Examination of Soil Samples from an Orange County, CA Site for Fairy Shrimp Cysts

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10(a)(1)(A) permit
TE835549-8
Effective to 3/9/2015

Introduction

Ecological Restoration Service was contracted by Dudek Engineering and Environmental, Encinitas, CA in November 2012 to process soil samples collected by from 34 seasonally ponding basins at the Newport Banning Ranch site, Orange County, CA, for determination of the presence of fairy shrimp cysts, and for culturing of *Branchinecta* cysts for identification to the species level of any cysts found.

Methods

Soil Processing for Cyst Presence

Ten individually packaged, approximately 100 ml dry soil samples per pool, collected by Thomas Liddicoat and Heather Moine from the Newport Banning Ranch site, were delivered to Ecological Restoration Service in November 2012. The samples were processed per the U.S. Fish and Wildlife Service (USFWS) April 19, 1996 Interim Survey Guidelines to Permittees for Recovery Permits under Section 10(a)(l)(A) of the Endangered Species Act for the Listed Vernal Pool Branchiopods, modified by Ecological Restoration Service as described below. Charles Black of Ecological Restoration Service is authorized by the U.S. fish and Wildlife Service to process dry samples for the presence of fairy shrimp cysts and to culture cysts to identify to species level as special conditions of his 10(a)(1)(A) permit. These samples were hydrated for approximately 2-10 hours in tap water, then washed through a set of sieves. Material passing through a Number 45 (.0139") USA Standard Testing Sieve, A.S.T.M.E.-11 specification and caught on a Number 70 (.0083") Sieve was rinsed into a container with approximately 50 ml of a saturated brine solution to float organic material, including fairy shrimp cysts. The material floating on the brine was decanted onto a paper filter on a filter funnel, and water was removed through the filter paper by vacuum suction. The material left on the paper was examined under a 6.3-570x power Olympus SZX9 Zoom Stereo Microscope. Distinctive fairy shrimp cysts, if present, were individually counted (if less than approximately 50) or estimated (for larger numbers) by examining ¼ or ½ subsections of the filter and multiplying the subset by the appropriate factor. The presences of ostracod shells and cladoceran ephippia were also noted in samples.

Results

Cyst Presence

Distinctive *Branchinecta* cysts were present in one or more samples from 26 of the 34 basins (Table 1). Numbers of cysts per sample (where present) ranged from 1 to an estimated 2800. Total numbers of cysts summed for all ten samples of individual basins ranged from 1 to an estimated 20,000. There was a positive relationship between the average number of cysts from all ten samples of a pool and the percentage of the ten samples that had cysts present (Figure 1).

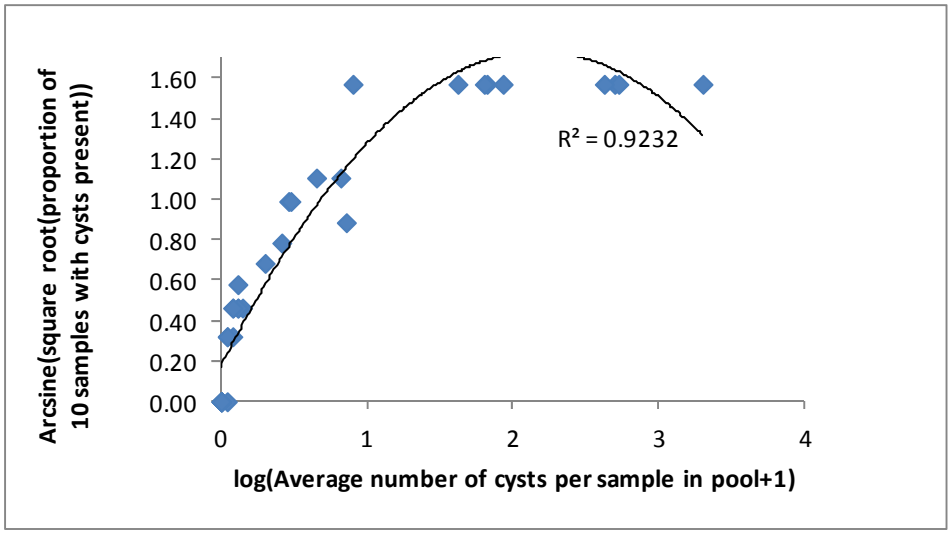

Figure 1 – Percentage of 10 samples per pool with cysts present versus average numbers of cysts per ten samples, values transformed for statistical purposes.

Other Aquatic Invertebrates

Ostracod shells were found in 15 basins (Table 2), all of which also had fairy shrimp present. Ostracod shells were not found in an additional 11 basins where *Branchinecta* cysts were found. Cladoceran ephippia were found in two basins (Table 2), both of which had ostracod shells and *Branchinecta* cysts.

Culturing of Cysts

Cysts recovered from sampling are undergoing culturing to identify them to species level, since the closely similar *Branchinecta sandiegonensis* and *Branchinecta lindahli* cannot be told apart. Due to a relatively high proportion of dormant and unviable cysts in most samples, I have found that usually at least 20 cysts are required to produce at least one identifiable adult. I would class the current basins in three classes: 8 pools with large numbers of cysts (> 100) where identification of species is likely; 5 pools with moderate numbers of cysts (< 100, > 20), where identification is possible; and 14 pools with low numbers of cysts (< 20) where successful culturing to adult size is unlikely. An additional complicating factor where identification of species is important for regulatory purposes, in areas where both the endangered *Branchinecta sandiegonensis* and the common *Branchinecta lindahli* are present, is that producing only one or a few shrimp through culturing may not capture the presence of the other species when it is in fact present, albeit in low numbers.

In extensive sampling of thousands of basins over the last twelve years, I have often found that pool soils that contain very low numbers of cysts frequently do not have hatched shrimp present even under good ponding conditions during winter rain periods. It is possible that cysts in these basins represent populations that were present under more favorable conditions in the past, and something about the basins has changed so that the few remaining cysts are unviable. Another alternative is that cysts have been translocated to basins by wind or other vectors, and conditions in these basins are not suitable for supporting fairy shrimp populations.

I certify that the information in this survey report and attached exhibits fully and accurately represent my work.

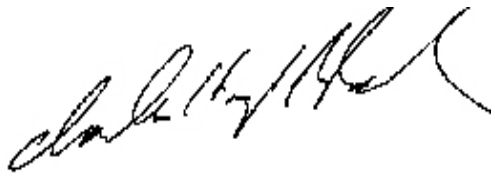

Table 1 - Numbers of Branchinecta cysts found in individual samples

Sample				Sample				Sample				Sample				Sample				Sample				Sample			
Pool		no	cysts	Pool		no	cysts	Pool		no	cysts	Pool		no	cysts	Pool		no	cysts	Pool		no	cysts	Pool		no	cysts
aa	a	1	0	dd	a	1	0	h	a	1	4	kk	a	1	1	n	a	1	1	q	a	1	0	w	a	1	0
aa	a	2	0	dd	a	2	6	h	a	2	1	kk	a	2	0	n	a	2	0	q	a	2	0	w	a	2	0
aa	a	3	0	dd	a	3	0	h	a	3	21	kk	a	3	1	n	a	3	0	q	a	3	0	w	a	3	0
aa	a	4	0	dd	a	4	0	h	a	4	160	kk	a	4	1	n	a	4	0	q	a	4	1	w	a	4	0
aa	a	5	0	dd	a	5	1	h	a	5	24	kk	a	5	3	n	a	5	0	q	a	5	0	w	a	5	0
aa	b	1	0	dd	b	1	0	h	b	1	21	kk	b	1	0	n	b	1	0	q	b	1	0	w	b	1	1
aa	b	2	0	dd	b	2	0	h	b	2	22	kk	b	2	7	n	b	2	0	q	b	2	0	w	b	2	0
aa	b	3	0	dd	b	3	0	h	b	3	360	kk	b	3	3	n	b	3	0	q	b	3	0	w	b	3	0
aa	b	4	0	dd	b	4	2	h	b	4	28	kk	b	4	3	n	b	4	0	q	b	4	0	w	b	4	0
aa	b	5	0	dd	b	5	7	h	b	5	12	kk	b	5	0	n	b	5	0	q	b	5	0	w	b	5	0
bb	a	1	0	ee	a	1	1	hh	a	1	1	l	a	1	0	nn	a	1	0	qq	a	1	0	x	a	1	8
bb	a	2	3	ee	a	2	0	hh	a	2	0	l	a	2	0	nn	a	2	0	qq	a	2	0	x	a	2	10
bb	a	3	1	ee	a	3	1	hh	a	3	0	l	a	3	0	nn	a	3	0	qq	a	3	0	x	a	3	10
bb	a	4	0	ee	a	4	3	hh	a	4	0	l	a	4	0	nn	a	4	0	qq	a	4	0	x	b	6	7
bb	a	5	0	ee	a	5	0	hh	a	5	0	l	a	5	0	nn	a	5	0	qq	a	5	0	x	b	7	6
bb	b	1	0	ee	b	1	0	hh	b	1	0	l	b	1	0	nn	b	1	0	qq	b	1	0	x	b	1	0
bb	b	2	0	ee	b	2	0	hh	b	2	2	l	b	2	0	nn	b	2	0	qq	b	2	0	x	b	2	2
bb	b	3	0	ee	b	3	5	hh	b	3	0	l	b	3	0	nn	b	3	0	qq	b	3	0	x	b	3	2
bb	b	4	0	ee	b	4	0	hh	b	4	0	l	b	4	0	nn	b	4	0	qq	b	4	0	x	b	4	0
bb	b	5	0	ee	b	5	0	hh	b	5	0	l	b	5	2	nn	b	5	0	qq	b	5	0	x	b	5	11
c	a	1	396	f	a	1	0	ii	a	1	1	ll	a	1	5	oo	a	1	0	r	a	1	0	y	a	1	2
c	a	2	600	f	a	2	0	ii	a	2	0	ll	a	2	20	oo	a	2	1	r	a	2	0	y	a	2	5
c	a	3	280	f	a	3	0	ii	a	3	2	ll	a	3	80	oo	a	3	4	r	a	3	0	y	a	3	5
c	a	4	360	f	a	4	0	ii	a	4	0	ll	a	4	20	oo	a	4	8	r	b	6	0	y	a	4	12
c	a	5	480	f	a	5	0	ii	a	5	0	ll	a	5	54	oo	b	1	1	r	b	7	0	y	a	5	7
c	b	1	560	f	b	1	0	ii	b	1	0	ll	b	1	52	oo	b	2	0	r	b	1	0	y	b	1	3
c	b	2	750	f	b	2	0	ii	b	2	0	ll	b	2	48	oo	b	3	0	r	b	2	0	y	b	2	9
c	b	3	480	f	b	3	0	ii	b	3	0	ll	b	3	70	oo	b	4	2	r	b	3	0	y	b	3	5
c	b	4	200	f	b	4	0	ii	b	4	0	ll	b	4	40	oo	b	5	3	r	b	4	0	y	b	4	6
c	b	5	860	f	b	5	0	ii	b	5	0	ll	b	5	20	oo	b	6	1	r	b	5	0	y	b	5	16
cc	a	1	1600	ff	a	1	0	jj	a	1	0	m	a	1	4	p	a	1	2	s	a	1	0	z	a	1	1
cc	a	2	2200	ff	a	2	0	jj	a	2	0	m	a	2	11	p	a	2	7	s	a	2	0	z	a	2	7
cc	a	3	2500	ff	a	3	0	jj	a	3	0	m	a	3	101	p	a	3	11	s	a	3	0	z	a	3	4
cc	a	4	2000	ff	a	4	0	jj	a	4	0	m	a	4	37	p	a	4	1	s	a	4	0	z	a	4	0
cc	a	5	2500	ff	a	5	0	jj	a	5	0	m	a	5	440	p	a	5	1	s	a	5	0	z	a	5	1
cc	b	1	2400	ff	b	1	0	jj	b	1	0	m	b	1	32	p	b	1	98	s	b	1	0	z	b	1	13
cc	b	2	1800	ff	b	2	0	jj	b	2	0	m	b	2	62	p	b	2	221	s	b	2	0	z	b	2	5
cc	b	3	1000	ff	b	3	0	jj	b	3	0	m	b	3	87	p	b	3	222	s	b	3	0	z	b	3	1
cc	b	4	1200	ff	b	4	0	jj	b	4	0	m	b	4	19	p	b	4	60	s	b	4	0	z	b	4	0
cc	b	5	2800	ff	b	5	1	jj	b	5	0	m	b	5	52	p	b	5	2	s	b	5	0	z	b	5	3
d	a	1	475	gg	a	1	0	k	a	1	1	mm	a	1	110	pp	a	1	0	v	a	1	0				
d	a	2	240	gg	a	2	0	k	a	2	1	mm	a	2	400	pp	a	2	0	v	a	2	0				
d	a	3	1230	gg	a	3	0	k	a	3	0	mm	a	3	500	pp	a	3	2	v	a	3	0				
d	a	4	1400	gg	a	4	1	k	a	4	0	mm	a	4	360	pp	a	4	39	v	a	4	0				
d	a	5	28	gg	a	5	0	k	a	5	0	mm	a	5	600	pp	a	5	15	v	a	5	0				
d	b	1	800	gg	b	1	0	k	b	1	0	mm	b	1	41	pp	b	1	0	v	b	1	0				
d	b	2	36	gg	b	2	1	k	b	2	0	mm	b	2	380	pp	b	2	1	v	b	2	0				
d	b	3	502	gg	b	3	0	k	b	3	0	mm	b	3	780	pp	b	3	2	v	b	3	0				
d	b	4	380	gg	b	4	0	k	b	4	0	mm	b	4	240	pp	b	4	0	v	b	4	0				
d	b	5	200	gg	b	5	0	k	b	5	1	mm	b	5	800	pp	b	5	3	v	b	5	0				

Table 2- Summary of cyst, ostracod, and cladoceran ephippia presence in pools

Pool	Total numbers of cysts present in 10 samples	Ostracods present	Cladoceran ephippia present
aa	0		
bb	4	x	
c	4966	x	x
cc	20000	x	
d	5291		
dd	16	x	x
ee	10		
f	0		
ff	1		
gg	2		
h	653		
hh	3		
ii	3	x	
jj	0		
k	3	x	
kk	19	x	
l	2	x	
ll	409		
m	845	x	
mm	4211	x	
n	1		
nn	0		
oo	20		
p	625		
pp	62		
q	1	x	
qq	0		
r	0		
s	0		
v	0		
w	1	x	
x	56	x	
y	70	x	
z	35	x	