

APPENDIX H
PRELIMINARY RESULTS OF FAIRY SHRIMP
GENETICS STUDY

MSCP vernal pool inventory
City of San Diego (USFWS)
Conservation genetics of the endangered fairy shrimp species Branchinecta sandiegonensis

Andrew J. Bohonak, Department of Biology, San Diego State University
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This is a preliminary report for the contract “*Genetic testing of the endangered fairy shrimp species Branchinecta sandiegonensis*” to A.J. Bohonak from the City of San Diego. This two year contract was set up in late 2002 and work for the project officially began January 1, 2003.

The information here supersedes that from reports submitted in November and December 2003, but should still be considered preliminary.

Motivation

Worldwide changes in land use (primarily agriculture and urbanization) have led to a global loss of temporary wetlands. In southern California, it is estimated that 95% of the vernal pools have been lost (Bauder 1998 and references therein). The threats to these naturally fragmented habitats are compounded by their inherent natural isolation at both local and regional scales. (Local metapopulations of ponds are found in areas where hydrologic conditions facilitate pool formation. Regionally, these pool complexes are separated kilometers or tens of kilometers by geologically unfavorable terrain.) Contemporary and historical connectivity between pools at these two scales is largely a matter of speculation (Bohonak & Jenkins, 2003). Because the continued loss of vernal pools may interact in complex ways with future climate change, there are many uncertainties concerning the persistence of vernal pool habitats, their associated ecosystem services and the endangered species they maintain (Pyke 2004).

Fairy shrimp (Crustacea: Branchiopoda: Anostraca) are relatively large crustacean zooplankton (> 10 mm) restricted almost entirely to temporary wetlands worldwide. Five Anostracan species are listed on the U.S. Endangered Species list, and several more are under consideration. Over 30 fairy shrimp are considered threatened or endangered worldwide. This project examined population genetic structure in the federally endangered fairy shrimp *Branchinecta sandiegonensis* in order to gain insight into contemporary and historical connectivity among pools and pool complexes, and make conservation recommendations.

To date, only allozymes have been used to study genetic structure in this species (Davies et al. 1997), and there are no DNA-level population genetic studies for any fairy shrimp. Davies et al. (1997) found significant genetic differentiation among 10 pools for *B. sandiegonensis* using allozymes, and evidence for a “temporal Wahlund effect” within pools. (The importance of overlapping generations created by the fairy shrimp cyst bank may be reflected by heterozygote deficiencies within each pond.) The goal of this study is to expand coverage to include the majority of the species range, including all pool complexes on City property. The choice of mitochondrial DNA sequence variation over allozymes for this study reflects the higher degree of precision that can be obtained with mtDNA sequencing. Also, sequence-level variation permits a wider range of analyses that can be used to unravel contemporary and historical processes such as allopatric isolation and gene flow.

Methods

Fairy shrimp were collected as adults or hatched from sediment samples by Marie A. Simovich (University of San Diego), a subcontractee and collaborator on this project. Simovich is permitted by USFWS for work on *B. sandiegonensis*, and Bohonak is listed under that permit. Individuals were collected from across southern California, identified to species according to Eriksen and Belk (1999), and stored in 95% ethanol or at -80° C until analysis. A map containing most of the collection locations is provided in Figure 1. We sampled additional ponds not specifically located on City of San Diego property, so that our results represent the dispersal biology and evolutionary history of this species across its entire range.

Protocol for amplifying a 658 bp portion of the mitochondrial gene cytochrome oxidase I (COI) was adapted from existing lab protocol for arthropods. (Bohonak has developed universal primers similar to LCO-1490 and HCO-2198 of Folmer et al. 1994). PCR products were cycle sequenced using BigDye v. 3 termination (Perkin-Elmer) and sequenced on an ABI 377 automated sequencer. Sequence alignments were conducted by eye using the program Sequencher. (Alignment is largely trivial, since COI is a protein-coding gene, and no insertions or deletions were detected.) Most individuals were cycle sequenced once in each direction.

For the final data set, evolutionary relationships among haplotypes will be determined using maximum parsimony with PAUP 4.0 (Swofford 2001), using Bayesian analysis with Mr. Bayes (Huelsenbeck and Ronquist 2004) and with network parsimony reconstruction as implemented in TCS (Clement et al. 2000). General population genetics summary statistics will be calculated using Arlequin v. 2.0 (Schneider et al. 2001). Geographic patterns will be interpreted using isolation by distance analyses (Bohonak 2002), standard phylogeography and nested clade analysis (Templeton 1998) using GeoDis (Posada et al. 2000). For this preliminary report on the preliminary data set, only a small subset of these analyses is presented.

Results

To date, DNA has been extracted from over 520 individual *B. sandiegonensis* from over 65 pools in 24 “pool complexes”. (A pool complex is a local metapopulation of hydrologically linked pools). An additional 50 fairy shrimp from other species have also been extracted for use in comparative studies and as outgroups. Because data collection will be ongoing for another seven months, I present here a preliminary summary of haplotype distributions within and among pools for 223 individuals that have been sequenced to date.

Haplotype distributions

From the 223 *B. sandiegonensis* sequenced, 39 unique haplotypes (“alleles”) have been found. Each of these alleles is a sequence that differs from all others by one or more base pairs. Table 1 summarizes allele distributions within and among pools, pool complexes and geographic regions. Unfortunately, there may be some confusion in nomenclature: the City’s labeling scheme calls geographic regions “Complexes” and local metapopulation of hydrologically connected pools “Sites”, although I prefer to call a local hydrologically linked set of pools a “complex. For clarity, Table 1 lists pools nested within complexes, nested within regions.

There are two dominant features in this data set. First, the numbers generally fall out along a diagonal, indicating that pool complexes are often fixed for unique haplotypes found nowhere else in the species. There is a high degree of endemism apparent within local groups of hydrologically linked pools, and genetic differentiation among regions is high. This is particularly obvious in areas such as Ramona, Otay Mesa, Otay Lakes and Marron Valley, which have less influence from development and recreation than sites in Mira Mesa and Del Mar.

Second, two groups of haplotypes can be distinguished: “A” and “B”. Haplotypes within group A or B differ from each other by relatively few mutational differences (<1% divergence). Divergence between A and B is much more pronounced (approximately 2.5% between pairs of alleles). This indicates that individuals from Group A and B have been isolated from one another biologically for tens of thousands or perhaps millions of years with little or no dispersal or hybridization.

Sample sizes are too small at this time to make definitive conclusions about variation within pools. However multiple alleles seem to be more prevalent in disturbed pools and those in suburban areas.

Geographic and phylogenetic analysis

A preliminary parsimony analysis was conducted with PAUP (heuristic search, 2000 random additions). Individuals from *B. lynchi*, *B. lindahli* and *B. coloradensis* were used as outgroups. A sample tree (1 of 98 equally parsimonious trees) is presented in Figure 2.

It is clear from this analysis that:

- 1) *B. sandiegonensis* appears to represent a monophyletic taxon (i.e., a “good species” from an evolutionary perspective), but this can only be verified by a complete genetic and morphological analysis of the genus.
- 2) There is considerable genetic variation within this species.
- 3) There is a deep split between clades “A” and “B”. Both clades are reciprocally monophyletic on all 98 maximally parsimonious trees.
- 4) Phylogenetic resolution with clades A and B will require additional analyses (e.g., nested clade analysis).
- 5) Clades A and B have unusual allopatric distributions (outlined in Figure 1), which do not correspond to any known current or past geologic features.
- 6) So far only 3 individual fairy shrimp violate the generalized distributions of clade A and B in Figure 1. (Note the outlying “1”s in Table 1.) Further analysis of these individuals will be undertaken to ensure that there were no contamination problems.

Preliminary conclusions

- The taxonomic status of *Branchinecta sandiegonensis* is uncertain, although it appears to be a “proper” monophyletic species at this time.
- There is high mtDNA divergence among hydrologically linked vernal pool “complexes”.

- It is obvious that gene flow between pool complexes is limited in areas that are less impacted by development and recreation (e.g., vernal pool complexes in Ramona, Otay Mesa, Otay Lakes and Marron Valley).
- Two major clades were found; their distribution does not correspond to any obvious contemporary geographic barrier.
- Evolutionary significant units “ESUs” that should be considered for conservation include the two major clades and individuals pool complexes.
- According to some interpretations of the ESU concept, every pool complex could be considered an ESU worthy of separate consideration. This will require additional genetic analyses with other markers.

Caveats

All conclusions should be considered preliminary at this time. However, the preliminary conclusions regarding low dispersal, high genetic endemism and ESU identification are unlikely to change with additional data. Additional sequences will help refine them quantitatively. The taxonomic status of *Branchinecta coloradensis* is outside the scope of this study and will have to be left for future work.

It is expected that this project will be completed by December 2004, with a final report presented in the spring of 2005. Results of this study will be disseminated to the scientific community through one or more peer-reviewed publications submitted beginning in 2004 or 2005.

Literature cited

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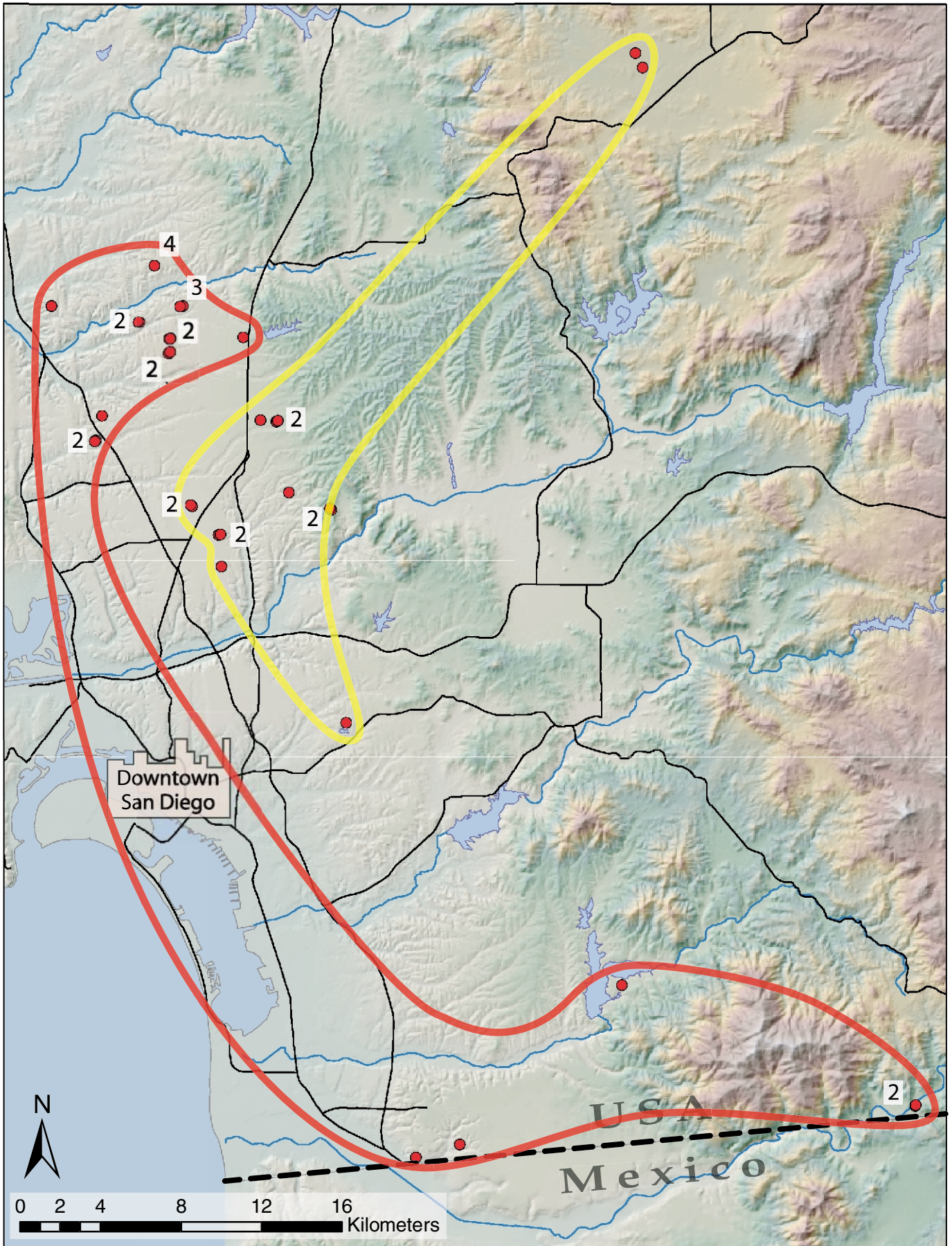
Table 1: Haplotype distributions within and among pool “complexes” (regions) and “sites” (local metapopulations of hydrologically linked pools).

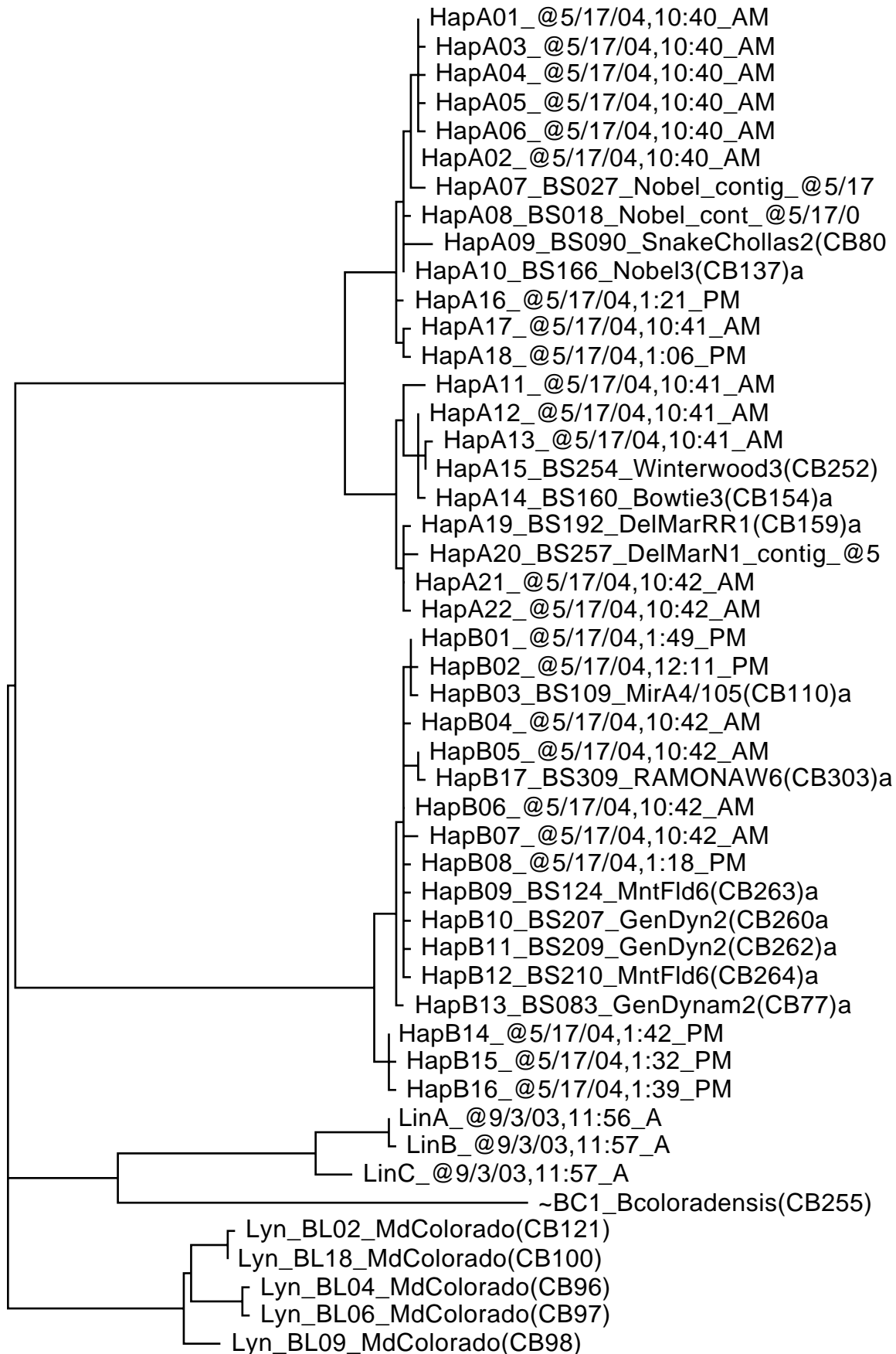
Figure 1: Geographic distribution of samples, with the primary distributions of clades A and B circled. Only 3 of 197 individuals violate these primary distributions (see Table 1). Red dots indicate ponds sampled.

Figure 2: Phylogenetic tree of preliminary data set. This is one of 98 equally parsimonious trees.

-			Haplotype																						
Region	Complex	Pond	A01	A11	A03	A02	A12	A13	A15	A06	A05	A07	A08	A10	A21	A22	A14	A04	A19	A20	A16	A09	A18	A17	
Carmel Mountain	Carmel Mountain	1	4																						
Mira Mesa	Brown	1	1	2																					
		2	3																						
		3	1	1	2																				
	Winterwood	1	2						1																
		2		1		1	1																		
		3	1							1															
		4					1																		
		5	2					1			2														
	Mesa Verde	1	1			4																			
		2	1			3																			
		3	5																						
	Maddox	1	2																						
2		3				1																			
4		3																							
7											2														
Cousins		1	4																						
Nobel Drive	Nobel Drive	1	1		7							1	1												
		2			4																				
		3			2										1										
	Eastgate	1	1													1									
		2			4																				
		4?			2																				
Del Mar	Bowtie	1	1												2	1									
		2													2	1									
		3					1							1		1									
	Del Mar Mesa East	2				1	1																		
		3					1																		
		4		3															1						
		5		1			2												1						
		mult. ruts													1										
		RR1		1			1								1	1			1						
	Del Mar Mesa North	1													1						1				
2		1												1											
3														2											
Otay Lakes	Otay Lakes	1																				5			
Otay Mesa	Snake Cholla	2																				5	1		
	Goat Mesa	2																					5		
Marron Valley	Marron Valley	3																						5	
		5																						5	
Mission Trails	Mission Trails	1																							
		3																						1	
MFGD	Sander	1																							
		2				1																			
	Montgomery Field	6																							
	General Dynamics	1																							
		2																							
Chollas	Chollas	2																							
Miramar	A4	103.5																							
		105																							
	AA9	MC4 K4																							
	AA10	MC5 MC6 68.3E																							
Ramona	Ramona	7																							
		17B W6																							
B.lindahli: Carmel Mountain	Carmel Mountain	1																							
		2																							
		4																							
		5																							
B.lindahli: Nobel drive	Pueblo	1																							
		2																							
B.lynchi: Mesa de Colorado	Mesa de Colorado	?																							
B.coloradensis	Mexican Cut	?																							
Grand Total			37	8	2	29	9	2	1	2	2	1	1	1	12	3	1	2	1	1	10	1	5	11	

-																										
Region	Complex	Pond	B07	B01	B02	B04	B09	B12	B13	BO6	B06	B08	B10	B11	B03	B14	B15	B16	B05	B17	LinA	LinB	LinC	x	Grand Total	
Carmel Mountain	Carmel Mountain	1																							4	
Mira Mesa	Brown	1																							3	
		2																							3	
		3																							4	
	Winterwood	1																								3
		2																								3
		3																								2
		4																								1
		5																								5
	Mesa Verde	1																								4
		2																								4
		3																								5
	Maddox	1																								2
		2																								4
4																									3	
7																									2	
Cousins	1																								4	
Nobel Drive	Nobel Drive	1																							10	
		2																							4	
		3																							3	
	Eastgate	1																								2
		2																								4
	4?																								2	
Del Mar	Bowtie	1																							4	
		2																							3	
		3																							3	
	Del Mar Mesa East	2																								2
		3																								1
		4																								4
		5																								4
		mult. ruts RR1																								1
	Del Mar Mesa North	1									1															3
		2																								2
3																									2	
Otay Lakes	Otay Lakes	1																							5	
Otay Mesa	Snake Cholla	2																							6	
	Goat Mesa	2																							5	
Marron Valley	Marron Valley	3																							5	
		5																							5	
Mission Trails	Mission Trails	1	2	2																					4	
		3	1	2																					4	
MFGD	Sander	1		1		4																			5	
		2		2		2																			5	
	Montgomery Field	6					1	1		2															4	
	General Dynamics	1		1						1	1					1	1								3	
	2								1	1	1				1	1								5		
Chollas	Chollas	2								2	1	2													5	
Miramar	A4	103.5		4						1															5	
		105		4												1									5	
	AA9	MC4																5								5
		K4			3													1								4
	AA10	MC5																2	2							4
MC6																	2	2	1						5	
68.3E																	2	2	1						5	
Ramona	Ramona	7																		5					5	
		17B																		5					5	
		W6																		3	1					4
B.lindahli: Carmel Mountain	Carmel Mountain	1																			1				1	
		2																			2	1			3	
		4																			4	6			10	
		5																			3	2			5	
B.lindahli: Nobel drive	Pueblo	1																					1		1	
		2																					1		1	
B.lynchi: Mesa de Colorado	Mesa de Colorado	?																						5	5	
B.coloradensis	Mexican Cut	?																						1	1	
Grand Total			3	17	2	6	1	1	1	8	3	2	1	1	1	6	12	2	13	1	10	9	2	6	250	





— 5 changes