Data Summary for the 2010 Field and Genetic Surveys of the Riverside Fairy Shrimp (*Streptocephalus woottoni*) in Southern California

Prepared for:

U.S. Fish and Wildlife Service

U. S. DEPARTMENT OF THE INTERIOR
U. S. GEOLOGICAL SURVEY
WESTERN ECOLOGICAL RESEARCH CENTER

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INTRODUCTION AND GOALS

The Riverside fairy shrimp, *Streptocephalus woottoni*, has been listed as federally endangered since 1993 and currently is presumed to occupy 60 or fewer pool complexes throughout southern California (San Diego, Los Angeles, Riverside, Orange, and Ventura counties) and Baja California, Mexico. Throughout its range, *S. woottoni* occupies deep pools >30 cm, in chaparral, coastal sage scrub, and grassland habitat. *Streptocephalus woottoni* often hatches later in the season than other fairy shrimp species because the deeper pools it inhabits require sufficient rainfall to fill (Ericksen and Belk 1999). A limited knowledge of its life- and natural-history exists. For example, a female can produce over 400 cysts in its lifetime (Simovich and Hathaway 1997) and only a subset of cysts hatches during any pool filling event. A high reproductive output and staggered hatching rate leads to a large cyst bank that, under natural conditions, facilitates this species to persist in a highly variable climate. *Streptocephalus woottoni* has experienced range-wide population decline, largely due to habitat loss and degradation such as altered hydrologic regimes and urbanization (U.S. Fish and Wildlife Service 2008). Because of its federally endangered status, impacts to *S. woottoni* populations and their vernal pool habitats should be minimized or compensated for by mitigation actions, such as habitat restoration or off-site habitat creation in areas with appropriate soil and hydrological conditions.

One challenge in restoring and maintaining viable populations of *S. woottoni* is to preserve genetic diversity and structure across the landscape. Preserving remaining genetic diversity across a species' distribution may be critical for long-term persistence, because this diversity represents the raw material for adaptation to differing local conditions and environmental change (Frankham 2005). Population genetics is designed to quantify genetic variability across space and time to assess natural processes including gene flow (migration and successful breeding), genetic differentiation, inbreeding, and selection. Previous research on fairy shrimp species has integrated genetic data to effectively quantify population differentiation, gene flow, and resolve taxonomic designations (Davies et al. 1997, Bohonak 1998, Fugate 1998, Ketmaier et al. 2008).

Genetic information may aid in implementing effective relocation and restoration actions for *S. woottoni*. For instance, genetic variability across populations may be significantly different such that introducing salvaged cysts from one location to another might disrupt local adaptation in the existing population, or translocated cysts may be less likely to successfully establish a new population. Although the Southern California Vernal Pool Recovery Plan mandates a better understanding of the genetics of fairy shrimp and other key species (U.S. Fish and Wildlife Service 1998), genetic data for *S. woottoni* are currently lacking. The need for inventory and monitoring of species composition and abundance across pool complexes is also outlined in the recovery plan. Genetic and geographic distribution data for *S. woottoni* may help inform recovery options for this species.

The primary objectives of this project were to survey known existing populations of *S. woottoni* across its range and to determine patterns of genetic diversity within and among these populations using the mitochondrial cytochrome oxidase I (COI) gene. Our survey data also provide information on the presence and abundance of *S. woottoni* cysts and adults within the recovery area. This study comprises the first range-wide data collection effort and analysis for *S. woottoni*.

METHODS

Site Sampling

Working with U.S. Fish and Wildlife Service (USFWS) biologists, we identified a subset of pools and pool complexes (local metapopulations of hydrologically linked pools), previously known or suspected to contain *S. woottoni* throughout the species range, as identified in the Five Year Review for this species (U.S. Fish and Wildlife Service 2008; Table 1). Efforts were made to contact landowners and gain permission to access all sites. Initial searches for landowner contact information included searches for public listings through USFWS records and personnel, public county records, and previous landowners. Once located, landowners were contacted via mail, email, phone, or a combination of these methods depending on available information. Written permission to access sites was acquired prior to accessing the sites. At sites where access was granted, most pools were sampled during the dry season, and a few were sampled during the wet season. We were also able to obtain cysts or soil samples taken previously from several pool complexes, some of which are now extirpated.

Dry season sampling consisted of collecting approximately 2 to 4 liters (l) of soil from each pool, depending on the pool size. Additional descriptive measurements were taken to assess pool size and other habitat characteristics. To extract cysts, 40 milliliter (ml) subsamples of soil were poured through a sieve and gently washed with water to remove larger substrate particles and organic debris. The remaining substrate was then searched for cysts using a dissecting microscope. Cysts were dried in collection tubes on filter paper and stored until ready for extraction. A detailed protocol for dry season sampling and soil sorting is in Appendix A. All remaining soil samples are being stored in the Simovich laboratory at the University of San Diego.

Wet season samples were collected after sufficient rainfall had inundated pools long enough for *S. woottoni* to hatch and mature (7 to 8 weeks from inundation depending on pool conditions; (Hathaway and Simovich 1996, Simovich and Hathaway 1997). Sites were monitored at regular intervals until mature adults were observed or until water levels decreased below capacity to support developing shrimp. Our sampling protocol for this study was modified from the *S. woottoni* protocol created by the USFWS but included habitat assessment and pool conditions (Appendix B). Voucher specimens were collected at Arnie's Point, Chiquita Ridge, and Johnson Ranch 2 and at the conclusion of our study will be deposited in the collections at the San Diego Natural History Museum or the Natural History Museum of Los Angeles County.

Table 1. Successful and attempted sampling localities for *S. woottoni*. Samples were successfully collected from 45 pools (representing 23 pool complexes) by sampling during the dry (dry) and wet (wet) seasons, soil from previous research or extirpated pools (soil), or cysts provided from extirpated pools (cysts). Access was not acquired for all sites due to un-locatable landowners, sites currently pending permission, or denial from the landowner. Not all sites sampled were found to contain cyst or adult *S. woottoni*.

Region	Pool Complex	Pool	Sampling Method	Sampled by	*S. woottoni* detected	Individuals sequenced	Notes
Los Angeles	LA International Airport (LAX)	EW14	Soil	This Study	No	No	Extinct, Soil stored 2005
Los Angeles	LA International Airport (LAX)	EW2	Soil	This Study	No	No	Extinct, Soil stored 2005
Orange	Saddleback Meadows	A	Dry	This Study	Yes	Yes	
Orange	Saddleback Meadows	B	Dry	This Study	Yes	Yes	
Orange	Saddleback Meadows	D	Dry	This Study	Yes	Yes	
Orange	Saddleback Meadows	E	Dry	This Study	Yes	Yes	
Orange	Saddleback Meadows	I	Dry	This Study	Yes	Yes	
Orange	El Toro		-	-	-	-	No permission granted
Orange	Foothills Parkway south		-	-	-	-	No permission granted
Orange	Radio Tower Road	North	-	-	-	-	No permission granted
Orange	Radio Tower Road	South	-	-	-	-	No permission granted
Orange	Whiting Ranch		-	-	-	-	No permission granted
Orange	Chiquita Ridge		Wet	This Study	Yes	Yes	
Riverside	Australia Pool		Dry	This Study	Yes	Yes	
Riverside	Clayton Ranch		Cysts	Bomkamp, 2009	Yes	-	Extinct, soil stored
Riverside	CNLM	2	Dry	This Study	No	No	
Riverside	CNLM	Stock Pond	Dry	This Study	Yes	Yes	
Riverside	Johnson Ranch		Soil	Simovich, 2004	Yes	Yes	Simovich sampled 2004
Riverside	March Air Reserve Base	3	-	This Study	-	-	No pool found, disced and graded
Riverside	March Air Reserve Base	6	-	This Study	-	-	No pool found, excavated training pit
Riverside	March Air Reserve Base	13	Dry	This Study	Unk.	No	1 *Streptocephalus* cyst recovered, failed to extract
Riverside	Pechanga Pool		-	-	-	-	No permission granted
Riverside	Rancho California Road Pool		Dry	This Study	Yes	Yes	
Riverside	Redhawk	11-5	Soil	Simovich, 2005	Yes	Yes	Extinct
Riverside	Redhawk	3-1	Soil	Simovich, 2005	Yes	Yes	Extinct
Riverside	Redhawk	9-1	Soil	Simovich, 2005	Yes	Yes	Extinct
Riverside	Schau Pool		Dry	This Study	Yes	Yes	
Riverside	Schleuniger Pool		Cysts	Bomkamp, 2009	Yes	Yes	
Riverside	Scott Pool		-	-	-	-	No landowner contact
Riverside	Skunk Hollow		Dry	This Study	Yes	Yes	
San Diego-N	Camp Pendleton 67	O	Soil	Simovich, 2004	Yes	Yes	
San Diego-N	Camp Pendleton 67	P	Soil	Simovich, 2004	Yes	Yes	
San Diego-N	Camp Pendleton 67	Q	Soil	Simovich, 2004	Yes	Yes	
San Diego-N	Camp Pendleton 67	V	Soil	Simovich, 2004	Yes	Yes	
San Diego-N	Camp Pendleton 68	E	Soil	Simovich, 2004	Yes	Yes	
San Diego-N	Camp Pendleton 68	X	Soil	Simovich, 2004	Yes	Yes	
San Diego-N	Camp Pendleton 89	D	Soil	Simovich, 2004	Yes	Yes	
San Diego-N	JJ2 (Poinsettia Train Station)		-	-	-	-	No permission granted
San Diego-N	MCAS Miramar AA1 east		-		-	-	No pool found; coordinates outside MCAS Miramar
San Diego-N	MCAS Miramar AA1 south	12	Dry	This Study	Yes	Yes	
San Diego-N	MCAS Miramar AA1 south	13	Dry	This Study	Yes	Yes	
San Diego-N	Ramona Airport	T	Wet	This Study	No	-	
San Diego-S	Otay Mesa East	79	Dry	This Study	-	-	Not sorted
San Diego-S	Otay Mesa East	86	Dry	This Study	-	-	Not sorted; no pool found, graded.
San Diego-S	Otay Mesa J11E Slump Pools	West Pool	Wet	This Study	No	-	
San Diego-S	Otay Mesa J15 Arnies Point	A	Wet	This Study	Yes	No	Not enough adult males present to take samples
San Diego-S	Otay Mesa J15 Arnies Point	B	Wet	This Study	Yes	Yes	
San Diego-S	Otay Mesa J26	East of Trust	Dry	This Study	Yes	Yes	
San Diego-S	Otay Mesa J29-30	J29	Soil	Simovich, 2003	Yes	Yes	Extinct
San Diego-S	Otay Mesa J29-30	J30 Cattle Tank	Dry	This Study	Yes	Yes	
San Diego-S	Otay Mesa J35 (Brown Field)		-	-	-	-	No permission granted
San Diego-S	Otay Mesa Lonestar	Cattle Tank	Dry	This Study	Yes	Yes	
San Diego-S	Otay Mesa SR125/J2?	2	Dry	This Study	-	-	Not sorted; inoculated with J29 soil
San Diego-S	Otay Mesa SR125/J2?	31	Dry	This Study	-	-	Not sorted; inoculated with J29 soil
San Diego-S	Otay Mesa SR125/J2?	32	Dry	This Study	Yes	Yes	Inoculated with J29 soil
San Diego-S	Otay Mesa SR125/J2?	35	Dry	This Study	-	-	Not sorted; inoculated with J29 soil
San Diego-S	Otay Mesa SR125/J2?	102	Dry	This Study	-	-	Not sorted; inoculated with J29 soil
Ventura	Tierra Rejada Preserve		-	-	-	-	Pool did not fill 2010, awaiting soil from USFWS

DNA Extraction and Sequencing

To extract DNA from cysts, we used a DNA IQ Kit (ProMega) using a protocol optimized for fairy shrimp (Steele et al. 2009). In order to make the embryo available for extraction, we first degraded cysts by soaking each cyst in bleach for 15 minutes (mins). The cysts were then transferred to a detergent (96.6 ml dH2O, 3.0 ml 4 M NaCl, 0.4 ml 10% Triton x-100) for 5 mins to remove all bleach and then placed individually in microcentrifuge tubes with 20 microliters (µl) of PBS buffer, 2.0 µl of 1 M DTT, and 2.0 µl of 20 mg/ml proteinase K. A dissecting needle was used to burst the embryo, after which 250 µl of lysis buffer and 2.5 ml of 1 M DTT were added to the tube and left to incubate overnight at 56 °C. On the following day, samples were briefly vortexed and 7 µl of resin was added to each sample, vortexed again for 5 s, and left to incubate at room temperature for 5 mins. Samples were then placed in a magnetic stand and the solution was pipetted out. A 100 ml volume of lysis buffer cocktail (100 µl lysis buffer: 1.0 µl 1 M DTT) was added to each tube, vortexed 2 s, and returned to the magnetic stand. The solution was pipetted out and 100 µl of 1 X wash buffer was added to each tube. The tubes were centrifuged again, returned to the magnetic stand, and the solution was pipetted out. The addition of wash buffer cleanse cycle was repeated twice and samples were then air-dried for up to 20 mins to remove any remaining solution. Elution buffer (100 µl) was added to each tube, vortexed 2 s, and incubated at 65 °C for 5 mins. Samples were then individually placed in the magnetic stand and the elution containing the DNA was removed and placed in a new microcentrifuge tube. Concentration of DNA for each sample was quantified using NanoDrop (Thermo Scientific, Wilmington, Delaware).

To extract DNA from adult shrimp, we used a DNEasy Blood and Tissue Extraction Kit, (Qiagen, Valencia, California). Briefly, shrimp samples were digested overnight in a solution of 180 µl Buffer ATL and 20 µl proteinase K at 56 °C. Samples were then centrifuged, then 200 µl of Buffer AL was added and the sample was again vortexed before adding 200 µl of 100% EtOH. Samples were vortexed, transferred to a spin column, and centrifuged for 60 s at 12000 rpm. The spin column was then transferred to a new microcentrifuge tube and 200 µl of Buffer AE was added and the spin column was incubated at room temperature for 60 s. The samples were centrifuged for 60 s at 12000 rpm and the spin column was discarded. To determine the concentration and quality of DNA, samples were tested using the NanoDrop (Thermo Fisher Scientific, Inc., Wilmington, Delaware) and/or run out on a gel.

We used polymerase chain reaction (PCR) to amplify an approximately 700 bp fragment of the mitochondrial Cytochrome Oxidase I (COI) gene, using universal primers LCO1490 and HCO2198 (Folmer et al. 1994). A total of 5.0 µl of template DNA was used in a 25.0 µl reaction. Cyst DNA extractions yielded varying concentrations of DNA, and samples were typically used at full concentration or diluted 1:4 using dH_2O. Template DNA from adult shrimp was typically diluted 1:10 or 1:15 using dH_2O. PCR reactions were conducted in 25.0 µl total volume consisted of 5.0 µl template DNA, 2.5 µl 10X buffer, 2.6 µl 50 mM $MgCl_2$, 2.5 µl 2.5 mM dNTPs, 1.4 µl of each primer, and 0.15 µl DNA Taq Polymerase. Cycling conditions were as follows: 94 °C for 2 min, 94 °C for 30 s, 48.6 °C for 40 s, 72 °C for 45 s, and a final extension at 72 °C for 7 min. Products were sent for sequencing to Genewiz (San Diego, California) and sequenced on an ABI 3730xl DNA Analyzer.

Sequence Analysis

All sequences were aligned by eye and base readings were reviewed using Sequencher 4.6 (Gene Codes Corp, Ann Arbor, MI). Unique haplotypes were determined using the program Collapse (Posada 1999). A parsimony network was built to determine the relationship among *S. woottoni* haplotypes in the program TCS (Clement et al. 2000).

The phylogenetic relationships among *S. woottoni* haplotypes and other North American *Streptocephalus* species were estimated using a Bayesian search method. COI sequences from seven North American and one African *Streptocephalus* species collected for a previous study were downloaded from Genbank (Daniels et al. 2004). These were combined with *S. woottoni* haplotypes and Bayesian searches were performed in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) using a general time reversible model with invariable sites and gamma distribution. The GTR+I+Γ model (Rodríguez et al. 1990) was determined to be the most appropriate model using AIC in MrModeltest 2.2 (Nylander et al. 2004). Tree searches were run for two million generations, sampling every 100 generations. The first 10% of trees were discarded as burn in, and the remaining trees retained. Two separate tree searches were performed to confirm stationarity. From the resulting 32,000 trees in the stationary distribution, a 50% majority rule consensus tree was built by rooting the tree with the African species, *S. namibiensis* and calculating average branch lengths. Branches containing posterior probabilities ≥ 95% were considered highly supported (Wilcox et al. 2002).

Global and pairwise estimates of genetic differentiation among pool complexes were examined using F-statistics, and an Analysis of Molecular Variance (AMOVA) was performed to determine the proportion of genetic variation partitioned within and among pool complexes and regions. An estimator of $F_{ST,}$ θ (Weir and Cockerham 1984), was calculated over all pool complexes. Population pairwise estimates of θ were also calculated among pool complexes. F-statistics and AMOVA were performed in Arlequin 3.5 (Excoffier and Lisdher 2010) and significance assessed with 10,000 randomizations of the AMOVA test-statistic.

RESULTS AND DISCUSSION

Site Visits

We attempted to gain samples from 58 pools throughout the range, representing 31 extant pool complexes and 4 extinct complexes for which soil has been preserved (Figure 1; Table 1). Locating, contacting, and obtaining site access permission from landowners was largely successful. During the time frame of this study we were unable to obtain permission to access and collect from nine pools. One pool (MCAS Miramar AA1 East; Table 1) could not be located. Geographic coordinates provided by USFWS placed this pool outside of MCAS Miramar.

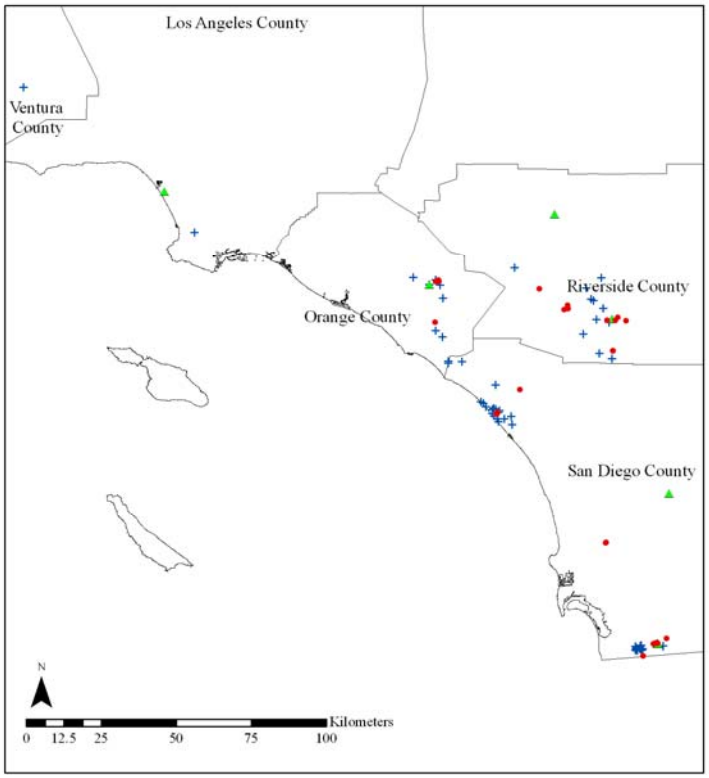


Figure 1. Sampling localities for *S. woottoni* throughout its range in southern California. Genetic data are reported for a majority of pools sampled (red circles). Sampled pools where *S. woottoni* were not detected are shown as green triangles and unsampled sites are shown as blue hatches.

Dry Season Sampling

During the dry season, a total of 26 pools were visited across 12 pool complexes (Table 2). Soil samples were collected for all pools sampled with the exception of March Air Reserve Base pools 3 and 6. Los Angeles International Airport (LAX) pools (not included in Table 2) were previously excavated due to airport expansion and soil samples were obtained from the storage facility. Field datasheets were not used for this site but soil samples were collected from Pools EW14 (grids 14, 28, and 58) and EW2 (grid 6). Figures showing the location of both pools within the LAX pool complex as well as the grid arrangement are shown in Appendix C. These figures were originally provided in the 2005 Salvage and Storage Methodology authored by Sapphos Environmental Inc.

For each pool sampled, GPS location, pool dimensions, vegetation, photos, and disturbances were recorded (collection data summarized in Table 2; see Appendix D for site photos). Pools occurred either on hardpan or claypan surfaces. Most pools contained less than 10% or more than 50% vegetative cover and there was variation in whether the vegetation was native or non-native. Efforts were made to document vegetation when recognizable and identification was certain. At most sites, more vegetative species were present than are listed in Table 2 (Appendix D).

The purpose of this study was not to assess population status, nor to definitely verify *S. woottoni* presence. However, we did note variation in habitat characteristics across sampled sites. Almost all surveyed pools had been deepened or modified by humans, likely as stock ponds. This modification may allow for *S. woottoni* to inhabit and persist in these pools, because they hold water for the length of time required for the species to complete its life cycle. One exception to this is Skunk Hollow, which is thought to be a naturally deep pool. Although disturbance and percent cover were not rigorously quantified, there appeared to be no association between cyst presence or density and percent native cover or presence/type of disturbance. Some pools appeared to be absent or graded flat (Table 2), and these sites likely require more investigation to determine whether *S. woottoni* is present.

Table 2. Summary data for dry season sampling. A total of 26 pools from 12 pool complexes were sampled in 2009. Pool conditions were highly variable, including level of disturbance, soil condition, and vegetation. Soil samples were not collected from March Air Reserve Base pools 3 or 6.

Pool Information			Collection Data						Pool Dimensions						Pool Vegetation					Habitat Conditions									Notes
Region	Pool Complex	Pool	Date	Time	Latitude	Longitude	Datum	Collectors	Maximum Depth (cm)	Length (m)	Width (m)	Surface area (m2)	Collected Soil Volume (L)	Pool Bottom	Vegetated	Bare	Native	Nonnative	Representative Plant Species	Undisturbed	Ungrazed	Disturbed	Grazed	Tire tracks	Cattle	Garbage	Discing/ plowing	Other	
Orange	Saddleback Meadows	A	11/20/2009	2:45pm	33.676933	-117.6203	WGS84	MS, AGV	n/a	42	20	800	2.5	claypan	85-90	10-15%	95-100	0-5%	*Baccharis salicifolia, Psilocarphus tenellus, Rumex crispus, Eleocharis montevidensis, Polypogon monspeliensis, Eremocarpus setigerus*				X						pool trampled, no soil cracking
Orange	Saddleback Meadows	B	11/20/2009	2:30pm	33.676367	-117.618175	WGS84	MS, AGV	n/a	7	12	75	2.5	claypan	85-90	10-15%	95-100	0-5%	*Polypogon monspeliensis, Eleocharis montevidensis, Eremocarpus setigerus, Baccharis salicifolia, Psilocarphus tenellus, Lolium multiflorum*	X	X								
Orange	Saddleback Meadows	D	11/20/2009	2:25pm	33.67808	-117.614622	WGS84	MS, AGV	n/a	27	22	560	2.5		0	100	0	0	*Baccharis salicifolia*				X		X				minimal soil cracking, soil broken and soft from ungulates
Orange	Saddleback Meadows	E	11/20/2009	1:15pm	33.68146	-117.61158	WGS84	MS, AGV	n/a	28	16	400		claypan	<5	100	<5		*Baccharis salicifolia*			X	X	X	X			X (digging)	heavily grazed, disturbed
Orange	Saddleback Meadows	I	11/20/2009	1:30pm	33.67594	-117.609935	WGS84	MS, AGV	n/a	20	30	570	2.5	claypan	0	100	0	0				X	X	X	X	X			minimal soil cracking
Riverside	Australia		12/3/2009	4:30pm	33.654373	-117.308723	WGS84	ML, CG	50	63	55	3200			0	100	0	0				X	X						Many shallow cracks
Riverside	CNLM	Stock Pond	12/3/2009	10:15am	33.568873	-117.0745	WGS84	MEL, CG, KK, LC	250	97	45.5	500	6	claypan	100	0	1	99	*Atriplex semibaccata, Salix nigra, Hordeum murinum, Xanthium strumarium, Bromus madritensis, Bromus diandrus, Rumex crispus*			X	X		X	X			Minimal soil cracking, thick clay layer 2-4" under surface, burrow mounds
Riverside	CNLM	2	12/3/2009	11:15am	33.564737	-117.0913	WGS84	MEL, CG, KK, LC	40	87.3	26.5	170	3	claypan	95-100	0-5	5-10%	90-95	*Heliotropium currasavicum, cryptantha intermedia, Hemizonia fasciculata, vinegar weed*									sheep possibly	
Riverside	Lonestar	Cattle Tank	11/19/2009	12:15pm	32.58122	-116.968118	WGS84	MS, AVG, MEL, KD	n/a	56	20.5	900	3	hardpan	50-60	0	90	10	*Eryngium aristulatum, Pogogyne nudiuscula, Isoetes sp.*	X									Deep soil cracking
Riverside	March Air Reserve Base	3	12/3/2009	3:00pm	33.8833	-117.265007	WGS84	ML, CG	n/a	n/a	n/a	n/a	0	hardpan	50	50	20-30	70-80	*Eremocarpus setigerus, Brassica nigra, Chenopodium album, Salsola tragus*		X	X				X		dogs	No cracking, depression barely visible but terrain not characteristic of vernal pool, vegetation disced, strips of old tarmac pavement present
Riverside	March Air Reserve Base	6	12/3/2009	3:30pm	33.905556	-117.263363	WGS84	ML, CG	n/a	n/a	n/a	n/a	0	hardpan	60	40	90-100	0-10%	*Hemizonia fasciculata, Eremocarpus setigerus, Chenopodium album*			X		X			X		Pools are now 3 excavated pits used for training
Riverside	March Air Reserve Base	13	12/3/2009	3:15pm	33.882745	-117.264567	WGS84	ML, CG	5	90	16	1100	2	hardpan	95-100	0-5	30	70	*Brassica nigra, Hemizonia fasciculata*			X							Adjcent to landing strip, water runoff might fill pool, footprint not visible
Riverside	Schau Pool		12/3/2009	5:00pm	33.592242	-117.235062	WGS84	ML, CG	n/a	3	4	10	1	hardpan	5-10%	90-95	0	5-10%				X	X	X					Multiple depressions that looked like pools, lots of vegetative growth
Riverside	Skunk Hollow		12/3/2009	11:30am	33.559382	-117.10717	WGS84	MEL, CG, KK, LC	70	530	160	84000	12	claypan	95-100	0-5	10-15%	85-90	*Avena fatua, Hemizonia fasciculata, Bromus spp., Cryptantha intermedia, Brassica nigra*										Soil cracking
Riverside	Rancho California Road Pool		12/3/2009	12:30pm	33.561312	-117.049855	WGS84	ML, CG	100	90	38	2800	3	claypan	0-1%	99-100	0	1%	*Brassica nigra, Eremocarpus setigerus*			X	X	X	X	X			Soil hard packed silt, heavily churned by cows
San Diego- N	AA1 south (MCAS Miramar)	12	11/23/2009	8:30am	32.88756166	-117.1102733	WGS84	AGV, MEL	50	90	60	300	4	hardpan	0	80	0	0											Man-made pool, shallow soil cracks. Rocky
San Diego- N	AA1 south (MCAS Miramar)	13	11/23/2009	8:30am	32.88763667	-117.1100017	WGS84	AGV, MEL	80	88	100	300	2.5	hardpan	0	80	0	0											Separated from pool AA1 south 13 by a berm at the south end
San Diego- S	East Otay Mesa	79	11/19/2009	12pm	32.56346	-116.90881	WGS84	MS, AVG, MEL, KD	n/a	8.8	5.7	50	2.5	claypan	50	0	5	0	*Eleocharis montevidensis, Polygonum lapathifolium, Atriplex sp.,*			X	X						Road pool, ostracods
San Diego- S	East Otay Mesa	86	11/19/2009	11:45am	32.56443	-116.913093	WGS84	MS, AVG, MEL, KD	n/a	n/a	n/a	n/a	1	claypan	80	20	0	100	*Rumex crispus, Lolium multiflorum, Tamarix xp.*			X	X						Pool in road, footprint hard to see, historically a 30' long pool
San Diego- S	J2 (Otay Mesa)	2	11/19/2009	3:45pm	32.582341	-116.957985	WGS84	MS, AGV, MEL, KD	n/a	16	14	190	2.5	hardpan	<5	95	50	50	*Baccharis salcifolia*			X	X	X	X				Consistently holds water and adult RFS, thin algal crust, restored pool innoculated with cysts
San Diego- S	J2 (Otay Mesa)	31	11/19/2009	2:30pm	32.582996	-116.95398	WGS84	MS, AGV, MEL, KD	n/a	24	16	340	2	hardpan	50	50	90	10	*Psilocarphus tenellus, Eleocharis montevidensis*										Algal crust
San Diego- S	J2 (Otay Mesa)	32	11/19/2009	2:51pm	32.582081	-116.954216	WGS84	MS, AGV, MEL, KD	n/a	16	13.5	130	1	hardpan	60	40	85	15	*Eremocarpus setigerus, Pogogyne nudiuscula*	X									
San Diego- S	J2 (Otay Mesa)	35	11/19/2009	2:30pm	32.582985	-116.954131	WGS84	MS, AGV, MEL, KD	n/a	18	9	13	2.5	hardpan	50	50	100	0	*Eryngium aristulatum, Pogogyne nudiuscula, Eleocharis montevidensis*										
San Diego- S	J2 (Otay Mesa)	102	11/19/2009	2:15pm	32.581803	-116.955221	WGS84	MS, AGV, MEL, KD	n/a	22.5	13.5	270	1	hardpan	50	50	90	10	*Eremocarpus setigerus, Pogogyne nudiuscula, Psilocarphus tenellus*										Man-made pool, soil cracked, regularly has *S. woottoni*, inoculated with Otay Mesa pool J29 cysts
San Diego- S	J26 (Otay Mesa)	East of Trust	11/19/2009	3:15pm	32.597928	-116.927713	WGS84	MS, AGV, MEL, KD	n/a	10	9	80	2.5	claypan	20	80	15	85	*Baccharis salcifolia, Eremocarpus setigerus, Brassica nigra*			X	X		X				Used as a reference pond, holds water early and longer than surrounding pools
San Diego- S	J30 (Otay Mesa)	Cattle Tank	11/19/2009	1:00pm	32.581977	-116.961615	WGS84	MS, AGV, MEL, KD	n/a	18.5	13.5	200	4	claypan	100	0	90	10	*Eremocarpus setigerus, Pogogyne nudiuscula*			X	X						First county record of *S. woottoni* from this pool

MEL= Megan Lahti (USGS), AGV= Amy Vandergast (USGS), CG= Carey Galst (USFWS), KK= Kim Klementowski (CNLM), LC= Lee Ann Carranza (CNLM), MS= Marie Simovich (USD), KD= Katie Davis (USD)

Wet Season Sampling

Southern California experienced an overall average amount of precipitation (97 to 101% average to date) from October 2009 through June 2010 based on weather stations at LAX and San Diego International Airport/Lindbergh field (NOAA; http://www.cnrfc.noaa.gov/monthly_precip.php). Across sites, 75 to 77% of the rainfall occurred from December through February and total precipitation ranged from 26.8 to 31.6 cm. For sites where access was granted before the onset of the wet season, we observed pools inundated with measureable levels of water from 11 February 2010 to 14 April 2010.

During the wet season, a total of 7 site visits were made to 5 pools across 4 pool complexes (Table 3; See Appendix E for site photos). *Streptocephalus woottoni* was observed at Arnie's Point and Chiquita Ridge while it was not observed at the Ramona Airport pool T and the Slump Pools. In field visits to the Ramona Airport site, we noted that neither the Ramona Airport T pool, nor surrounding pools appeared to be deep pools typical of *S. woottoni* habitat (Table 3; Appendix E).

We observed adult shrimp beginning 10 March 2010, although land managers reported seeing *S. woottoni* hatchlings at least 1 wk prior. *Streptocephalus woottoni* densities were greater than 1 shrimp /m^2 at both Chiquita Ridge and Arnie's Point and sex distribution of males to females was 1:4 at Arnie's point (sex ratios not estimated at Chiquita Ridge). Aquatic conditions where shrimp occurred ranged from 217 to 521 µS conductivity, 154 to 368 ppm total dissolved solutes, 95.9 to 230 ppm salinity, 7 to 8.49 pH, 14.8 to 15.5 °C water temperature, and 10 to 100% turbidity. Western toad (*Anaxyrus boreas*), spadefoot (*Spea hammondi*), and pacific chorus treefrog (*Pseudacris regilla*) tadpoles were present at both Arnie's Point and Chiquita Ridge. No major disturbances were noted at any pools sampled during the wet season. Field datasheets can be found in Appendix E.

Cyst Sorting and Cyst Densities

In addition to sites from which soil was obtained during this study, soil samples obtained previously by M. Simovich from an additional 13 pools (7 complexes) were sorted for cysts (Table 4). In some instances, we used cysts from older soil samples (also collected previously by M. Simovich) of the same sites that were sampled during this study. At the time this report was written, soil samples from East Otay Mesa Pools 79 and 86 have not been sorted and/or analyzed. We were unable to definitively detect the presence of *S. woottoni* cysts from March Air Reserve Base pool 13, CNLM Pool 2, and LAX pools EW2 and EW14. Adult shrimp were not observed at Ramona Airport pool T or at JllE West Pool during the wet season. *Streptocephalus woottoni* cyst densities varied from 0 (Saddleback Meadows B) to 17 (Rancho California Road Pools) cysts/ml of soil but averaged 1.52 cysts/ml. Broken cysts accounted for over half (58%) of the cysts sorted and overall variability in the proportion of broken cysts across sites ranged from 12% (Skunk Hollow) to 100% (LAX pool EW2, Otay Mesa J29, and Australia). The proportion of broken cysts varied independently of the soil collection date. The presence of cladocerans (water fleas) and ostracods were noted during cyst sorting.

Table 3. Summary data for wet season sampling. A total of 7 visits were made to 5 pools from 4 pool complexes in 2010. Adult *S. woottoni* were observed at J15 (Arnie's Point) pools A and B and Chiquita Ridge.

County	Pool Complex	Pool	Date	Personnel	Latitude	Longitude	Datum	Max Pool Depth (cm)	Mean Pool Depth (cm)	Pool Width (m)	Pool Length (m)	Pool Area (m^2)	Conductivity (us)	TDS (ppm)	Salinity (ppt)	pH	Temperature (°C)	Turbidity (% visibility)	Sweep Length (m)	Sweep Volume (cm^3)	# *S. woottoni* (Female, Male)	# *Branchinecta* sp. (Female, Male)	Notes Summary
San Diego north	Ramona Airport	T	2/11/2010	MEL	33.039105	-116.9219933	WGS84	18	6	8	23	160	91.7	65.2	51.9	9.1	21.9	90%	8	1409	0	0	20% water surface covered with veg along periphery, uplands grassy, >500 tadpoles, Cladocerans and osctracods, thin algae covering pool bottom
San Diego north	Ramona Airport	T	3/4/2010	MEL, SAH	33.039105	-116.9219933	WGS84	22	10	10	37	370	91.6	65.8	48	7.7	12.8	90%	2	352.4	0	15 (13, 2)	GPS locality provided by FWS is in a drainage that potentially connects with the measured pool in a wet year
San Diego south	J15 (Arnie's Point)	A	3/10/2010	MEL, SAH	32.546548	-116.998655	WGS84	20	12	13	22	250	231	165	104	8.49	15.5	10%	88	15506	10 (8, 2)	3 (3, 0)	Water muddy/silty, warm, many smaller and more shallow pools in complex
San Diego north	Ramona Airport	T	4/14/2010	MEL, SAH	32.546735	-116.9982017	WGS84	dry	dry	dry	dry	dry	dry	dry	dry	dry	dry	dry	dry	dry	dry	dry	Dry
San Diego south	J11E (Slump Pools)	West	4/14/2010	MEL, SAH	32.547447	-117.014813	WGS84	40	20	60	25	1200	304	216	135	7.64	18.1	25%	n/a	n/a	0	>32 (30, 2)	Difficult to navigate/see with dense grassy vegetation; visual detection ~40mins by 2 people
Riverside	Chiquita Ridge		3/11/2010	MEL, JS	33.553123	-117.622365	WGS84	18	14	60	40	200	217	154	95.9	7	14.8	100%	n/a	n/a	>100	>100	Visual detection; tadpoles, pool drying up, lots of vegetation in pool including upland plants such as mustard and invasive grasses
San Diego south	J15 (Arnie's Point)	B	4/14/2010	MEL, SAH	32.546735	-116.9982017	WGS84	33	20	10	10	80	521	368	230	7.92	14.8	10%	8	2374	121 (95, 26)	0	<50 HYRE tadpoles, very silty/muddy, minimal vegetation

MEL= Megan Lahti (USGS), SAH= Stacie Hathaway (USGS), JS= Jonathan Snapp-Cook (USFWS)

Table 4. Cyst sorting and analysis data. During cyst sorting, the number of intact cysts and broken cysts was recorded, as well as the presence of cladocerans and ostracods. Soil samples were collected up to 19 years ago (1991) and as recently as 1 year ago (2009). For each site, we extracted cysts and recorded the rate of PCR failure.

Sites sampled																		
Region	**Pool Complex**	**Pool**	**Species**	**# Intact Cysts**	**# Broken Cysts**	**Proportion broken**	**Sorted Soil Volume (ml)**	**Intact Cyst Density (cysts/ml)**	**Date Collected**	**Date Sorted**	**Sorter**	**Cladocerans**	**Ostracods**	**# Cysts Extracted**	**# PCRs**	**# of Samples that Failed to PCR**	**Proportion Failed PCR Samples**	**Cysts Remaining**
Orange	Saddleback Meadows	A	*S. woottonii*	30	13	0.30	440	0.07	11/20/2009	3/30/2010	YM			6	6	1	0.17	yes
Orange	Saddleback Meadows	B	*S. woottonii*	2	5	0.71	1000	0.00	11/20/2009	4/26/2010	YM			2	2	0	0.00	no
Orange	Saddleback Meadows	D	*S. woottonii*	32	37	0.54	200	0.16	11/20/2009	5/4/2010	YM			8	8	4	0.50	yes
Orange	Saddleback Meadows	E	*S. woottonii*	44	345	0.89	40	1.10	11/20/2009	5/10/2010	YM			5	5	1	0.20	yes
Orange	Saddleback Meadows	I	*S. woottonii*	30	86	0.74	200	0.15	11/20/2009	5/11/2010	YM			8	8	4	0.50	yes
Los Angeles	LAX*	EW2	*S. wootonii*	0	0	n/a	40	n/a	7/18/2005	6/28/2010	YM			0	n/a	n/a	n/a	n/a
			Branchinecta sp.	0	8	1.00	40	0.00										
Los Angeles	LAX*	EW14	*S. wootonii*	0	0	n/a	40	n/a	7/18/2005	6/28/2010	YM			0	n/a	n/a	n/a	n/a
			Branchinecta sp.	8	150	0.95	40	0.20										
Riverside	Australia Pool		*S. woottonii*	33	193	0.85	480	0.07	12/3/2009	5/25/2010	YM	x	x	5	5	1	0.20	yes
			Branchinecta sp.	0	414	1.00	480	0.00										
Riverside	CNLM	2	*S. woottonii*	0	0	n/a	120	n/a	12/3/2009	4/26/2010	YM			n/a	n/a	n/a	n/a	n/a
Riverside	CNLM	Stock Pond	*S. woottonii*	5	27	0.84	1000	0.01	12/3/2009	4/26/2010	YM			2	2	0	0.00	no
Riverside	March Air Reserve Base	13	*Streptocephalus sp.*	1	1	0.50	1300	0.00	12/3/2009	3/2/2010	YM			0	n/a	n/a	n/a	no
			Branchinecta sp.	316	899	0.74	1300	0.24										
Riverside	Rancho California Road Pool		*S. woottonii*	680	722	0.51	40	17.00	12/3/2009	1/5/2010	YM			13	13	6	0.46	yes
Riverside	Schau Pools	Conserved Pool	*S. woottonii*	30	115	0.79	120	0.25	12/3/2009	6/1/2010	YM			6	6	0	0.00	yes
Riverside	Skunk Hollow (Sample 1)		*S. woottonii*	146	20	0.12	40	3.65	6/11/2004	9/24/2009	MEL	x		17	17	5	0.29	yes
			Branchinecta sp.	82	15	0.15	40	2.05										
Riverside	Skunk Hollow (Sample 2)		*S. woottonii*	132	98	0.43	120	1.10	6/11/2004	10/22/2009	YM			10	10	5	0.50	yes
			Branchinecta sp.	139	106	0.43	120	1.16										
Riverside	Skunk Hollow (Sample 3)		*S. woottonii*	196	210	0.52	200	0.98	12/3/2009	12/23/2009	YM			0	n/a	n/a	n/a	yes
			Branchinecta sp.	52	216	0.81	200	0.26										
San Diego-N	MCAS Miramar AA1	12	*S. woottonii*	30	552	0.95	280	0.11	11/23/2009	6/17/2010	YM		x	7	7	0	0.00	yes
			Branchinecta sp.	55	713	0.93	280	0.20										
San Diego-N	MCAS Miramar AA1	13	*S. woottonii*	6	138	0.96	520	0.01	11/23/2009	6/28/2010	YM			4	4	0	0.00	no
			Branchinecta sp.	129	1533	0.92	520	0.25										
San Diego-S	Otay Mesa J26-29		*S. woottonii*	106	136	0.56	40	2.65	10/28/1999	10/28/2009	YM			8	8	6	0.75	no
			Branchinecta sp.	6	35	0.85	40	0.15										
San Diego-S	Otay Mesa J29-30 (Sample 1)*	J29	*S. woottonii*	187	125	0.40	40	4.68	11/15/1991	10/27/2009	YM			15	15	9	0.60	yes
			Branchinecta sp.	29	59	0.67	40	0.73										
San Diego-S	Otay Mesa J29-30 (Sample 2)*	J29	*S. woottonii*	0	8	1.00	520	0.00	12/17/2003	10/20/2009	YM	x	x	0	n/a	n/a	n/a	n/a
			Branchinecta sp.	995	791	0.44	520	1.91										
San Diego-S	Otay Mesa J29-30	J30	*S. woottonii*	231	70	0.23	120	1.93	10/25/1999	9/25/2009	YM	x		19	19	4	0.21	yes
			Branchinecta sp.	6	13	0.68	120	0.05										
San Diego-S	Otay Mesa Lonestar	Lonestar	*S. woottonii*	53	142	0.73	40	1.33	11/19/2009	12/14/2009	YM			10	10	3	0.30	yes
			Branchinecta sp.	77	103	0.57	40	1.93										
San Diego-S	Otay Mesa SR125	32	*S. woottonii*	31	11	0.26	360	0.09	11/19/2009	12/9/2009	YM	x		10	10	3	0.30	yes
			Branchinecta sp.	205	221	0.52	360	0.57										

Table 4 continued.

Region	Pool Complex	Pool	Species	# Intact Cysts	# Broken Cysts	Proportion broken	Sorted Soil Volume (ml)	Intact Cyst Density (cysts/ml)	Date Collected	Date Sorted	Sorter	Cladocerans	Ostracods	# Cysts Extracted	# PCRs	# of Samples that Failed to PCR	Proportion Failed PCR Samples	Cysts Remaining
Sites not sampled but soil provided by M. Simovich																		
Riverside	Johnson Ranch	2	*S. woottonii*	61	13	0.18	120	0.51	7/11/2000	10/26/2009	YM			19	19	7	0.37	yes
Riverside	Redhawk*	3-1	*S. woottonii*	153	54	0.26	80	1.91	11/1/2005	11/18/2009	YM			13	13	3	0.23	yes
Riverside	Redhawk*	9-1	*S. woottonii*	30	17	0.36	400	0.08	11/1/2005	11/20/2009	YM			13	13	7	0.54	yes
Riverside	Redhawk*	11-5	*S. woottonii*	37	48	0.56	320	0.12	11/1/2005	11/10/2009	YM			13	13	4	0.31	yes
			Branchinecta sp.	0	1	1.00	320	0.00										
San Diego-N	Camp Pendleton 67	O	*S. woottonii*	5	2	0.40	40	0.13	6/17/2004	8/14/2004	M. Kowalski	x	x	4	4	1	0.25	no
San Diego-N	Camp Pendleton 67	P	*S. woottonii*	26	4	0.15	80	0.33	6/17/2004	8/20/2004	K. Davis	x	x	9	9	0	0.00	no
San Diego-N	Camp Pendleton 67	Q	*S. woottonii*	20	0	0.00	40	0.50	6/17/2004	8/19/2004	K. Davis	x	x	15	15	1	0.07	no
San Diego-N	Camp Pendleton 67	V	*S. woottonii*	9	2	0.22	40	0.23	6/17/2004	8/24/2004	J. Garcia			6	6	0	0.00	no
San Diego-N	Camp Pendleton 68	E	*S. woottonii*	23	1	0.04	40	0.58	6/18/2008	7/9/2008	M. Abcede			8	8	0	0.00	no
San Diego-N	Camp Pendleton 68	X	*S. woottonii*	26	1	0.04	40	0.65	6/17/2004	8/24/2004	J. Garcia	x		13	13	1	0.08	no
San Diego-N	Camp Pendleton 89	D	*S. woottonii*	48	0	0.00	40	1.20	6/22/2004	10/10/2004	J. Ahner		x	11	11	0	0.00	yes
Riverside	Clayton Ranch*†		*S. woottonii*						9/26/2003	11/30/2009	F.Wegscheider			19	19	3	0.16	yes
Riverside	Schleuniger Pool†		*S. woottonii*						11/14/2009	11/17/2009	F.Wegscheider			3	3	1	0.33	no
Soil Samples collected but not sorted and/or analyzed																		
San Diego-S	Otay Mesa East	79	soil not sorted						11/19/2009	n/a								
San Diego-S	Otay Mesa East	86	soil not sorted						11/19/2009	n/a								

* Pools are extirpated

† cysts provided by Frank Wegscheider and Tony Bomkamp

YM= Yara Matta (USD), MEL = Megan Lahti (USGS)

DNA Extractions and Amplifications

In total, we successfully obtained samples for DNA analysis from 32 pools representing 20 pool complexes (Figure 1). We sequenced 5 to 10 individuals, including both cysts and adults, from each pool whenever possible. But for some sites, the number of cysts or adult shrimp that could successfully be sequenced was a limiting factor. Often, DNA from cyst extractions was negligible, degraded, or failed to sequence.

In some cases soil is being stored for long periods of time to facilitate future restoration or mitigation efforts. Therefore, we examined the failure rate of PCR amplification by collection year. Although not a definitive test of cyst viability, amplification rate may provide an indirect measure of cyst viability, since amplification failure can be caused by DNA degradation. The amplification failure rate of cysts varied across sites and showed a positive correlation with time since soil collection (Spearman Rank-Order Correlation Coefficient: $n = 31$, $df = 29$, $r_s = 0.528$, $\text{p-value}_{(1\text{-tailed})} = 0.0005$; Figure 2). Other factors that may have affected PCR amplification rates could include genetic variability at the primer binding sites that impeded priming, variation in extraction and PCR chemicals and technical error. Detected genetic variation was very low overall and failed PCR reactions were always repeated multiple times, often until the template DNA was depleted. Furthermore, we would anticipate that amplification failures due to these causes would not be correlated with cyst age. Increased amplification failure rates in older samples strongly suggest DNA degradation and may indicate loss of viability in samples stored for long periods of time. We recommend that direct cyst viability experiments be conducted for soils and cysts stored for mitigation purposes.

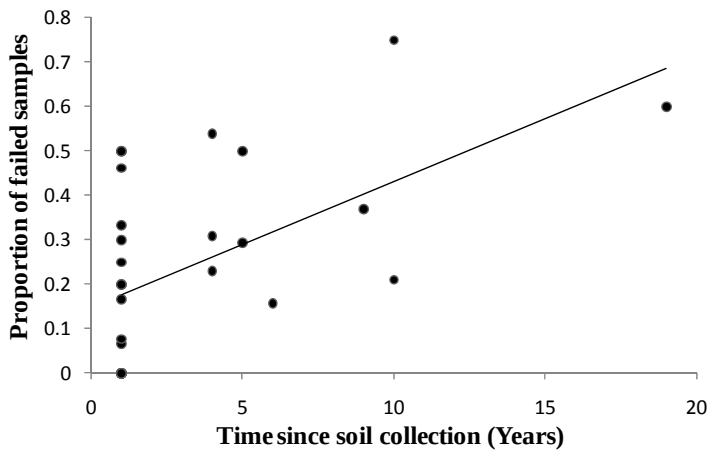


Figure 2. Scatterplot showing a positive correlation between soil age and the proportion of cysts that failed to amplify. Significance of correlation (p=0.0085) suggests that cyst DNA may degrade with time in storage.

Genetic Results

In total we sequenced 179 individuals from 32 pools comprising 20 pool complexes throughout most of the species' range (Figure 1; Table 5). At the time of this report, we were unable to obtain cysts or adults from the northern-most portion of the range, in Los Angeles and Ventura Counties, or from the southern-most localities known in Baja California, Mexico.

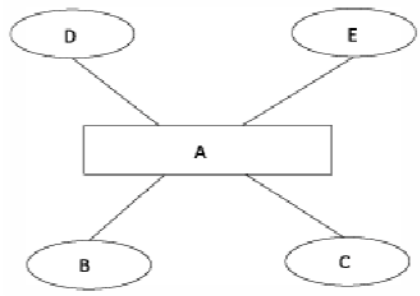


Figure 3. Haplotype network for *S. woottoni*. Throughout its range, *S. woottoni* shows low haplotype variability and all of the variation occurs in San Diego County.

The mtDNA COI sequences were clipped and 647 bases were analyzed for each *S. woottoni* sample. Genetic variability was very low overall. We detected five unique haplotypes, with pairwise sequence divergence among haplotypes ranging from 0.15% to 0.3% (1 to 2 base changes, Figure 3). Haplotype A was the most common, found in all regions and in all but two pools sampled. Haplotype B was limited to Otay Mesa pools in south San Diego County. Haplotype C was a singleton found in the Lonestar pool in Otay Mesa. Haplotype D was limited to pool 89D on Camp Pendleton, and haplotype E was a singleton found in Camp Pendleton pool 68X (Table 5). All genetic variability was limited to San Diego County, and all sampled pools in Riverside and Orange Counties were fixed for haplotype A.

The amount of genetic variation at this locus is low compared to that reported in other population level studies of fairy shrimp. The co-occurring endangered species, *Branchinecta sandiegonensis*, the San Diego fairy shrimp, exhibited much higher genetic diversity at the same COI gene region, with 50 unique haplotypes found across 30 sampled pool complexes (Bohonak 2005). Average pairwise divergence across *B. sandiegonensis* haplotypes was 1.65% and maximum pairwise divergence reached 3.04% (Bohonak 2005). Similarly, in a genus-wide study of *Streptocephalus*, the lowest levels of intraspecific sequence divergence in the mtDNA 12S rRNA gene were reported in *S. woottoni* (0.31%), as compared to 0.9% in *S. namibiensis*, 9.32% in *S. texanus*, 11.49% in *S. purcelli*, 12.02% in *S. mackini* and 13.07% in *S. dorothae* (Daniels et al. 2004). Several processes may explain low variation within *S. woottoni*. First, the species may have undergone a recent range expansion into its current range. Second, the species may have undergone a recent selective sweep at the mtDNA locus. Third, mutation rates in the COI gene may be orders of magnitude lower in *S. woottoni* than other *Streptocephalus* or co-occurring *Branchinecta* species. Genetic signature of a recent range expansion in *S. woottoni* is consistent with the occurrence of this species almost entirely in modified pools. Based on our observations in this and other surveys, and previous published studies of *S. woottoni*, almost all currently known localities of this species are pools that have been deepened or enhanced as wallows or stock ponds (Eng et al. 1990, Simovich and Fugate 1992, Brown et al. 1993). Previously, other authors have noted that the range of other *Streptocephalus* species may be expanding due to the creation of man-made temporary pond habitats (Belk 1977, Wiman 1979). Widespread creation and modification of pools into cattle tanks in southern California likely coincided with the spread of ranching, after Spanish colonization in the 1700s.

Table 5. mtDNA COI haplotype frequencies for *S. woottoni* throughout southern California. Low haplotype diversity was detected overall, although genetic structure among pools was evident among pools in San Diego County.

				Haplotype Frequencies				
County/Region	**Complex**	**Pool**	**N**	**A**	**B**	**C**	**D**	**E**
Orange	Chiquita Ridge	-	5	1.00				
Orange	Saddleback	A	5	1.00				
Orange	Saddleback	B	2	1.00				
Orange	Saddleback	D	4	1.00				
Orange	Saddleback	E	4	1.00				
Orange	Saddleback	I	4	1.00				
Riverside	Australia	-	4	1.00				
Riverside	Clayton Ranch*	-	16	1.00				
Riverside	CNLM stock pond	-	2	1.00				
Riverside	Johnson Ranch	2	10	1.00				
Riverside	Rancho California	-	3	1.00				
Riverside	Redhawk*	11-5	2	1.00				
Riverside	Redhawk*	3-1	4	1.00				
Riverside	Redhawk*	9-1	3	1.00				
Riverside	Schau	-	5	1.00				
Riverside	Schleuniger Pool	-	2	1.00				
Riverside	Skunk Hollow	-	10	1.00				
San Diego-N	Camp Pendleton 67	O	3	1.00				
San Diego-N	Camp Pendleton 67	P	8	1.00				
San Diego-N	Camp Pendleton 67	Q	11	1.00				
San Diego-N	Camp Pendleton 67	V	6	1.00				
San Diego-N	Camp Pendleton 68	E	8	1.00				
San Diego-N	Camp Pendleton 68	X	8	0.88				0.12
San Diego-N	Camp Pendleton 89	D	11				1.00	
San Diego-N	MCAS Miramar AA1	12S	5	1.00				
San Diego-N	MCAS Miramar AA1	13S	4	1.00				
San Diego-S	Otay Mesa J15 Arnie's Point	B	5	1.00				
San Diego-S	Otay Mesa J26	J26	2		1.00			
San Diego-S	Otay Mesa J29-30*	J29	6	0.33	0.67			
San Diego-S	Otay Mesa J29-30	J30	10	0.10	0.90			
San Diego-S	Otay Mesa Lonestar	-	4	0.25	0.50	0.25		
San Diego-S	Otay Mesa J2 (SR125 mit)	32	3	1.00				
* Extinct pool complexes, cysts sampled from preserved soil.								

The Bayesian phylogenetic analysis revealed that all *S. woottoni* haplotypes are more closely related to each other than to the other sampled North American species, with a posterior probability of 1.0. This suggests that *S. woottoni* is a monophyletic species in comparison to the sampled taxa; however, low posterior probabilities at most higher nodes make it difficult to assess its relationship to other *Streptocephalus* species (Figure 4). Additional gene and outgroup sampling may help to resolve these relationships.

Despite low levels of genetic variability, haplotype frequencies among complexes differed. The AMOVA showed that most genetic variability was partitioned among pool complexes (64.65%), with 18.01% partitioned among regions (Table 6). Pool complexes were significantly differentiated from one another (F_{ST} = 0.789), and contained significantly high levels of genetic variation within them (F_{IS} = 0.826), suggesting that some pools within complexes also vary in haplotype frequencies (Table 7). Pairwise comparisons among pool complexes show that Otay Mesa Lonestar, J26, and J29-30 and Camp Pendleton 89 are significantly differentiated from most other pool complexes (Table 8).

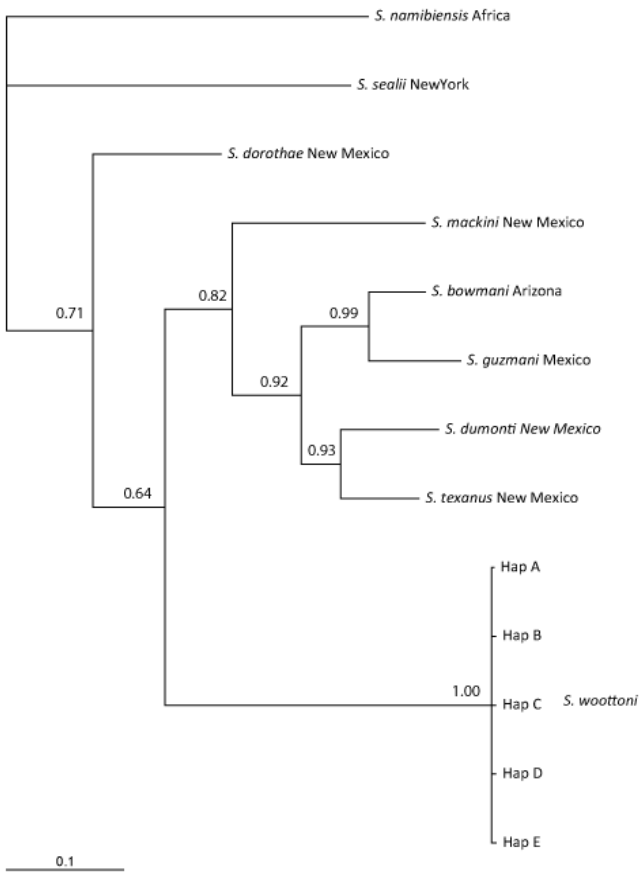


Figure 4. Bayesian phylogenetic tree for North American *Streptocephalus*. Posterior probabilities ≥0.95 are considered highly supported. *Streptocephalus woottoni* is monophyletic although its relationship to other *Streptocephalus* species is not well resolved.

Lack of geographically extensive variation in the mtDNA COI gene across the range should not be taken as definitive evidence that populations are currently connected by high levels of gene flow. On the contrary, in areas where genetic variation was detected, haplotype frequencies varied significantly across even geographically proximate pools, suggesting limited gene flow.

Limited gene flow may allow for adaptation to local pool conditions and cautions against moving cysts among pool complexes for restoration activities. An additional consideration is that our current genetic information is from a single mtDNA gene. Mitochondrial DNA variation may not be indicative of processes in the nuclear genome. Further genetic analysis of nuclear markers is warranted to address whether genetic differentiation exists in the northern portion of the species range and to differentiate between possible signatures of selective sweeps or range expansion in mtDNA. Preliminary screening of nuclear genetic markers conducted in the Bohonak laboratory suggests that ISSRs (Inter Simple Sequence Repeats) may be variable across the species range. Single nucleotide polymorphisms (SNPS) or microsatellite markers would also be appropriate marker choices for further determining population genetic structure.

Table 6. AMOVA analysis showing the partitioning of genetic variation among pools based on geographic locality.

Source of Variation	d.f.	Sum of Squares	Variance Components	% of Variation
Among Regions	3	8.647	0.02979	18.01
Among Complexes within Region	16	13.126	0.10696	64.65
Within Complexes	159	4.562	0.02869	17.34
Total	178	26.335	0.16545	

Table 7. Genetic structure within and among populations of *S. woottoni.* F_{CT} is a measure of differentiation among regions, F_{ST} is a measure of differentiation among complexes within regions, and F_{IS} is a measure of differentiation among individuals within a complex.

Fixation Indices	F-statistic	p-value
F_{CT} (among regions)	0.18006	0.1388
F_{ST} (among complexes within region)	0.78848	$\leq$ 0.0000
F_{IS} (within complexes)	0.82656	$\leq$ 0.0000
*Significance tested with 10,100 permutations		

Table 8. Population pairwise F_{ST} among sampled pool complexes.

Pool Complex	Chiquita Ridge	Saddleback	Australia Pool	Clayton Ranch	CNLM Stock Pond	Johnson Ranch	Rancho California	Redhawk	Schau	Schleuniger	Skunk Hollow	Miramar AA1	C. Pend 67	C. Pend 68	C. Pend 89	Arnies Point	Lonestar	J26	J29-30
Chiquita Ridge	-																		
Saddleback	0	-																	
Australia Pool	0	0	-																
Clayton Ranch	0	0	0	-															
CNLM Stock Pond	0	0	0	0	-														
Johnson Ranch	0	0	0	0	0	-													
Rancho California	0	0	0	0	0	0	-												
Redhawk	0	0	0	0	0	0	0	-											
Schau	0	0	0	0	0	0	0	0	-										
Schleuniger	0	0	0	0	0	0	0	0	0	-									
Skunk Hollow	0	0	0	0	0	0	0	0	0	0	-								
Miramar_AA1	0	0	0	0	0	0	0	0	0	0	0	-							
C. Pend 67	0	0	0	0	0	0	0	0	0	0	0	0	-						
C. Pend 68	-0.0687	0.11848	-0.10891	0.09312	-0.31765	0.02946	-0.17483	0.01562	-0.0687	-0.31765	0.02946	0.01562	0.22995	-					
C. Pend 89	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0.90705**	-				
Arnies Point	0	0	0	0	0	0	0	0	0	0	0	0	0	-0.0687	1	-			
Lonestar	**0.28571**	**0.63285**	0.22222	**0.59199**	-0.01818	**0.47269**	0.13402	**0.44493**	**0.28571**	-0.01818	**0.47269**	**0.44493**	**0.76493**	**0.30435**	**0.8247**	**0.28571**	-		
J26	**1**	**1**	1	**1**	1	**1**	1	**1**	**1**	1	**1**	**1**	**1**	**0.8124**	**1**	**1**	-0.01818	-	
J29-30	**0.70944**	**0.8152**	**0.69524**	**0.8**	0.65343	**0.76012**	**0.67785**	**0.75172**	**0.70944**	0.65343	**0.76012**	**0.75172**	**0.87056**	**0.68258**	**0.89403**	**0.70944**	0.15823	-0.19885	-
SR125 Mitigation	0	0	0	0	0	0	0	0	0	0	0	0	0	-0.17483	1	0	0.13402	1	0.67785

bold indicates p-values < 0.05

SUMMARY OF CONCLUSIONS AND RECOMMENDATIONS

1. Our site visits revealed some locations where pools previously listed as occupied by USFWS now appear to be absent or graded, or were not typical of *S. woottoni* habitat and *S. woottoni* was not observed. Efforts should be made to verify *S. woottoni* presence at these sites, visit all remaining sites, and update the current status of all known inhabited pool locations. Furthermore, because of the ephemeral nature of these pools, efforts should be made to annually monitor sites for filling and presence of adult shrimp.

2. Our PCR failure rates were significantly greater for cysts stored for longer periods of time. This may indicate a loss of cyst viability over time. We recommend that more definitive viability testing (such as hatching experiments) be conducted on soils stored for long periods of time for mitigation and restoration purposes.

3. *Streptocephalus woottoni* represents a monophyletic taxon (i.e. a unique species) in this dataset.

4. All detected population genetic variability was located in San Diego County pools, namely those surveyed on Camp Pendleton (San Diego north) and Otay Mesa (San Diego south). These geographic regions in particular should continue to be protected in order to preserve known genetic diversity.

5. Significant genetic differentiation was detected among some pool complexes on Otay Mesa and Camp Pendleton, suggesting that gene flow (defined as dispersal and successful breeding) is limited among even geographically proximate pool complexes.

6. Genetic variation in the surveyed gene was low compared to that found in other co-distributed taxa and congeners. Additional nuclear markers should be developed and studied in this species to determine whether population genetic structure exists in the northern portion of the species range, and to differentiate between signatures of range expansion or selective sweeps in mtDNA.

REFERENCES

Belk, D. 1977. Zoogeography of the Arizona fairy shrimps (Crustacea: Anostraca). Journal of the Arizona Academy of Science **12**:70-78.

Bohonak, A. J. 1998. Genetic population structure of the fairy shrimp Branchinecta coloradensis (Anostraca) in the Rocky Mountains of Colorado. Canadian Journal of Zoology **76**:2049-2057.

Bohonak, A. J. 2005. Conservation genetics of the endangered fairy shrimp species Branchinecta sandiegonensis. Appendix to MSCP Vernal Pool Inventory., City of San Diego and US Fish and Wildlife Service.

Brown, J. W., H. A. Wier, and D. Belk. 1993. New Records of Fairy Shrimp (Crustacea: Anostraca) from Baja California, Mexico. The Southwestern Naturalist **38**:389-390.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: A computer program to estimate gene genealogies. Molecular Ecology **9**:1657-1659.

Daniels, S. R., M. Hamer, and C. Rogers. 2004. Molecular evidence suggests an ancient radiation for the fairy shrimp genus Streptocepalus (Branchiopoda: Anostraca). Biological Journal of the Linnean Society **82**:313-327.

Davies, C. P., M. A. Simovich, and S. A. Hathaway. 1997. Population of genetic structure of a California endemic branchiopod, *Branchinecta sandiegonensis*. Hydrobiologia **359**:149-158.

Eng, L. L., D. Belk, and C. H. Eriksen. 1990. California Anostroca: distribution, habitat and status. Journal of Crustacean Biology **10**:247-277.

Ericksen, C. and D. Belk. 1999. Fairy Shrimps of California's Puddles, Pools, and Playas. Mad River Press, Eureka, California.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology **3**:294-299.

Frankham, R. 2005. Stress and adaptation in conservation genetics. Journal of Evolutionary Biology **18**:750-755.

Fugate, M. L. 1998. Branchinecta of North America: population structure and its implications for conservation practice. Pages 140-146 *in* C. W. Witham, E. T. Bauder, D. Belk, W. R. Ferren, Jr., and R. Ornduff, editors. Ecology, conservation and management of vernal pool ecosystems. California Native Plant Society, Sacramento

Hathaway, S. A. and M. A. Simovich. 1996. Factors affecting the distribution and co-occurrence of two southern Californian anostracans (Branchiopoda), *Branchinecta sandiegonensis* and *Streptocephalus woottoni*. Journal of Crustacean Biology **16**:669-677.

Huelsenbeck, J. P. and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics **17**:754-755.

Ketmaier, V., D. Pirollo, E. De Matthaeis, R. Tiedemann, and G. Mura. 2008. Large-scale mitochondrial phylogeography in the halophilic fairy shrimp Phallocryptus spinosa (Milne-Edwards, 1840) (Branchiopoda: Anostraca). Aquatic Sciences **70**:65-76.

Nylander, J. A. A., F. Ronquist, J. P. Huelsenbeck, and J. L. Nieves-Aldrey. 2004. Bayesian phylogenetic analysis of combined data. Systematic Biology **53**:47-67.

Posada, D. 1999. Collapse. Dept. of Zoology, Brigham Young University, Provo, UT.

Rodríguez, F., J. F. Oliver, A. Marín, and J. R. Medina. 1990. The general stochastic model of nucleotide substitution. Journal of Theoretical Biolology **142**:485-501.

Simovich, M. A. and M. L. Fugate. 1992. Branchiopod diversity in San Diego County, California, USA. Transactions of the Western Section of the Wildlife Society **28**:6-14.

Simovich, M. A. and S. A. Hathaway. 1997. Diversified bet-hedging as a reproductive strategy of some ephemeral pool anostracans (Branchiopoda). Journal of Crustacean Biology **17**:38-44.

Steele, A. N., M. A. Simovich, D. Pepino, K. McNamara Schroeder, A. G. Vandergast, and A. J. Bohonak. 2009. Optimization of DNA extraction methods for encysted embryos of the endangered fairy shrimp, *Branchinecta sandiegonensis*. Conservation Genetics **10**:1777-1781.

U.S. Fish and Wildlife Service. 1998. Vernal Pools of southern California Recovery Plan. U.S. Fish and Wildlife Service, Portland, Oregon.

U.S. Fish and Wildlife Service. 2008. Riverside fairy shrimp (Streptocephalus wootoni) 5-year review: summary and evaluation. Calsbad FWO, Carlsbad, CA.

Weir, B. S. and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution **38**:1358-1370.

Wilcox, T. P., D. J. Zwickl, T. A. Heath, and D. M. Hillis. 2002. Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. Molecular Phylogenetics and Evolution **25**:361-371.

Wiman, F. 1979. Hybridization and detection of hybrids in the fairy shrimp genus *Streptocephalus*. American Midland Naturalist **102**:149-156.

Appendix A

Dry season sampling and cyst sorting protocol for the Riverside fairy shrimp (*Streptocephalus woottoni*)

Soil Collection

We will sample dry vernal pool soils, following standard methods developed by Dr. Simovich to maximize the ability to collect cysts in a given pool. These are as follows:

- For pools of under 9 m^2, we will sample up to 1L.
- For pools between 9 m^2 and 400 m^2, we will sample 2-2.5 L (filling approx. 1/2 gallon ziplog bag).
- For pools greater than 400 m^2, we will sample up to 4 L (filling approx. 1 gallon ziploc bag).

Care will be taken not to take so much soil as to affect the hydrology or cyst bank. Soil will be taken from the deepest spots, areas of cracked soil, or containing algal crust and ostracod shells. We will choose several spots if the pool has multiple low spots (tire tracts etc.) From each spot chosen, several flakes will be lifted using a small hand trowel, approximately 3 to 5 cm thick. The amount of soil and location of soil samples are both important to maximize our ability to collect the desired amount of cysts for genetic study.

Bags will be returned to the lab and stored in dry storage. Bags will *not be sealed tightly* (to avoid mold or steaming). If soils must be taken while still damp, upon return to the lab the bags will be immediately emptied onto plates and soils are allowed to air dry before storage or analysis.

Cyst Sorting

Cysts will removed, identified and counted utilizing the following protocol in the Simovich lab:

1. First soil is homogenized in the collection bag by gently breaking up clumps by hand.
2. Next 40 ml of soil are poured through a 500 µm (No. 35) sieve on top of a 180 µm (No. 80) sieve and gently washed until only the larger grains are left. If ostrocods are present these are noted.
3. The bottom sieve is then removed, and systematically searched for cysts under a dissection microscope.
4. If cysts are present, they are placed on a piece of damp filter paper and set inside a glass vial. The number of cysts placed in a vial is noted.
5. If no cysts are found in the first 40mL, the process is repeated for additional 40 ml aliquots until at least 30 cysts are found, or the bag of soil is depleted. The total amount of soil sieved will be recorded.
6. If multiple genera are noted, (*Branchinecta* and *Streptocephalus*), a separate vial is used for each type.
7. Vials are left open to dry for two days and then capped if no moisture is found.
8. Sieves are thoroughly washed and air dried between samples to avoid cross contamination.

Riverside fairy shrimp genetic study, dry season data sheet 2009–2010

Site: _____ **Date:** _____/_____/_____

Pool ID: _____ **Time:** _____

Collectors: (Lead) _____ **(Others)** _____

GPS lat: _____ **GPS lon:** _____ **datum:** NAD83

Photos (__overall pool __overall vegetation __close-up of soil) Notes: _____

_____

Pool Sketch:
include scale, measurements of pool size (**length/width**), indication of **North**, sample location(s)

Soil Collection Guidelines	
Pool Size	**Material Collected**
< 9m^2	≤ 1L
9 – 400m^2	2 – 2.5L
> 400m^2	≤ 4L

Pool Depth: _____cm (estimated max.)

Pool Length: _____m **Pool Width:** _____m

Surface Area: _____m^2 (estimated max.)

Amount of Soil Collected: _____L

Pool Bottom Surface:
__hardpan __claypan __cobbly/rocky __lava flow __other_____

Vegetation in Pool:
% Bare ground_____ % Vegetated ground_____
% Native_____ % Nonnative_____
--__mulefat __wooly marbles __lolium __Orcutt's grass --list others_____

_____

Habitat Conditions: (check all that apply)
--__undisturbed or __disturbed: __tire tracks __garbage __discing/plowing
--__ungrazed or __grazed: __cattle __horses __sheep __other___

Visible Threats/Land Use: _____

_____

_____

Management Recommendations: _____

_____

_____

Appendix B

Wet season sampling protocol for the Riverside fairy shrimp (*Streptocephalus woottoni*)

Shrimp Collection

Study pools will be checked after each significant rain event and monitored every 10-14 days until adults are detected. Faunal samples, pool physical attributes and water chemistry measurements will be taken at 10-14 day intervals (see attached data sheet). The *Streptocephalus woottoni* populations in each pool will be assessed and the developmental stage and percent of reproductive adults will be noted. When adult *S. woottoni* are noted to be present, fairy shrimp will be sampled using an aquarium or dip net with mesh size less than 1/8th inch. The number of sweeps will be dependent on pool size, and sweeps will be conducted to sample representative portions of the bottom, edges, and vertical water column of the pool. The volume of the sample taken will be determined from the aperture of the net used and the number and length of sweeps. *Streptocephalus woottoni* will be counted and densities calculated for the volume of water sampled.

Up to 3 adult males and 3 female *S. wootoni* will be sampled and retained as museum voucher specimens and up to 10 males will be retained for DNA analysis (but in total, no more than 10% of the population present will be taken from any pool),). Sampled fauna will be anesthetized with carbonated water, preserved in the field with alcohol and returned to the laboratory. In the laboratory, samples will be cleaned of debris and algae and field identifications verified..
Care will be taken to avoid transfer of cysts, soil and other materials from one pool to another. We will remove mud, snails, algae, and other debris from nets, boots, and all other surfaces. We will rinse cleaned items with water before leaving each study site. This cleaning will be done away from the immediate vicinity of ponds, streams, or wetlands. All field equipment will be thoroughly cleaned using a 5% bleach solution followed by a tap water rinse upon return to the laboratory.

RIVERSIDE FAIRY SHRIMP GENETICS STUDY:
Wet Season Sampling Data Sheet (2010)

SITE:_____ Date:_____
Pool ID: _____ Photos:_____ Collectors: _____
GPS_N:_____ GPS_W:_____ Datum:_____
Notes:_____
_____
_____
_____

Pool Sketch:

Pool Depth (Max):_____
Pool Depth (Avg):_____
Pool Width:_____
Pool Length:_____
Pool Area:_____

WATER QUALITY

Conductivity:_____µS
TDS:_____ppm
Salinity: _____ppt
pH:_____
Temperature:_____°C
Turbidity:_____
_____

S. woottoni
Observed:_____
Collected (Females):_____
Collected (Males):_____

Branchinecta sp.
Observed:_____
Collected (Females):_____
Collected (Males):_____

SHRIMP SAMPLING

Net Aperture:_____

Sweep Length:_____
S. woottoni:♀_____♂_____
Branchinecta: ♀_____♂_____

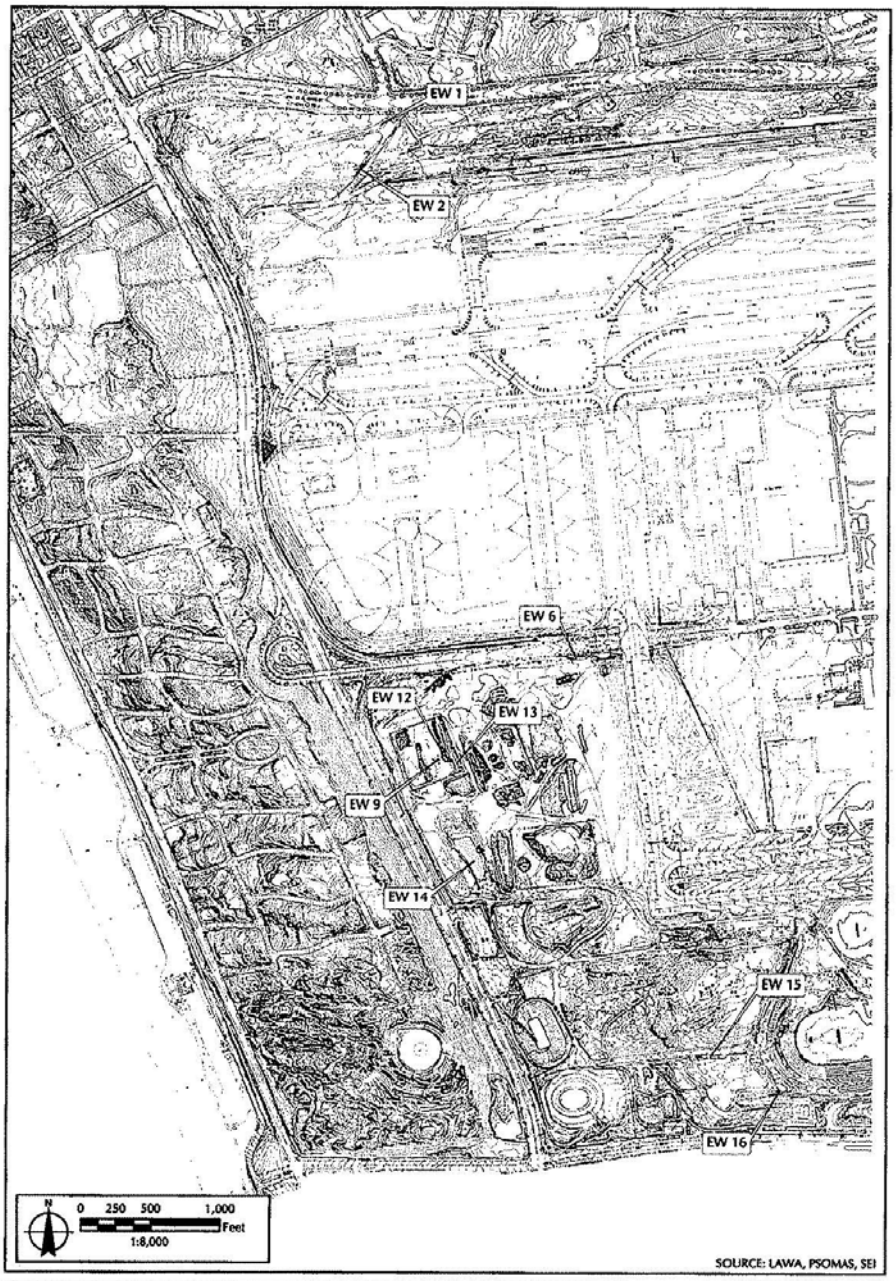
Sweep Length:_____
S. woottoni:♀_____♂_____
Branchinecta: ♀_____♂_____

Sweep Length:_____
S. woottoni:♀_____♂_____
Branchinecta: ♀_____♂_____

Sweep Length:_____
S. woottoni:♀_____♂_____
Branchinecta: ♀_____♂_____

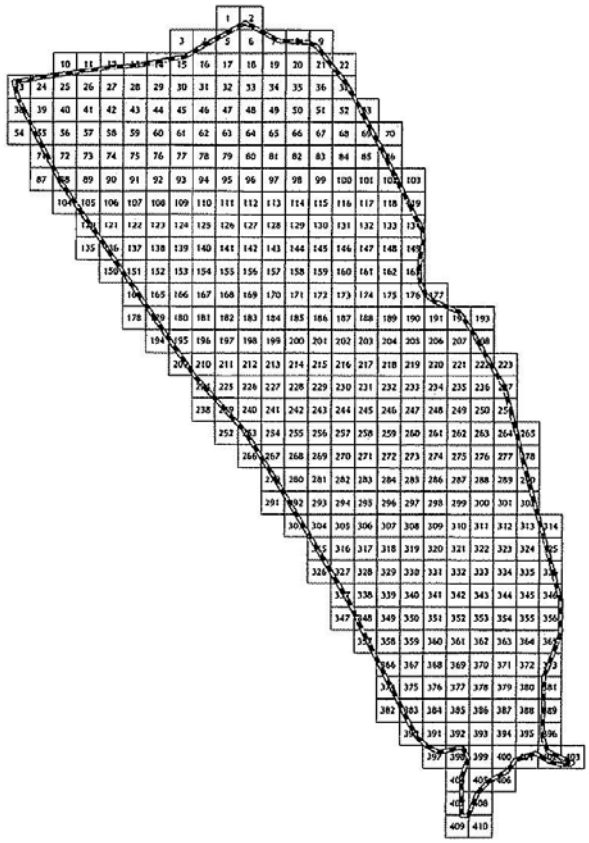
Appendix C

LAX pre-excavation pool localities and soil sample localities from Sapphos Environmental Inc., 2005 Memorandum for the record (MFR).



Ephemerally Wetted Pools

ATTACHMENT A
Ephemerally Wetted Pool Index Map



N

0 250 500 1,000 Feet

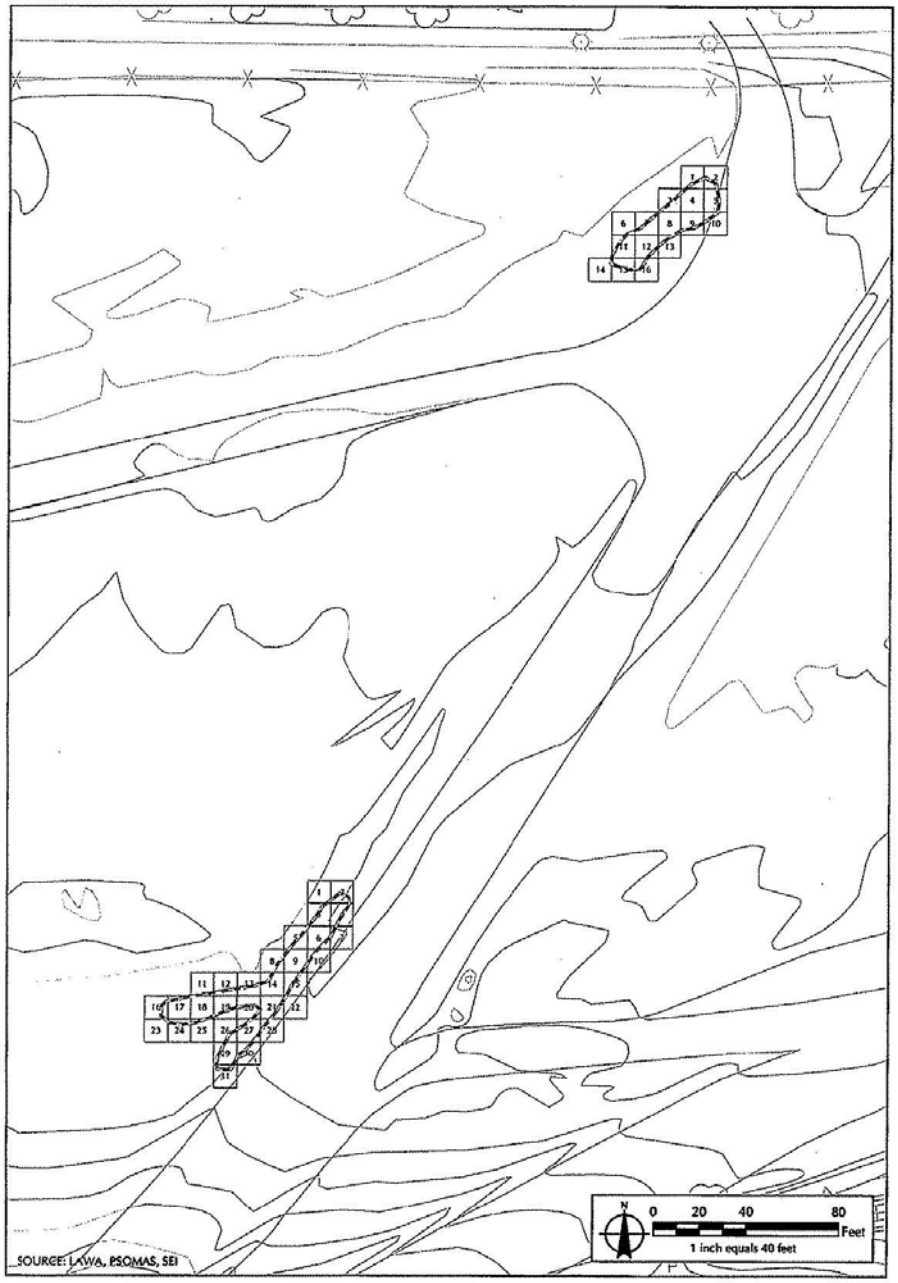
1 inch equals 40 feet

SOURCE: LAWA, PSOMAS, SEI

81 Foot Contour of EW-14

10 Foot Grid

ATTACHMENT A-1
EW-14 Ten Foot Grid Index



EW Boundary 10 Foot Grid

ATTACHMENT A-2
EW-1 and EW-2 Ten Foot Grid Index

Appendix D

Site photos from dry season sampling of the Riverside fairy shrimp (*Streptocephalus woottoni*)

a.) Pool 79, Otay Crossings

b.) Cattle Pond, Media Road

c.) Pool 86 (Map A), Otay Crossings

d.) Cattle Tank, J30

e.) Pool 102, SR125

f.) Pool 31, SR125

g.) Pool 35, SR125

h.) Pool 32, SR125 (pool photo corrupted)

i.) Pool 2, SR125

j.) J26 Prison Complex (outside fence)

k.) Pool E, Saddleback Meadows

l.) Pool I, Saddleback Meadows

m.) Pool D, Saddleback Meadows

n.) Pool B, Saddleback Meadows

o.) Pool A, Saddleback Meadows

p.) Road Pool, Rancho California

q.) Pool AA12, Miramar AFB

r.) Pool AA13, Miramar AFB

s.) Pool 2, CNLM

t.) Skunk Hollow, CNLM

u.) Stock Pond, CNLM

v.) Pool 6, March AFB (C. Galst photo)

w.) Pool 3, March AFB (C. Galst photo)

x.) Pool 13, March AFB (C. Galst photo)

y.) Australia Pool, Lake Elsinore

z.) Conserved Pool, Schau property

Appendix E

Site photos from wet season sampling of the Riverside fairy shrimp (*Streptocephalus woottoni*)

Pools sampled during the wet season from a) Riverside and b-d) San DiegoCounties.

a.) Arnie's Point

b.) West Pool 11E, Slump Pools

c.) Pool T, Ramona Airport

d.) Chiquita Ridge, Rancho Mission Viejo