that causes water to collect and pond. Vernal pools/swales may occur singly, but more typically occur in vernal pool/swale complexes, due to the local hydrology, geology, and topography. Initially, the dry soil in vernal pools/swales becomes wet and starts to saturate during the fall and early winter rains. The second stage in a typical vernal pool cycle is characterized by peak rainfall and inundation of the vernal pools/swales. Vernal pools may remain inundated until spring or early summer, sometimes filling and emptying numerous times during the wet season. The vernal pools gradually dry down during the spring, quite often forming the unique "bathtub ring" of flowers from endemic vernal pool plants blooming profusely at the pool margins. This drying down stage is typified by the production of seeds in the endemic plants and the dispersal of animals from the vernal pools. These pools eventually dry down totally, with the onset of drought conditions. During this final stage, early season and shallow-rooted plants turn brown, and the soil dries and may crack. With average rainfall patterns, vernal pools are typically characterized by a predominantly annual plant community dominated by wetland species.

Note: At this time, vernal pool-associated activities not directed toward the listed species, such as botanical surveys and wetland delineations, are not considered to require a permit. However, persons conducting such activities should minimize any potential impact on the vernal pool branchiopods or plants by reducing the amount of walking through vernal pools to the lowest extent practical. Persons conducting projects that require permits (e.g., branchiopod or amphibian surveys) should also minimize walking through the pools.

#### I. Survey Approval

Unless otherwise authorized by the U.S. Fish and Wildlife Service (Service) in writing, these Guidelines shall be utilized for all surveys conducted for the listed vernal pool branchiopods. Any deviations from the methods prescribed by these Guidelines must be approved by the Service before surveys are conducted. The permittee shall provide the appropriate Service Field Office (see XI, Service Contact section) with all of the following information in writing for each project site at least 10 working days prior to the anticipated start date of survey work:

- a. The precise location of the project site clearly delineated on either an original or high quality copy of a U.S. Geological Survey topographic map (exact scale, 7.5 minute, 1"=2000 ft.). The map should contain the project name, type of project by category [the categories are: development, mitigation banking, or other (specify)], the estimated area (acreage) of the project site and an estimated number or area (acreage) of pool/swales on the site, quad name, and county name;
- b. Names of all vernal pool biologists and associated personnel with reference to their section 10(a)(1)(A) permit number; and
- c. A written request to commence wet season or dry season sampling for each project to

be surveyed for the listed vernal pool branchiopods.

## II. Sampling Survey Completion

a. Once initiated, surveys conducted pursuant to these Guidelines may be suspended prior to completion if:

1. the presence of one or more of the five listed branchiopods on the subject site is determined through identification at any point within the wet season survey cycle; or
2. it is agreed that one or more of the listed vernal pool branchiopods are present on the subject site.

b. Permission to dry season survey for the listed vernal pool branchiopods requires the completion of both the full wet season survey and the dry season survey, including the complete analysis of all dry soil samples (see V).

c. A complete survey consists of sampling for either:

1. two full wet season surveys done within a 5-year period; or
2. two consecutive seasons of one full wet season survey and one dry season survey (or one dry season survey and one full wet season survey).

d. Each vernal pool/swale in a vernal pool/swale complex shall be surveyed as per these Guidelines. However, in the case of a large vernal pool/swale complex, the Service may authorize a representative portion or portions of the vernal pool/swale complex to be surveyed as per these Guidelines.

## III. Notification of Presence

Should the permittee determine that any of the five listed vernal pool branchiopods are present at a site, the appropriate Service Field Office (see XI, Service Contact section) shall be notified within 10 working days by letter or telephone.

## IV. Wet Season Surveys

Wet season survey sampling shall not be conducted at any project site unless the permittee receives prior permission from the Service (see I (c)).

#### a. Survey Initiation, Frequency, and Termination

1. Surveyors should visit sites after initial storm events to determine when pools/swales have been inundated. A pool/swale is considered to be inundated when it holds greater than 3 cm of standing water 24 hours after a rain event.
2. Pools/swales shall be adequately sampled once every two weeks, beginning no later than two weeks after their initial inundation and continuing until they are no longer inundated, or until they have experienced 120 days of continuous inundation.
3. In cases where the pools/swales dry and then refill in the same wet season, sampling shall be reinitiated within eight days of refilling every time they meet the 3 cm of standing water criteria and shall continue until they have experienced 120 days of continuous inundation, or until they are no longer inundated.
4. If a vernal pool/swale has already experienced 120 days of continuous inundation, but then dries down and subsequently refills in the same wet season, surveys must be re-initiated in accordance with IV(a)(3) above, each time the vernal pool/swale refills and meets the 3 cm of standing water criteria.
5. Once initiated, surveys conducted pursuant to these Guidelines may be suspended prior to completion if the presence of one or more of the five listed branchiopods on the subject site is determined through identification at any point within the wet season survey cycle

#### b. Survey Sampling

At each wet season visit, representative portions of the pool/swale bottom, edges, and vertical water column shall be adequately sampled using a seine, dip net or aquarium net appropriate for the size of the pool or swale. Net mesh size shall not be larger than (1/8) inch. Seines shall be examined and emptied of material at least once every five linear meters.

#### c. Voucher Specimens

1. Voucher specimens shall be collected only once for each individual vernal pool/swale and shall be accessioned to either the California Academy of Sciences (CAS) or the Natural History Museum of Los Angeles County (LACM) (see VIII).
2. Voucher specimens of all listed vernal pool branchiopods captured shall be collected and all other specimens shall be returned in good condition to the vernal pool/swale where they were found as quickly as possible.
3. No more than 20 specimens of each species of listed vernal pool branchiopods from each pool/swale, or less than 10% of the subpopulation present in the pool/swale,

whichever is the lesser amount, shall be retained and preserved as voucher specimens.

4. Only sexually mature, adult branchiopods shall be used for purposes of voucher specimens for species identification. The Service will not accept species identifications made using immature specimens.
5. The sample of 20 voucher specimens shall include no less than three specimens of either sex.

## V. Dry Season Surveys

Dry season soil sampling shall not be conducted at any project site unless the permittee receives prior written permission from the Service (see I (c)).

### a. Soil Collection

Soil shall be collected when it is dry to avoid damaging or destroying cysts which are more fragile when wet. A hand trowel or similar instrument shall be used to collect approximately one liter volume sample per pool/swale of the top 1-3 cm of pool sediment. Whenever possible, soil samples shall be collected in chunks. The trowel shall be used to pry up intact chunks of sediment, rather than loosening the soil by raking and shoveling which can damage cysts.

In southern California there are a number of federally listed plant species (*Orcuttia californica*, *Pogogyne abramsii*, and *Pogogyne nudiscula*) that often co-occur with the fairy shrimp. Removal of soil could damage populations of these plants by inadvertently removing seed. Dry sampling should be minimized or avoided within those vernal pools/swales that are known to, or may, contain these species. The permittee shall contact the Carlsbad Field Office (see XI, Service Contact section) regarding the distribution of these listed plants species prior to conducting dry sampling in Los Angeles, Orange, Riverside and other southern California counties.

### b. Soil Sample Volume

Each soil sample from the 10 soil sample locations shall be labeled, stored, and analyzed individually.

1. A total of 10 soil samples of approximately 100 ml each shall be taken from each pool/swale, for a total soil sample volume of approximately one liter per pool/swale.
2. In the case of a very large playa, dry lake, or vernal pool, the Service may authorize the removal of more than one liter of soil.

3. If a pool has a diameter of less than three meters, the total soil sample taken shall not exceed ½ liter in volume per pool, and the 10 soil samples shall be approximately 50 ml each in volume.

c. Soil Sample Locations

A total of 10 soil samples shall be collected from the following locations within each pool/swale sampled:

1. Starting with one soil sample taken from the edge of the pool/swale, at least four soil samples shall be taken from equidistant points along the longest transect of the pool/swale.
2. Starting with one soil sample taken from the edge of the pool/swale, at least four soil samples shall be taken from equidistant points along the widest transect of the pool/swale.
3. If neither the longest or the widest transect encompasses the deepest part (or parts) of the pool/swale, then at least two soil samples shall be taken from the deepest part (or parts) of the pool/swale..

d. Soil Storage

1. The soil samples from each soil sample location shall be stored in separate bags, labeled with the specific location within the pool/swale from where each soil sample was taken. A sketch of the pool/swale showing the specific location of each soil sample shall be included in the 90-day report.
2. Soil samples containing any residual moisture initially shall be adequately ventilated and allowed to air dry thoroughly before storage of the sample. The bags containing the soil samples shall be kept out of direct sunlight in order to avoid excessively heating the sample.
3. All soil samples shall be retained and stored as directed in V(d)(1) and V(d)(2) above until the Service is able to provide direction in species-level identification of the cysts of all the aforementioned branchiopod species.

e. Soil Sieving

1. The soil samples shall not be ground, crushed, or otherwise manipulated in order to expedite the sieving process. A relatively short period of pre-soaking the soil sample may be helpful/necessary in order to facilitate the sieving process. Small aliquots (approximately 50 ml in volume) of soil shall be gently washed with water through a graded series of U.S. standard eight inch soil sieves ending in mesh sizes 300 micron (um), and 150 micron (um).

2. Sieves must be thoroughly rinsed and visually inspected for any cysts adhered to the sieves prior to the start of sieving. This process must be repeated for each individual soil sample location. Sieves shall also be rinsed and thoroughly inspected upon completion of sieving soil samples.

#### f. Soil Examination

1. Washed and sieved soil fractions from the 300 um and 150 um sieves shall be examined under a dissecting microscope for tadpole shrimp and fairy shrimp cysts. The process shall be repeated until all individual soil samples have been examined. All sieved material shall be processed and dried as quickly as possible, preferably within one hour from the initial wetting.

Note: Do not return soil to survey sampling site.

2. All fairy shrimp and tadpole shrimp cysts shall be removed from the soil, separated by cyst type into labeled vials, allowed to air-dry, and then stored dry.

#### g. Cyst Density

Cyst density information for each soil sample location shall be calculated by dividing the total number of cysts recovered by the total amount of soil from the individual aliquots from that soil sample location. Total cyst density information for each soil sample location shall be reported for each species in terms of: none; 1-25 cysts/100 ml soil; 26-50 cysts/100 ml soil; 51-100 cysts/100 ml soil; 101-199 cysts/100 ml soil; or more than 200 cysts/100 ml soil.

#### h. Cyst Identification

Each fairy shrimp and tadpole shrimp cyst type shall be identified to genus by a qualified biologist. The Service may require an independent review by a crustacean biologist(s) of any vernal pool branchiopod or cyst identification.

There are two options when a branchiopod cyst identification is made to genus:

1. the survey, pursuant to these Guidelines, may be suspended if it is agreed one or more of the listed species are present on the project site; or
2. one subsequent complete wet season sampling survey shall be conducted to complete survey requirements.

#### VI. Cyst Voucher Specimens

A representative sample of each cyst type from each pool/swale shall be accessioned to either CAS or LACM (see VIII).

## VII. 90-Day Reports

### a. U.S. Fish & Wildlife Service

The permittee shall provide the appropriate Service Fish and Wildlife Office (listed in the Service Contact section) with all of the following information in writing, using the appropriate Vernal Pool Data Sheet where applicable as the reporting form, no more than 90 calendar days after completing the last field visit of the season at each project site:

1. The location of the project site clearly delineated on an original or high quality copy of a U.S. Geological Survey topographic map (exact scale, 7.5 minute, 1"=2000 ft.). The location of the listed vernal pool branchiopods is to be included on the 7.5 minute maps in as precise a manner as possible (e.g., lat/long or location within a section).
2. Five color photographic 35mm slides and/or 3" x 5" photographs of each project site taken during sampling in the wet season; this is to include two slides and/or photographs taken from standing position that portray the general landscape of the site [i.e., two photos from an opposing axis of the site (e.g., north and south compass headings)]; and three slides and/or photographs of representative vernal pools, swales, and other areas within the site sampled for the five listed vernal pool branchiopod species. The following information shall be legibly written on each slide/photograph with permanent ink: precise location of the project site, direction from which photograph was taken, date of photograph, initials of photographer, and initials of the scientific names of any of the five listed vernal pool branchiopod species that were found at the depicted site.  
Note: Slides and/or photographs only need to be submitted once per project site.
3. The estimated number of individuals of any of the listed vernal pool branchiopods observed in each pool/swale shall be reported in terms of an order of magnitude (e.g., 10's, 100's, 1000's).  
*(Refer to the Vernal Pool Data Sheet)*
4. The number of individuals of any of the listed vernal pool branchiopods or cysts preserved from each pool/swale and the name of the institution in which they are accessioned.  
*(Refer to the Vernal Pool Data Sheet)*
5. A qualitative description of the vernal pool/swale community. A general list of amphibian species and non-listed vernal pool crustacean species (by common and/or scientific name) encountered at the project site is desirable. For purposes of this permit a full survey for these species is not required. However, if more detailed information is collected, it shall be included in the Vernal Pool Data Sheet.  
*(Refer to the Vernal Pool Data Sheet)*

6. Data collected during each field visit, including: date, air temperature, water temperature, weather conditions (e.g., sunny, overcast), maximum depth of each pool/swale, and size (area in square meters) of each pool/swale.  
*(Refer to the Vernal Pool Data Sheet).*
7. (Optional) water chemistry data collected during each field visit, including: alkalinity (total: ppm or mg/l), conductivity (uMHO), dissolved oxygen (ppm or mg/l), dissolved NH<sub>4</sub> (ppm or mg/l), pH, salinity (ppt), total dissolved solids (TDS, ppm), and turbidity.  
*(Refer to the Vernal Pool Data Sheet)*

b. California Department of Fish & Game

1. Permittees should consult with the California Department of Fish and Game (916/653-4875) to determine their responsibilities under the California Endangered Species Act and the California Fish and Game Code.
2. The permittee shall supply the California Department of Fish and Game (Natural Diversity Data Base, California Department of Fish and Game, 1807 13<sup>th</sup> Street, Suite 202, Sacramento, California 95814; telephone 916/322-2494) with completed California Native Species Field Survey Forms, no more than 90 calendar days after completing the last field visit of the season at each project site.

VIII. Accessioning Voucher Specimens

- a. All vernal pool branchiopod voucher specimens (including individuals collected and cysts) shall be accessioned into either the California Academy of Sciences (CAS) or the Natural History Museum of Los Angeles County (LACM). All specimens shall be preserved according to the accession standards of the repository which will accession and maintain the specimens. The October 1995 CAS and September 1995 LACM standards are attached to these Interim Survey Guidelines.
- b. All vernal pool branchiopod voucher specimens (including individuals collected and cysts), along with a copy of the Vernal Pool Data Sheet containing all of the items listed in VII (a), shall be permanently deposited in the CAS or LACM within 90 calendar days of the completion of the field survey and the Service shall be supplied with the CAS or LACM catalog numbers given to the specimens.
- c. The permittee shall supply the CAS or LACM with a photocopy of their section 10(a)(1)(A) permit to validate that the specimens supplied to them were taken pursuant to a permit. The Service will likely consider refusal by the CAS or LACM to accession any listed branchiopod specimens to be a violation by the permittee of their section 10(a)(1)(A) permit (e.g., if due to improper preservation/storage).

California Academy of Sciences (CAS)  
Department of Invertebrate Zoology and Geology, Golden Gate Park,  
San Francisco, California 94118; telephone (415) 750-7082

Natural History Museum of Los Angeles County (LACM)  
Crustacea Section, Invertebrate Zoology, 900 Exposition Boulevard,  
Los Angeles, California 90007; telephone (213) 744-3450

IX. Additional information, limitations, and caveats with respect to these Guidelines are as follows:

a. From time to time, specific circumstances may justify or necessitate revision of these Guidelines, on a case-by-case basis. At the discretion of the Service, such a variance may be allowable under these Guidelines if:

1. the permittee explains to the Service in writing why the variance to the Guidelines is needed and justified; and
2. the Service concurs, in writing, with the variance requested by the permittee.

b. The Service reserves the right to reject vernal pool branchiopod surveys conducted under these protocols as inadequate if:

1. survey methods used are inconsistent with these Guidelines, unless prior written permission (see I, Survey Approval) has been obtained; or
2. other information indicates that the survey is inadequate as determined by the Service.

#### X. Permit Infractions

The Service may consider any of these actions to be a violation by the permittee of their section 10(a)(1)(A) permit:

- a. falsification of any reporting or information;
- b. failure to follow the stated Guidelines sampling methodologies;
- c. failure to obtain prior permission to commence wet season surveys or failure to obtain written permission to commence dry season surveys (see section I (c));
- d. failure to notify the Service within 10 days of a determination of presence of one or more of the listed vernal pool branchiopods on a survey site;
- e. failure to accession voucher specimens or improperly accessioned voucher

specimens;

f. failure to file completed 90-day reports with the Service within 90 calendar days after completing the last field visit of the season at each project site; or

g. failure to file completed Natural Diversity Data Base forms with the California Department of Fish and Game within 90 calendar days after completing the last field visit of the season at each project site.

Violation(s) of a section 10(a)(1)(A) permit may result in its non-renewal, suspension or revocation.

#### XI. Service Contact

For the Central Valley hydrographic basin and the coast ranges north of the Santa Cruz County line, the Sacramento Fish and Wildlife Office (2800 Cottage Way, W-2605, Sacramento, California 95825; telephone 916/414-6600) should be contacted regarding vernal pool branchiopod issues.

For areas from Santa Cruz County south to Ventura County, contact the Ventura Field Office (2493 Portola Road - Suite B, Ventura, California 93003; telephone 805/644-1766).

For areas from Los Angeles County south to the U.S.- Mexico border, contact the Carlsbad Field Office (2730 Loker Avenue West, Carlsbad, California 92008; telephone 619/431-9440).



**U.S. Fish and Wildlife Service Vernal Pool Data Sheet  
Wet Season Survey**

Note: Please fill out the required information completely for each site visit.

Species Observations: state none or estimate # of individuals present in terms of an order of magnitude (e.g., 10's, 100's, 1000's)

Anostracans:  
(note reproductive status)

Notostracans:  
(note reproductive status)

Species Observations (Optional) :

Cladocerans:	yes	no	Insects: (adult or larvae)		
Conchostracans:	yes	no	Anisoptera:	yes	no
Copepods:	yes	no	Zygoptera:	yes	no
Ostracods	yes	no	Hydrophilidae:	yes	no
Fish	yes	no	Dytiscidae:	yes	no
Frogs	yes	no	Corixidae:	yes	no
Salamanders	yes	no	Notonectidae:	yes	no
Waterfowl	yes	no	Belostomatidae:	yes	no
Other (specify) _____			Other (specify) _____		

Voucher Specimens

Specimens shall be preserved according to the standards of the institution in which they will be accessioned.

<u>Species</u>	<u># Individuals</u>	<u>Accession/Catalog #</u>	<u>Pool #</u>
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# **Collection, Preservation, Handling, and Accessioning Information for Small Crustaceans**

Crustacea Section, Invertebrate Zoology  
The Natural History Museum of Los Angeles County  
900 Exposition Boulevard  
Los Angeles, California 90007

## **Collection Data**

To the extent possible, the following data should be included. The Museum reserves the right to refuse acceptance of any specimens without a minimum of usable, legible collection data. Archival quality materials (including glass vials, permanent ink or pencil and permanent label paper, and glass outer jars with screw-top polypropylene lids) should always be used (see below). All collections should include the following information:

- Taxon name: (Lowest available or known, down to species where possible)
- Date: (day, month, year)
- Time of Day: (if known)
- Detailed Location:
- Latitude and Longitude:
- Specific habitat information:
- Name of collector:
- Collecting method(s) / device(s):
- Preservative used:
- Notes: (to include any observations on behavior, co-occurring species, etc.)

## **Preservation**

Ideally, even small crustaceans should be initially fixed in 5 to 10% formalin (37% formaldehyde in solution, as commercially purchased, mixed with 90-95% water). As an alternative, 100% ethyl alcohol, although not a fixative and so not as good for long term tissue preservation, can sometimes be used (not recommended for animals longer than 20 mm total length). With either method, specimens should be transferred to 70% ethyl alcohol (ethanol) after a minimum of 8 hours of fixation. The 70% ethanol to tissue ratio should be approximately 3 to 1 for long term storage.

## **Storage**

Archival quality materials (including glass vials, permanent ink or pencil and permanent label paper, and glass outer jars with screw-top polypropylene lids) should always be used. Specimens should be placed in small glass vials completely filled with 70% ethanol and plugged with cotton (not foam). Vials are then inverted and stored in a slightly larger outer storage jar of glass or plastic, also filled with 70% ethanol and fitted with a polypropylene-closure lid. Labels are ideally situated in the outer jar containing the vial rather than in the shell vial, never on the outside of the jar or affixed to the lid.

**Shipping**

Specimens should be shipped in plastic, leak-proof bottles, jars, or vials, and must be adequately cushioned by bubble-wrap, plastic peanuts, etc. to ensure their safe arrival. It is preferred that specimens that are designated types or vouchers be sent by registered or certified mail, although this is at the discretion of the sender. Use the complete address given at the top of this document.

**Cost of Specimen Accessioning**

Because of the rising costs of accessioning and maintaining valuable collections, the Natural History Museum reserves the right to charge on a per-sample basis for accessioning collections. This fee varies with the size of the collection, duration of the project, and availability of Museum staff at the time of deposition. The fee may be waived at the discretion of the Curator or Collections Manager of Crustacea and may fluctuate depending upon our evaluation of lots received.

**Accessioning Information**

The Natural History Museum of Los Angeles County will accept for permanent care and curation selected collections of Crustacea, including those from vernal pools and other ephemeral freshwater habitats and representatives of threatened or endangered taxa. The Museum is willing to act as the repository for collections acquired during USFWS or other surveys.

To be accepted for accessioning, the collections must be in reasonably good shape, meaning that the animals themselves must not be overly deteriorated and that all previously stated collecting, preserving, and labeling protocols have been followed. Furthermore, all collections must be accompanied by a detailed list of the specimens being sent.

The Museum reserves the right to charge an accessioning fee to cover the costs of accessioning any and all deposited specimens. This fee may be waived at the discretion of the curator in charge of the Museum Section that will be overseeing the accessioning and curation of the collection.

The Museum reserves the right to decide whether an incoming collection should be stored topically vs. separated and stored according to taxonomic divisions (i.e. storing all members of one family together rather than keeping all collections from one site together).

The Museum further reserves the right to decide which specimens will be kept and maintained for long term storage and which may be passed on to other institutions in exchange or as long term loans for research purposes.

**For further information contact:** (Or write to the address given above)

Dr. Gary Pettit, Collections Manager, Crustacea	213-744-3450 fax 746-2999
Dr. Joel W. Martin, Curator of Crustacea	213-744-3440 fax as above

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

October 1995

### **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 (CASIZG). However, only those specimens which have been properly fixed, preserved and documented will be accepted for archival. 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 CASIZG.

Any questions regarding these standards and protocols may be directed to Dr. Robert Van Syoc, Senior Collection Manager of invertebrates at CASIZG (415-750-7082). Visits to the collection to deposit potential voucher specimens must be at least 7 days in advance. Specimens may be shipped to CASIZG, but shipments with damaged specimens or broken containers will not be accepted. Each shipment must be accompanied by a packing list of specimens sent. CASIZG catalog numbers will be assigned by CAS staff and notification sent to you by U. S. mail. This will be done in an expeditious manner, but staffing limitations may cause delays. Therefore, allow several days for notification of CASIZG catalog numbers.

#### Materials required:

- 100% or 95% non-denatured ethanol
- 75% non-denatured ethanol (diluted from 100% or 95% with de-ionized or distilled water)
- 2 dram, 4 dram, 6 dram glass shell vials
- 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 (8 oz. bottles) or 85 mm diameter (32 oz. bottles), with foam or plastic liners
- Nalgene polypaper
- Dot-matrix printer and alcohol-proof ink ribbons, or technical pen with alcohol-proof ink, or #2 pencil

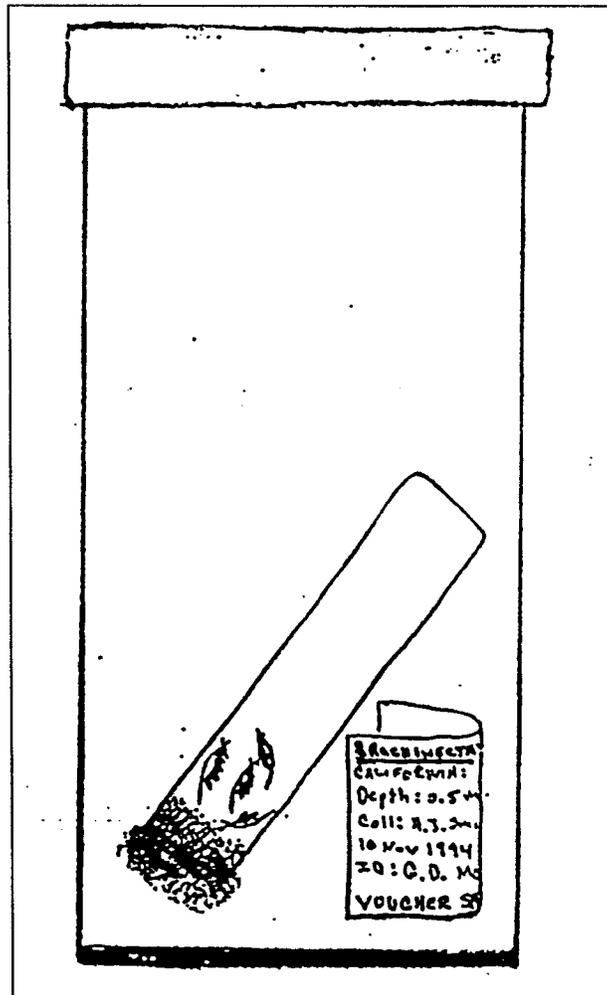
## Standards and Protocols:

1. Vernal pool crustaceans must be fixed in 100-95% non-denatured ethanol and preserved for archival in 75% non-denatured ethanol. Enough 100-95% ethanol should be used in the initial fixation to insure proper fixing of tissues. A ratio of at least 10 parts 100-95% ethanol to 1 part tissue is required for initial fixation. A ratio of at least 3 parts 75% ethanol to 1 part tissue is required for preservation.
2. All specimens must be sorted by collecting event (each locality/date/time of collection). They must be identified to species level, each species from each collecting event placed into a vial or vials in it's own 8 or 32 oz. bottle (use the smaller size if possible).
3. Specimens are placed into 2 dram, 4 dram, or 6 dram glass shell vials filled with 75% 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 75% ethanol (Fig. 1). 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 which 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 75% 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.
4. The 8 or 32 oz. glass bottle is capped with the foam or plastic lined, screw-top polypropylene closure.
5. 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 printed using a dot-matrix printer with alcohol-proof ink ribbon. Alternatively, it 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.

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
- 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"

Fig. 1: 8 oz. bottle containing inverted glass vial plugged with cotton.  
Note label inside jar, but not inside vial.



**List of potential (not specifically required) vendors of some required materials.**

Glass vials: S-930	Acme Vial and Glass 1601 Commerce Way Paso Robles, CA 93446 (805) 239-2666
Glass bottles: and polypropylene lids	California Glass 155 98th Ave. Oakland, CA 94603 (510) 635-7700
Polypropylene lids:	Berlin Packaging 7900 Edgewater Dr. Oakland, CA 94621 (510) 562-7201
Cotton: non-sterile	California Medical Supplies 3315 Broadway Oakland, CA (510) 885-5105
Nalgene Polypaper:	VWR Scientific (415) 468-7150 (800) 932-5000
Alcohol-proof ("non-bleeding") printer ribbons:	Automated Office Products Inc. 9700-A Martin Luther King Jr. Hwy. Lanham, MD 20708
Non-denatured ethanol 190 or 200 proof (95% or 100%)	Gold Shield Chemical 3111 Depot Rd. Hayward, CA 94545 510-782-2040

Materials may be obtained from other sources, but should conform to the specific standards listed above. CASIZG will not act as a supplier of materials.

Field survey forms and information regarding the California Natural Diversity Database can be accessed on the internet at <http://www.dfg.ca.gov/whdab/cnddb.htm>.

State of California • The Resources Agency  
Department of Fish and Game • Wildlife & Habitat Data Analysis Branch  
The California Natural Diversity Database  
Commonly Asked Questions

What is the California Natural Diversity Database (CNDDDB)?

The CNDDDB is a program within the Department of Fish and Game's Habitat Conservation Division and within the Wildlife & Habitat Data Analysis Branch. The CNDDDB's mission is to track the location and condition of California's many species of rare and sensitive plants, animals, and natural communities (e.g., marshes, riparian systems, desert scrub, etc.). These species and natural communities are collectively referred to as "elements."

The CNDDDB includes in its inventory all federally and state listed plants and animals, all species that are candidates for listing, all species of special concern, and those species that are considered "sensitive" by government agencies and the conservation community. This is a computerized inventory and information is available for a fee in hard copy and digital forms. The CNDDDB is a dynamic system with information continually being added and upgraded. The CNDDDB contains over 36,000 locational records for over 3,000 elements.

How is CNDDDB information set up or organized?

CNDDDB data are organized geographically and taxonomically. Information is retrieved by United States Geological Survey (USGS) map sheet (e.g., typically 1:24,000, 1:100,000, or 1:250,000 scale), or by taxa. Most CNDDDB clients request information for USGS 7.5U minute quads. The approximately 49 square miles covered by a single USGS 7.5U minute quad is the smallest area for which we will perform a data retrieval. Due to the nature of our inventory, it is important that our clients query surrounding quads as well as the quads on which their project site or area of interest is located.

What types of information can I obtain?

**OVERLAYS & TEXT** We can produce computer generated overlays for the 1:24,000-, 1:100,000-, or 1:250,000-scale base maps. Text reports are included only with the USGS 7.5' scale overlays. An Element Table on the USGS 7.5' (1:24,000) overlay provides the following information for each element occurrence: a map feature number, occurrence number, map index number, element code, accuracy class, element count, the common and scientific names, numbers of components, and legend. Most of our clients request overlays for USGS 1:24,000 and 1:100,000 scale maps (map index numbers only with legend for 1:100,000 scale). Overlays cost \$30 (standard vellum media) per quad which include text reports, except for RAREFIND subscribers (see below).

RAREFIND 2 - We can also make our data available via a microcomputer database application called RAREFIND 2. You can obtain our entire statewide data set. RAREFIND 2 is available on a yearly subscription basis. The cost for the statewide data set is \$1,250 for government agencies and nonprofit conservation organizations or \$2,500 for commercial clients. A subscription includes an initial set of data with the RAREFIND 2 application followed by an updated data set 6 months later. The GIS digital layer is also available to subscription customers. Renewing customers are given a 10% discount.

RAREFIND 2 subscribers are also afforded a special overlay price of \$20 per quad. RAREFIND 2 is a compiled, stand alone application that requires a 386 or higher series PC, with 560 K free DOS memory and 100 MB free hard disk space. RareFind 2 will run on M S-DOS version 5.0 or higher, Windows 3.1, Windows 95 or NT. No additional software is required.

How do I order information from the CNDDDB?

It is easy to request information from the CNDDDB. Call one of our Information Services staff at (916) 324-3812 to place your order by phone or e-mail us at [kbates@dfg.ca.gov](mailto:kbates@dfg.ca.gov). It is most helpful to have the name or names of the 7.5' quad maps for which you want information contact us. We will give you a cost estimate before we proceed with your request.

How long does it take to get information from the CNDDDB?

The usual turn around time for data requests is one to two weeks. We ask you to remember that this is a computerized system and it does go down from time to time. Such unforeseen event can interfere with our normal response time.

How do I pay?

You are invoiced directly from our accounting department after the products have been sent to you. You do not need to pay up front; but invoices are due and payable upon presentation—no terms are available. Delinquent accounts will be denied additional services until the balance has been paid.

NOTE: There is a 50% cancellation charge if you cancel your order after we have already processed your request and generated our products. There is a no-return policy on products already delivered.

Why is there a charge for this information?

Our enabling legislation requires that we "insure cost-sharing by all who use the CNDDDB, and develop a fee structure to recover actual costs" for its use. The Department of Finance has determined that this will include not only direct costs for generating and distributing our data, but will also include some program overhead. We recover about \$225,000 per year in fees, which amount to about 25% of our program costs.

The Wildlife & Habitat Data Analysis' Mission and Vision:

“ ..to provide high quality scientific information, tools, and expertise needed by Department employees, other public agencies, private organizations, and the public for making informed conservation decisions regarding our biological resources. ...to serve as the State's clearinghouse for biological data and center for conducting conservation analyses at statewide, regional, and local scales and actively acquire, integrate, improve, and distribute biological resource data and associated spatial data in support of conservation policy and planning needs.”

NDDDB Questions & Answers.wpd//Rev:1/26/00  
(from <http://www.dfg.ca.gov/whdab/cnddb.htm>.)