



United States Department of the Interior



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Survey Guidelines for the Listed Large Branchiopods

Introduction

The endangered Conservancy fairy shrimp (*Branchinecta conservatio*), longhorn fairy shrimp (*Branchinecta longiantenna*), vernal pool tadpole shrimp (*Lepidurus packardii*), 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 Agate Desert Region of Oregon and in California's Central Valley, Coast Ranges, and a limited number of sites in and south of the Transverse Ranges. 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, Los Angeles, and San Diego counties, California, and northwestern Baja California, Mexico. The San Diego fairy shrimp (*Branchinecta sandiegonensis*) was listed under the Act on February 3, 1997 (62 Federal Register 4925). This species inhabits San Diego and Orange counties in California, and northwestern Baja California, Mexico. These six species, hereafter referred to as the listed large branchiopods, are fully protected under the Act. Surveys for all of these species should follow the methods described in these Survey Guidelines for the Listed Large Branchiopods (Guidelines). It is expected that the Guidelines will be revised in the future as additional information becomes available.

The U.S. Fish and Wildlife Service (Service) has published recovery plans for vernal pool species in Southern California (Service 1998a) and in Northern California and Southern Oregon (Service 2005, 2012). These recovery plans list actions that will assist in the recovery of the vernal pool species, which include separate actions to develop survey guidelines and to conduct directed species status surveys or monitoring surveys. These Guidelines were created to provide a method to best detect the presence of the listed large branchiopods in a vernal pool (or similar feature as described in the next section). The Service believes that strict adherence to the Guidelines during status surveys will result in an increased probability of detection of the listed large branchiopods if they are present at a particular vernal pool, or similar natural or artificial habitats that support vernal pool characteristics and organisms. Please note that the Guidelines when considered alone are not intended to provide proof of the absence of any of the large branchiopod species at any location. Further, the Guidelines are intended to provide data for use in the recovery of the listed large branchiopods and are not intended as guidelines for other processes.

The Guidelines are issued as guidance to section 10(a)(1)(A) permittees. Because taking (killing, injuring, harming or harassing) endangered or threatened 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 large branchiopods. Failure to obtain this permit may

result in violation(s) of section 9 of the Act. Additionally, violation(s) of a section 10(a)(1)(A) permit may result in its non-renewal, suspension or revocation.

Background

Vernal pools and swales (low lying trough-like depressions) are ephemeral wetlands that form in areas with Mediterranean climates that have shallow depressions underlain by a substrate of hardpan, clay, basalt, or similar restrictive layer near the surface that restricts water percolation [California Department of Fish and Wildlife (CDFW) 1998]. They may be characterized by a barrier to overland flow that causes water to collect and pond. Although there are many types of seasonally inundated wetlands, vernal pools are so named for their endemic floras (*e.g.*, *Downingia* sp., *Eryngium* sp., *Plagiobothrys* sp., *Psilocarphus* ssp.) which are adapted to periodic or continuous inundation during the winter and spring and desiccation during the summer and fall (Stone 1990). Vernal pools are typically classified by the local geology and type of substrates found within and around the vernal pools (Zedler 1987, CDFW 1998). Vernal pools/swales may occur singly, but more typically occur in vernal pool/swale complexes, due to local hydrology, geology, and topography. In an intact vernal pool, the first few rains of the season wet the surface, sometimes ponding water for a few hours to days before percolating into the subsoil. Once the interstitial areas within the subsoil, above the impervious layer (*i.e.*, hardpan, claypan, bedrock etc.), are filled with water; the basin will start to inundate (Zedler 1987, Hanes *et al.* 1990). Precipitation events and other environmental stimuli help break the dormancy of many of the large branchiopod eggs (also known as embryonic eggs, resting eggs, or cysts) (Erikson and Belk 1999). Large branchiopod eggs are generally concentrated in the upper one centimeter of soil, usually within the organic layer deposited by algae and decaying vascular plant material. Once ponding occurs, the eggs (except for *Streptocephalus wootonii*) normally hatch within a few days. Hatching may not always occur in a single wet season (Eriksen and Belk 1999). 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, like the California tiger salamander (*Ambystoma californiense*) from the vernal pools (Zedler 1987, Stone 1990). These pools eventually dry down completely, with the onset of drought conditions. During this final stage, early season and shallow-rooted plants turn brown, and the substrate dries and may crack. With average rainfall patterns, vernal pools are typically characterized by a predominantly annual plant community dominated by seasonally astatic wetland species (Stone 1990). Vernal pools are found in California, Southern Oregon, and northern Baja California, Mexico. Other types of wet lands can also provide habitat for listed large branchiopod species if they exhibit similar hydrologic conditions. These include naturally occurring seasonal wetlands, which are typically a component of vernal pool complexes. Vernal swales are vegetated drainage ways with undefined beds and banks. Although similar to vernal pools in vegetation composition and soil characteristics, vernal swales differ from vernal pools in that they generally convey water rather than pond it. Additionally, other artificial topographic features can mimic the ephemeral aquatic habitat of the natural vernal pools and may serve as habitat for a variety of aquatic invertebrates including fairy shrimp (Sutter 1998). These vernal pool mimics, which occur where vernal pools and vernal pool species are normally found, may include such anthropogenic features as tire ruts, agricultural and construction ditches, borrow pits, cattle troughs, and cement culverts.

Some terms used in the Guidelines are defined as follows:

Branchiopod - any crustacean of the class (or subclass) Branchiopoda, having flattened, foot-like or paddle-like appendages that are used for locomotion, filter feeding, and respiration. Includes fairy shrimp, tadpole shrimp, and clam shrimp.

Feature – a single instance of listed large branchiopod habitat, such as a vernal pool, swale, or other inundated depression.

Habitat - the place or type of site where a plant or animal naturally or normally lives and grows.

Permittee - a person possessing a valid 10 (a)(1)(A) recovery permit for listed large branchiopods.

Potential listed large branchiopod habitat - any seasonally inundated depression that on average ponds water at a sufficient depth and duration for a listed large branchiopod to complete its lifecycle. Generally these habitats occur within the California Floristic Province at elevations below 1,707 meters in the Coast range (CNDDDB #178) and below 914 meters elevation for the rest of California (CNDDDB #244) and Oregon. Habitats that swiftly flow water (*e.g.*, creeks, streams, and ephemeral drainages) or habitats that are semi-to-permanently inundated and support perennial population of predators (*e.g.* bullfrogs, fish, and crayfish), generally do not provide suitable habitat for listed large branchiopods.

Vernal Pool Complex (CDFW 1998) – Vernal pools may occur singly or in complexes. A complex is a set of naturally occurring pools in close proximity. Intervening non-pool terrain within a vernal pool complex is commonly referred to as upland and often includes wetland or partially wetland swales that can interconnect pools within the complex. Pools can usually be distinguished from uplands by a distinct change in vegetation and soils characteristics. Individual features within a complex have the potential to be hydrologically interconnected during some wet seasons.

Wet season - that portion of year when precipitation generally occurs, usually in California and Southern Oregon during the period from October to June.

Survey Methods

Note: At this time, activities associated with potential branchiopod habitat that are not directed toward listed large branchiopod species, such as botanical surveys and wetland delineations, do not require a permit. However, persons conducting such activities should minimize any potential impact on the listed large branchiopods or listed plants by reducing the amount of walking through the potential habitat to the lowest extent practical. Persons conducting projects that require permits (*e.g.*, branchiopod or amphibian surveys) should also minimize walking through potential large branchiopod habitat since walking through wet pool sediments and substrate can damage branchiopod eggs, vernal pool plants and their seeds.

I. SURVEY APPROVAL

These Guidelines shall be utilized for all surveys conducted for listed large branchiopods unless otherwise authorized in writing by the Service. 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 15 working days prior to the anticipated start date of survey work:

- a. The location of the survey area clearly delineated on a high-quality 7.5 minute U.S. Geological Survey (USGS) topographic map at exact scale (*i.e.*, 1:24,000 or 1"=2000 ft) and, if possible, equivalent GIS product. Name of USGS map shall be identified. If the USGS map name is unavailable, graticules or tics shall be used to depict the map

location, with the PROJECTION and DATUM of the coordinate system identified. 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 of features or total area (acreage) of potential branchiopod habitat on the site, quadrangle name, and county name. This map will normally only be required for the initial survey request; however, the Service field office may request updated copies for additional follow-on surveys;

- b. Names of all permitted biologists who will be conducting the surveys in independent status with reference to their section 10(a)(1)(A) permit number; and
- c. A written request to commence wet season and dry season sampling for each project to be surveyed for listed large branchiopods. Authorization must be obtained from the appropriate Service field office prior to conducting surveys. Authorization for both the wet and dry season survey for a single project may be given in a single authorization letter from the appropriate Service field office.

Note: Some preserves (including conservation banks and mitigation banks) established to protect listed large branchiopod habitat have Service-approved monitoring plans that require surveys for listed large branchiopods as part of the monitoring efforts. The approval of these monitoring plans by the Service is sufficient to conduct the surveys per the methods in the Service-approved plan, and no further authorization from the Service (*e.g.*, on an annual basis) is needed to proceed with the required recurring surveys. However, any variation to the monitoring protocol outlined in the Service-approved plan will then require approval by the appropriate Service field office.

II. SAMPLING SURVEY COMPLETION

- a. Once initiated, surveys conducted pursuant to these Guidelines should be continued until the surveys are completed in order to provide the greatest probability of the detection of all listed large branchiopod species at the feature. However, with written concurrence from the Service the survey may be suspended prior to completion if the presence of one or more of the six listed large branchiopods is determined through identification at any point within the wet season survey cycle, or if new information or changing conditions demonstrate that it is not possible for the specific feature to support one or more of the listed large branchiopods.
- b. A complete survey consists of one wet season survey and one dry season survey conducted and completed in accordance with these guidelines and conducted within a 3-year period. The order of the surveys is not important.
- c. Results of wet season or dry season surveys conducted for a single feature do not extrapolate to other features that may separately co-occur, such as in a vernal pool complex.

III. NOTIFICATION OF PRESENCE

Should the Permittee determine that any of the six listed large branchiopods are present at a previously undocumented site; the appropriate Service Field Office (see XI, Service Contact section) shall be notified within 10 working days by electronic mail, letter, or telephone.

IV. WET SEASON SURVEYS

Wet season survey sampling shall not be conducted at any feature unless the Permittee receives prior authorization from the Service (see I (c)).

a. Survey Initiation

Surveyors should visit sites after initial storm events to determine when known or potential listed large branchiopod habitat has become inundated. Appropriate habitat is considered to be inundated when it holds greater than 3 cm of standing water 24 hours after a rain event.

b. Frequency and Termination

Because of differing climatic conditions occurring throughout the range of the listed large branchiopods sampling frequency and termination differ for the following three Survey Zones derived from geographic subdivisions of the California Floristic Province (Baldwin et al. 2012):

1. Survey Zone A (Southern Oregon, Sacramento Valley, San Francisco Bay Area, North Coast Ranges, Northern Sierra Valley Foothills, Cascade Range foothills, and South Coast Ranges; See county list in Section XII and Figure 2).
 - i. All potential habitat must be adequately sampled at 14-day intervals after initial inundation of habitat.
 - ii. Sampling will continue within each potential habitat until it dries or a minimum of 90 consecutive days of inundation has occurred.
 - iii. Sampling will be reinitiated within 14 days of an individual habitat drying and inundating during the same wet season.
2. Survey Zone B (San Joaquin Valley, Central and Southern Sierra Nevada foothills and Tehachapi Mountains; See county list in Section XII and Figure 2).
 - i. All potential habitat must be adequately sampled at 10-day intervals after initial inundation of habitat
 - ii. Sampling will continue within each potential habitat until it dries or a minimum of 90 consecutive days of inundation has occurred
 - iii. Sampling will be reinitiated within 10 days of an individual habitat drying and inundating during the same wet season
3. Survey Zone C (Southwestern California including South Coast, Channel Islands (U.S. only), Transverse and Peninsular Ranges; See county list in Section XII and Figure 2).
 - i. All potential habitat must be adequately sampled at 7-day intervals after initial inundation of habitat
 - ii. Sampling will continue within each potential habitat until it dries or a minimum of 120 consecutive days of inundation has occurred (based on the life cycle of *Streptocephalus woottoni*)(Hathaway and Simovich 1996)
 - iii. Sampling will be reinitiated within 7 days of an individual habitat drying and inundating during the same wet season

4. If a feature has already experienced the minimum allotted days of continuous inundation (90 or 120 days), but then dries down and subsequently refills in the same wet season, surveys must be re-initiated in accordance with IV(b)(1),(2), or (3) above, each time the feature refills and meets the 3 cm of standing water criterion.
5. A single wet season survey may be considered unreliable as a method to detect listed large branchiopods at any location if moderate to extreme drought conditions persist through the wet season as determined by the National Oceanic and Atmospheric Administration (NOAA) records of the Palmer drought indices (NOAA 2014). A single wet season survey may also be considered unreliable if no branchiopods are found and a subsequent dry season survey detects branchiopod resting eggs.

If a single wet season is determined to be unreliable by the Service then the next wet season with sufficient rainfall may be used to complete survey requirements. Precipitation timing during the wet season and water temperatures within a feature may also be considered by the Service in a determination of the reliability of a wet season survey.

6. Once initiated, surveys conducted pursuant to these Guidelines should be continued until completed in order to ensure the maximum probability for the detection of all listed large branchiopod species at the site. However, in some cases, as determined by the Service, the Guidelines may be suspended prior to completion if the presence of one or more of the six listed large branchiopods on the subject site is determined through identification at any point within the wet season survey cycle.
7. In order to increase the probability of detection of all possible co-occurring species (*e.g.*, not all species may hatch within a single year), the Service may request one subsequent complete wet season sampling survey be conducted to complete survey requirements. This would likely be required if the dry season survey results indicated branchiopods were present where a previous wet season survey found no branchiopods.

c. Wet Season Survey Sampling Method

At each wet season visit, representative portions of the bottom, edges, and vertical water column of the feature shall be adequately sampled using a seine, dip net or aquarium net appropriate for the size of the feature. The standard wet season vernal pool data sheet (Appendix 1) will be used to record the appropriate data from each feature. Following are some sampling tips to improve the detection of large branchiopods:

1. View the habitat for large branchiopods prior to entering it. It is generally much easier to capture large branchiopods if you can see them first.
2. Don't plow the pool with the net. Gently bounce the dip net along the pool bottom in front of you as you walk forward. Alternatively, you can move the dip net in a mild zigzag pattern in front of yourself while walking gently through the pool. If no branchiopods are captured, swirl the net in an approximate 3 foot diameter circle

creating a whirlpool. This eddy will pull benthic dwelling organisms, such as tadpole shrimp and California tiger salamander (*Ambystoma californiense*), from the bottom sediments up in to the water column. Then move the net in the opposite direction of the eddy to capture specimens.

3. Frequently view the contents of the net. This is especially important when filamentous algae and other plants occur in high concentrations. Seines shall be examined and emptied of material at least once every 3 to 5 linear meters. Care should be taken such that nets are not clogged with debris, thus pushing the water and possibly large branchiopods ahead of the net rather than capturing them.
4. Use appropriate net mesh sizes and apertures for the habitat sampled. A plankton net with a plastic reservoir works well for the earliest rounds of sampling, when the large branchiopods are small and hard to see. Then switch to a 1/16 inch (.16 cm) diameter mesh net with a frame width of at least 4 to 5 inches (approx. 10-13 cm), but preferably 7 to 12 inches (approx. 18-30 cm). Seines may be appropriate to use when sampling larger playa type habitats. Net mesh size for dip nets and seine nets shall not be larger than 1/16 inch (.16 cm).
5. Adjust your sampling for specific weather conditions. Since large branchiopods are poikilotherms (cold-blooded), move the net a little slower when the water is cold (<5° C) and a little faster when the water is warm (20° C). On days when warm water conditions prevail, make sure to sample the deep holes and hoof prints. Many large branchiopod species, such as *L. occidentalis*, tend to avoid the surface and seek refuge in the slightly cooler waters these areas provide.
6. Additional information on pool conditions, particularly those conditions that might be detrimental to branchiopod populations (trash, algal bloom, abundance of predators, etc.), shall also be recorded. *[If possible it is best to record all organisms observed and their abundance as per the data sheet. Changes in trends of common species may be just as important as those for listed species and/or may indicate environmental factors that may eventually affect listed species. See section VII(a)6 below.]*
7. The permittee shall disinfect sampling and field gear after each use within single isolated vernal pools (not within a vernal pool complex) or after sampling within a single complex of closely spaced vernal pools. In areas where *B. sandiegonensis* and *B. lindahli* co-occur the Service recommends disinfecting nets between each pool sampled:
 - i. Remove mud, snails, algae, and other debris from nets, boots, vehicle tires, and all other surfaces. Rinse cleaned items with sterilized (e.g., boiled or treated) water before leaving each survey site.
 - ii. Boots, nets, hands, etc. shall be scrubbed with either a bleach solution (0.5 to 1.0 cup per 1.0 gallon of water), Quat-128™ (1:60), or a 3 to 6 percent sodium hypochlorite solution and thoroughly rinsed clean with water. Cleaning equipment in the immediate vicinity of a pond or wetland shall be avoided (e.g., clean in an area at least 31 meters from aquatic features). Care shall be taken so that all traces of the disinfectant are removed before entering the next aquatic habitat.

- iii. In remote locations, clean all equipment as described above upon return to the lab or base camp. Elsewhere, when washing machine facilities are available, remove nets from poles and wash with bleach on the delicates cycle, within a protective mesh laundry bag.
- iv. Used cleaning materials (liquids, etc.) shall be disposed of safely at the lab. Used disposable gloves shall be retained for safe disposal in sealed bags.

Note: The Service acknowledges the threatened/endangered California tiger salamander (*Ambystoma californiense*) use some of the same habitats that are potential habitat for listed large branchiopods for a portion of their life cycle, and that the Permittee may incidentally capture adults or larvae of this species while sampling potential habitat for branchiopods. Focused surveys for the California tiger salamander require a section 10(a)(1)(A) recovery permit specific for this species. Therefore, any California tiger salamander incidentally captured during a survey shall be returned in good condition to the feature where they were found as quickly as possible, and sampling within that feature shall cease. The Permittee shall report the sighting to the Service within 15 days and will request advice on how to proceed for the survey. The California species of concern, western spadefoot toad (*Spea hammondi*), may also be encountered during surveys and shall be handled in accordance with State regulations. All California tiger salamanders and western spadefoot toads encountered incidentally during wet season surveys shall be recorded on the appropriate Listed Large Branchiopod Data Sheet and submitted with the required 90-Day Report, including the numbers of each life stage of the species netted.

d. Identification/Voucher Specimens

1. For all survey zones except areas within the jurisdiction of the Carlsbad Fish and Wildlife Office:
 - i. Voucher specimens should be collected only once for each individual feature sampled during a single wet season and deposited at one of the institutions in Section VIII below. Note that a single feature would include all habitat that is hydrologically connected and biologists should verify the extent of each surveyed feature to prevent taking duplicate voucher specimens. Additional voucher specimens may be taken from individual features if previously undetected species are found or at the direction of the appropriate Service field office.
 - ii. Voucher specimens of all listed large branchiopods captured shall be collected and all other non-voucher specimens shall be returned in good condition to the feature where they were found as quickly as possible.
 - iii. No more than 20 specimens of each species of listed large branchiopod from each feature, or less than 10% of the estimated subpopulation present in the feature, whichever is the lesser amount, shall be retained and preserved as voucher specimens. We anticipate that primarily males will be collected for identification, but a sample should include no less than three specimens of either sex, and both male and female taxonomic

characteristics shall be observed for proper identification. Identification shall be made only by biologists with valid 10(a)1(A) permits for the listed large branchiopods.

2. For areas within the jurisdiction of the Carlsbad Fish and Wildlife Office:
 - i. Specimens should be collected from each individual feature sampled during a single wet season and deposited at one of the institutions in Section VIII below. Note that a single feature would include all habitat that is hydrologically connected at the time of sampling and biologists should verify the extent of each surveyed feature to prevent taking duplicate voucher specimens. Additional specimens may be taken from individual features if new cohorts of branchiopods are observed, previously undetected species are found, or at the direction of the appropriate Service field office.
 - ii. Incidentally captured branchiopods that will not be collected as voucher specimens shall be returned in good condition to the feature where they were found as quickly as possible.
 - iii. For a standard survey, up to 20 specimens of each species of listed large branchiopod from each feature, or less than 50% of the estimated subpopulation present in the water column, whichever is the lesser amount, may be retained and preserved as voucher specimens during each sampling visit. For a standard survey we anticipate that primarily males will be collected for identification, but a sample should include no less than three specimens of either sex, and both male and female taxonomic characteristics shall be observed for proper identification. - Identification shall be made only by biologists with valid 10(a)1(A) permits for the listed large branchiopods.

The size of a given feature and the number of branchiopod species and individuals it supports can vary greatly. To afford survey biologists flexibility in conducting effective surveys under varying conditions, additional survey guidance is included below.

- A. With written authorization from the Carlsbad field office, once the expected listed branchiopod(s) have been documented within a given feature, surveys may cease for that feature.
- B. With written authorization from the Carlsbad field office, a single voucher only of each listed species found within a vernal pool complex (as determined by the FWO) may be taken (e.g., to minimize take where branchiopod numbers are limited).
- C. Multiple similar branchiopod species may occupy the same feature in varying numbers. To increase the detectability of a rare species under these circumstances, where feasible, we recommend that 30 specimens, or less than 50% of the estimated subpopulation present in the water column, whichever is the lesser amount, be retained and preserved for identification during each sampling visit where

new cohorts of branchiopods are observed (e.g., within Zone C, *B. lindahli* may occur with *B. lynchi* or *B. sandiegonensis*). As only a limited number of branchiopod eggs hatch during a given wet season we do not anticipate any negative effects to the diversity of fauna within the pool resulting from the collection of additional specimens to allow for accurate documentation of rare species. However, requests to conduct surveys at the same location over multiple wet seasons should be carefully reviewed by the Carlsbad field office.

D. Where multiple species within a genus co-occur, hybridization may be observed. Proper identification of hybrids may require the collection of approximately 10 to 15 additional female specimens (e.g., hybrids between *B. lindahli* and *B. sandiegonensis* are more easily identified with female specimens). In Orange and San Diego County, if any variability in the characteristics of *B. lindahli* and *B. sandiegonensis* is observed, those specimens should be scored relative to a morphological hybrid index (Simovich *et al.* 2013). A revised version of the morphological hybrid index shall be made available on the Service's website.

3. 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.
4. Procedures for submitting voucher samples to several institutions can be found at the links provided in section VIII below.
5. A portion of voucher specimens may also be submitted to an appropriate institution (contact the Service field office) for identification using DNA.

V. DRY SEASON SURVEYS

Dry season soil/substrate sampling shall not be conducted at any project site unless the Permittee receives prior authorization from the Service (see I (c)).

a. Soil/Substrate Collection

Soil (substrate) shall be collected when it is dry to avoid damaging or destroying listed large branchiopod eggs (also known as cysts or resting eggs). A hand spade or similar instrument shall be used to collect the sample at each feature taking from the top 1-3 cm of pool sediment. Whenever possible, substrate samples shall be collected in chunks. The spade shall be used to pry up intact chunks of sediment, rather than loosening the substrate by raking and shoveling which can damage eggs.

In California there are a number of federally listed plant species that often co-occur with the listed large branchiopods. Please refer to the recovery plans for information regarding listed vernal pool associated plants (Service 1998a, 2005). Although incidental take prohibitions do not apply to listed plants, except on land under Federal jurisdiction (Service 1994), the Permittee must comply with State laws regarding take of State rare and listed plants while sampling for listed large branchiopods.

The Permittee shall contact the appropriate field office (see XI, Service Contact section) and conduct research regarding the distribution of listed vernal pool associated plant species prior to conducting dry season sampling.

b. Soil/Substrate Sample Volume

1. The number of soil/substrate samples and the amount of soil/substrate collected per feature shall be proportional to the size of the feature. Unless the feature is very small [less than 25 square feet (2.5 square meters)], 10 samples, comprising no more than 100 ml each, which combined total one liter, should be sufficient for a feature size of roughly 250 square feet (24 square meters). Table 1 below lists the appropriate number and volume of soil/substrate sampling required per feature size. For small features, the first survey should be limited to a single core sample at the pool's low spot. If no cysts are found, a follow-up survey can be conducted using the full 1 liter of sediment that is described below (Bohanak and Simovich 2013).

Table 1. Number and Volume of Soil Samples Required Based on Feature Size			
Feature Size		Minimum Number of Samples	Sample Volume (in ml)
Square Meters	Approx. Acreage		
< 2.5	0.0005	As appropriate	25-50
2.5 - 24	0.005	10	50-100
25 - 235	0.05	25	50-100
236 - 2300	0.5	50	50-100
2300 - 23,225	5.0	100	50-100

2. Unless authorized by the Service, no more than 10% of the sampled feature's surface area shall be disturbed while collecting soil/substrate, and no more than 2 liters per 10 square meters of pool surface area (as a ratio) shall be taken as soil/substrate samples from a single feature (Service 1998b).
3. Soil/substrate samples from the same feature can be combined in the same storage container.
4. Tools and other gear used to remove dry season soil/substrate samples should be disinfected between isolated vernal pools or after sampling within a complex of closely spaced vernal pools.

c. Soil/Substrate Sample Locations

Soil/Substrate shall be collected mainly from the lowest topographic areas within the feature sampled. Areas within the feature that show evidence of past aquatic invertebrate and vertebrate accumulations/deposits, such as seed shrimp and tadpole shrimp carapaces, should be priority sampling areas.

d. For monitoring populations, the goal is to establish a relative estimate of population size that can be compared within pools over time, and hopefully between pools.

1. Recommended protocol: two perpendicular transects should be set up using string, such that they meet in the pool's center (deepest spot). One of the transects should pass over the pool's second lowest point. Five core samples 2" in diameter and 2" deep should be taken as follows: one in the pool center, and one radiating in each of the four directions on the transect lines: 1.0 m from the pool center. Deviations from this protocol may be authorized by the appropriate Service field office.

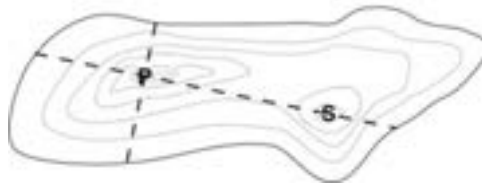


Figure 1. Schematic of transect layout. P represents the primary deep spot, and S represents the secondary deep spot.

e. Soil/Substrate Storage

1. The soil/substrate samples taken from each feature shall be stored in a separate container with the collection date, location of feature, and name of collector (Permittee).
2. Soil/substrate samples containing any residual moisture shall be adequately ventilated and allowed to air dry thoroughly before storage of the sample. Soil/substrate samples shall be stored in a dry location and kept out of direct sunlight in order to avoid excessively heating the sample until delivery to the Permittee who will conduct the sample analysis.

VI. DRY SEASON SOIL/SUBSTRATE SAMPLE PROCESSING AND EXAMINATION FOR LISTED LARGE BRANCHIOPODS

Only biologists who hold a valid 10(a)1(A) recovery permit for the listed large branchiopods with additional terms and conditions included in their permit specifically for conducting processing, isolating, and identifying listed large branchiopod eggs are authorized to conduct this procedure. There are different procedures used by branchiopod biologists to isolate branchiopod eggs from substrate samples. Biologists may use those techniques that they have found to be most successful in isolating whole, intact (undamaged) branchiopod eggs. The following methods have been used to isolate branchiopod eggs by some biologists. Deviations from these methods are at the discretion of the permitted biologist. The standard dry season vernal pool data sheet (Appendix 2) is recommended to record the appropriate data from each feature.

a. Soil/Substrate Preparation

1. Soil samples should be allowed to hydrate in either water (filtered or distilled preferred) or a minimum of 5% brine solution containing lukewarm water and table salt (NaCl). The substrate material shall be gently worked by hand to breakdown any persistent soil structure. Iodized salt will not be used for this procedure.

b. Soil/Substrate Sieving

1. Small aliquots (approximately 50 ml in volume) of the organic material rising to the top of the brine solution should be skimmed off and placed in a 710-micron diameter pore-size sieve (No. 25 U.S. standard eight inch soil sieve) which has been stacked atop a 75-micron diameter pore-size sieve (No. 200 U.S. standard eight inch soil sieve).
2. The organic material placed in the top sieve shall be flushed with lukewarm tap water or brine to strain out the larger material and carry the eggs and smaller particles into the mesh of the 75 micron screen.
3. During the sieving process the organic material samples shall not be ground, crushed, or otherwise manipulated in order to expedite the sieving process.
4. All sieves used for processing soil/substrate samples will be thoroughly cleaned and visually inspected prior to and after completion of the procedure to ensure no branchiopod eggs are adhered to the surfaces of the sieves.

c. Soil/Substrate Examination

1. The soil/substrate retained from the 75-micron diameter pore-size sieve shall be removed, thinly spread into petri dishes (or other suitable container), and viewed under a dissection microscope for large branchiopod eggs. Alternatively, the intact resting eggs may be fragile and removing from the sieve may result in damage. In that case the sample should remain in the sieve and viewed as a unit under the dissecting scope. Generally, viewing should be performed while the sieved product is still moist in order to maximize detectability of the large branchiopod eggs due to increased contrast with background material. These eggs

should not be manipulated or handled if at all possible to avoid damaging the egg structure and identifiable characteristics.

2. All mineral material retained in the brine solution should then be processed and viewed under the dissecting microscope in the same manner as the organic material to ensure that all remnant organic material, including large branchiopod eggs, have been viewed. .
 3. Unless being used for hatching experiments all branchiopod eggs may be removed from the soil/substrate, separated by egg type, and placed in labeled vials. Filled vials should be allowed to air dry and then stored dry with proper labeling. Alternatively, entire petri dishes with sieved contents may be labeled and stored when dry. The storage of eggs in their dry natal substrate is at the discretion of the permitted biologist.
- d. Large Branchiopod Egg Abundance.
- Information concerning the relative amount of eggs viewed within each soil/substrate sample processed should be estimated and included in final reporting. Total egg abundance information for each soil feature shall be reported for each species in terms of: none (no eggs found in sample); low abundance (estimate of 1-10eggs/100 ml soil); medium abundance (estimate of 11-50 eggs/100 ml soil; and, high abundance (estimate of more than 50 eggs/100 ml soil).
- e. Large Branchiopod Egg Identification
- Each large branchiopod egg type shall be identified to genus by the qualified biologist. Except for *Branchinecta* genus, no further identification will be made beyond the genus. The Service may require an independent review by a crustacean biologist(s) of any listed large branchiopod egg identification.
- f. There are two options after a *Branchinecta sp.* egg is identified:
1. The egg(s) may be sent to an appropriate facility for DNA analysis and identification to species. This is the only method that can be used to differentiate between *Branchinecta* species.
 2. The egg(s) still within natal substrate may be subject to hydration and incubation (culturing) in order to hatch out the eggs and raise the individual *Branchinecta sp.* to sexually mature adults that can be identified to species. This activity can be conducted only by a biologist who is in possession of a valid 10(a)1(A) permit with special terms and conditions for this procedure. However, absence of the listed *Branchinecta sp.* at any location cannot be assumed based on hydration and incubation.
- g. Large branchiopod egg voucher specimens
- If available, a representative sample of eggs from each species of branchiopod found in each feature shall be deposited in one of the facilities and in the manner listed in section VIII below.

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 Sheets where applicable as the reporting form, no more than 90 calendar days after completing the last field visit of the wet season at each project site, or 90 days after completion of the analysis of dry season soil/substrate samples:

1. The location of the survey area clearly delineated on a high-quality 7.5 minute U.S. Geological Survey (USGS) topographic map at exact scale (*i.e.*, 1:24,000 or 1"=2000 ft) and, if possible, equivalent GIS product. Name of USGS map shall be identified. If the USGS map name is unavailable, graticules or tics shall be used to depict the map location, with the PROJECTION and DATUM of the coordinate system identified.
2. The location of the listed vernal pool branchiopods depicted on the USGS map in as precise a manner as possible, or on additional 1:24,000 or larger (*e.g.*, 1:5,000) scale maps, with scale identified on each map. It may be necessary to prepare multiple maps to accurately convey all information. Occurrences shall be labeled such that the reader can associate the map with the findings in the report. Maps shall be either topographic with elevation contours identified, or aerial photography with locator information identified. Surface features on the occurrence map(s) shall be readable and not masked out by other supplied information. A North Arrow shall be included on all maps. Service field offices may request to be provided with a final copy of GIS data compiled as part of the survey including applicable "shape" files with appropriate metadata.
3. A minimum of 5 color digital photographs taken from each project site during sampling in the wet season; this is to include two 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 photographs of representative vernal pools, swales, and other areas within the site sampled for the six listed large branchiopod species. The following information shall be provided with each photograph: 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 six listed vernal pool branchiopod species that were found at the depicted site.

Note: Photographs only need to be submitted once per project site unless they are used to demonstrate variable conditions throughout the season.

4. 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)
5. The number of individuals of any of the listed vernal pool branchiopods or eggs preserved from each pool/swale and the name of the institution to which they were provided.

(Refer to the Vernal Pool Data Sheet)

6. 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, For taxonomic descriptions of co-occurring aquatic invertebrates the following references are instructive: McCafferty and Provonsha 1983, Merritt et al. 2008, and Thorpe and Rogers 2011).

7. Data collected during each field visit should include: 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. Also, any additional information not required but obtained including water chemistry data (*e.g.*, alkalinity conductivity, dissolved oxygen, dissolved NH₄, pH, salinity total dissolved solids and turbidity), terrestrial vertebrate species viewed, etc.
(Refer to the Vernal Pool Data Sheet).

8. For wet season surveys only Permittees must clearly state in the introduction of the 90-day report if vernal pool conditions were not suitable for conducting surveys during the season.

9. Reports of monitoring surveys conducted on preserves as part of Service approved management and monitoring plans may be turned in to the appropriate field office as part of the annual reporting.

10. Each report shall include the following certification statement and be signed by each surveyor(s) performing activities in independent status pursuant to a recovery permit: "I certify that the information in this survey report and attached exhibits fully and accurately represents my work." The date of signature and the surveyor's permit number shall be included.

b. California Department of Fish & Wildlife

1. Permittees should consult with the California Department of Fish and Wildlife (916-445-3764) 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 Wildlife [California Natural Diversity Data Base (CNDDDB), California Department of Fish and Wildlife, 1807 13th 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. The record of an occurrence of a species at a single location needs only one entry in the CNDDDB, unless additional entries are requested by the Service or the CDFW.

VIII. PROVIDING VOUCHER SPECIMENS TO A REPOSITORY

- a. All vernal pool branchiopod voucher specimens collected (both adult wet preserved individuals and dry resting eggs) shall be submitted to the collection of either the California Academy of Sciences (CAS), the Natural History Museum of Los Angeles County (NHM of Los Angeles), or other institution designated by the appropriate Service Field Office. All specimens shall be preserved according to the standards of the repository which will accession and maintain the specimens. Review these standards and instructions before proceeding with the field work – they describe acceptable preservation methods.
- b. All vernal pool branchiopod voucher specimens collected (both adult wet preserved individuals and dry resting eggs), along with a copy of the Vernal Pool Data Sheet shall be permanently deposited in the CAS or NHM of Los Angeles (or other approved institution) within 90 calendar days of the completion of the field survey and the Service shall be supplied with the CAS or NHM of Los Angeles (or other institution) catalog numbers given to the specimens.
- c. The Permittee shall supply the CAS or NHM of Los Angeles (or other approved institution) 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 NHM of Los Angeles to accession any listed vernal pool 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
Department of Invertebrate Zoology and Geology
55 Concourse Drive, Golden Gate Park
San Francisco, CA 94118
Telephone: (415) 379-5256
Link: http://www.fws.gov/sacramento/es/Survey-Protocols-Guidelines/Documents/CASIZ_Fairy_Shrimp_Vouchers_July_2014.pdf

Natural History Museum of Los Angeles County,
Research and Collections, Crustacea
attention Adam Wall, Curatorial Assistant
900 Exposition Boulevard
Los Angeles, California 90007
Tel. 213 763-3450
email: awall@nhm.org
Link: <http://research.nhm.org/mbc/vernalpools/>

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 a deviation from 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 consider 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 section I(c)] 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 methods without authorization from the appropriate field office to deviate from the methods;
- c. Failure to obtain prior written authorization from the appropriate field office to commence either wet season surveys or dry season surveys [see section I (c)];
- d. Failure to notify the Service within 10 days of the 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 Wildlife 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 Southern Oregon, contact the Roseburg Field Office (RFO), 2900 N.W. Stewart Parkway Roseburg, Oregon 97471; telephone 541/957-3474).

For Del Norte, Humboldt and Mendocino Counties, contact the Arcata Fish and Wildlife Office (AFWO), 1655 Heindon Road, Arcata, California 95521 (telephone: 707-822-7201; fax: 707-822-8411).

For the Central Valley hydrographic basin and the coast ranges north of the Santa Cruz County line and south of the Mendocino County line (but including the Russian River area of southwestern Mendocino County), including the Bay Delta, contact the Sacramento Fish and

Wildlife Office (SFWO), 2800 Cottage Way, W-2605, Sacramento, California 95825 (telephone: 916-414-6600).

For areas from Los Angeles County north and west of the Santa Monica pier, west of the 405 freeway, north of the San Gabriel Mountains, and west of the San Andreas Rift Zone; Ventura County; Santa Barbara County; areas in San Luis Obispo County west of the Carrizo Plain; and Monterey, San Benito, and Santa Cruz Counties in their entirety, contact the Ventura Fish and Wildlife Office (VFWO), 2493 Portola Road, Suite B, Ventura, California 93003 (telephone: 805-644-1766).

For areas from Los Angeles County east of the Santa Monica pier and east of the 405 freeway, south of and including the San Gabriel Mountains, and east and north of the San Andreas Rift Zone; in Kern County south and east of the Tehachapi Mountains and east of the Piute and Scodie Mountains; in Inyo County east of the Owens Valley; then south to the U.S. border with Mexico including San Bernardino, Riverside, Orange, Imperial, and San Diego Counties in their entirety, contact the Carlsbad Fish and Wildlife Office (CFWO), 2177 Salk Avenue, Suite 250, Carlsbad, California 92008 (telephone: 760-431-9440).

XII. COUNTIES ASSIGNED TO SURVEY ZONES (Also see Figure 2 next page)

- A. Survey Zone A includes the following geographic areas: Southern Oregon, Sacramento Valley, San Francisco Bay Area, North Coast Ranges, Northern Sierra Valley Foothills, Cascade Range foothills, and South Coast Ranges.

Survey Zone A includes the following counties: Santa Barbara, San Luis Obispo (except Carrizo Plain), Monterey, San Benito, Santa Cruz, Santa Clara, San Mateo, Alameda, Contra Costa, Solano, Sacramento, Amador, Alpine and all counties northwards, including Jackson County, Oregon.

- B. Survey Zone B includes the following geographic areas: San Joaquin Valley, Central and Southern Sierra Nevada foothills, and Tehachapi Mountains.

Survey Zone B includes the following counties: San Joaquin, Calaveras, Alpine, Stanislaus, Tuolumne, Merced, Mariposa, Madera, Fresno, Kings, Tulare, Kern, Carrizo Plain in San Luis Obispo County, Mono, and Inyo.

- C. Survey Zone C includes the following geographic areas: Southwestern California including South Coast, Channel Islands, Transverse and Peninsular Ranges.

Survey Zone C includes the following counties: Ventura, Los Angeles, San Bernardino, Riverside, Orange, San Diego and Imperial.

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Dr. Pete Balfour, Dr. Andrew Bohonak, Tara Collins, Dr. Michael Fugate, Dr. Brent Helm, D. Christopher Rogers, Dr. Marie Simovich, Carol Witham, Todd Wood.

Appendix 1. U.S. Fish and Wildlife Service – Data Sheet for Wet Season Surveys For Listed Large Branchiopods

Site or Project Name:	County:	Quad:	Township:	Range:	Section:
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SURVEYOR / Permit Number:

Date:	Time:	Weather Conditions:
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Feature ID #	UTM (Northing , Easting, Datum)	Temp (°C)		Depth (cm)		Surface Area (m x m)		Crustaceans					Insects			Platyhelminths (flatworms)	Habitat Condition	Notes / Voucher information
		Air	Water	Average	Est. Max.	Present	Est. Max.	Anostracans	Notostracans	Copepods	Ostracods	Cladocera	Coleoptera	Hemiptera	Diptera Culicidae			

Notes: Fill in abbreviated names of Anostracans and Notostracans, for all others indicate presence with a check mark. Anostracan and Notostracan Abbreviations: Use first two letters of genus and species name (e.g., LIOC = *Lindleriella occidentalis*, BRLI = *Branchinecta lindahli*).
 For habitat conditions use two letter abbreviation as follows: NP = Natural Pool, CP = Constructed Pool; UD = undisturbed, D = disturbed: with TT = tire tracks, T = trash, P = plowed; G = grazed, UG = ungrazed by: C = cattle, H = horses, S = sheep; AB = Algal blooms present.
 (Estimate grazing regime by height of grasses and forbs and density of hoof prints) LG = light grazing, MG = moderate grazing, HG = heavy grazing.

