Monitoring and Managing California Endemic Large Branchiopods

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ABSTRACT. Twelve of the 37 species of large branchiopods (i.e., fairy shrimp, tadpole shrimp, and clam shrimp) that occur in California are considered endemic. Of these endemics, six are listed under the federal Endangered Species Act as either threatened or endangered. These California endemics inhabit temporary wetlands, chiefly vernal pools. Over 90% of the California Central Valley vernal pools have been lost. Of the less than 10% of vernal pools that still remain in the Central Valley, only 30% are under some form of protection (e.g., conservation easement, deed restriction). Often these protected lands support endemic large branchiopods whose stewards are charged with their long-term monitoring and management. Given the differing life histories among these endemics, the task of applying management decisions based on monitored population trends can be problematic. Therefore, the monitoring techniques discussed are simple to execute, easy to repeat, and cost-effective to obtain useful data; they include semi-quantitative wet-season sampling for large branchiopods and other aquatic invertebrates, micro-and-macroscopic hydrophyte monitoring, water quality parameters and disturbances. Management decisions concerning grazing, exotic plants and wildlife, "edge effects," and predicted future impacts on large branchiopods from global warming and climate change are examined.

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INTRODUCTION

California supports 37 species of large branchiopods (LB's, as defined by Belk, 1996) with 12 endemic or near-endemic species, including 10 fairy shrimp, one tadpole shrimp, and one clam shrimp species (Helm and Noyes, 2016). Six of these endemics are listed under the federal Endangered Species Act of 1973 as amended, as threatened (FT) or endangered (FE) (U.S. Fish and Wildlife Service [USFWS] 1993, 1994, 1997). The list below shows the authority and year of publication for each of these 12 species.

- Vernal pool fairy shrimp, *Branchinecta lynchi* Eng, Belk & Eriksen, 1990 (FT)
- Conservancy fairy shrimp, *Branchinecta conservatio* Eng, Belk & Eriksen, 1990 (FE)
- Longhorn fairy shrimp, *Branchinecta longiantenna* Eng, Belk & Eriksen, 1990 (FE)
- Midvalley fairy shrimp, *Branchinecta mesovallensis* Belk and Fugate, 2000
- California fairy shrimp, *Linderiella occidentalis* (Dodds), 1923
- Mono Lake brine shrimp, *Artemia monica* Verrill, 1869
- San Francisco brine shrimp, *Artemia franciscana* Kellogg, 1906
- San Diego fairy shrimp, *Branchinecta sandiegonensis* Fugate,1993 (FE)
- San Rosa Plateau fairy shrimp, *Linderiella santarosae* Thiéry and Fugate,1994

- Riverside fairy shrimp, *Streptocephalus woottoni* Eng, Belk & Eriksen, 1990 (FE)
- California clam shrimp, *Cyzicus californicus* (Packard), 1883
- Vernal pool tadpole shrimp, *Lepidurus packardi* Simon, 1886 (FE)

Although there are numerous causes for reductions in these species' populations and distributions, the major contributor has and continues to be habitat loss (USFWS 1993, 1994, 1997; King, 1998), in particular, the loss of vernal pools. Approximately 7,000,000 acres of vernal pool terrain in the Central Valley (CV) of California were present before European arrival (Holland, 2009). Of the less than 10% of vernal pool terrains that still remain in the CV, only 30% are under some form of protection (e.g., conservation easement, deed restriction) (Witham et al., 2014). The loss of endemic LB habitat in the CV continues to this day. Habitat mapping of CV vernal pool terrain found 764,868 acres of terrain in 2012 compared to the 807,820 acres of terrain mapped in 2005 – a net loss of 42,952 acres in just seven years. Between 2005 and 2012, 5.3% of all known vernal pool terrain was lost (Witham et al., 2014). Furthermore, 95% of this habitat loss was due to unregulated agricultural conversions, versus only 5% to urban/industrial conversions.

The primary goal of this paper is to contribute to the existing knowledge concerning monitoring and management of California endemic LB's, in particular those that are federally listed, and to assist in their protection, conservation, and recovery. Since the emphasis is on endemic LB species occurring in vernal pool terrains, the two brine shrimp species that live in hypersaline habitats are excluded.

If a land steward's goal is to maintain or increase endemic LB occurrences and abundances, then some form of monitoring is required. The results of this monitoring should inform what maintenance and management activities are deemed necessary. This adaptive management approach as defined by Holling (1978) is a systematic approach for improving resource management by learning from management outcomes. According to Johnson (1999), "The overall goal of adaptive management is not to maintain an optimal state of the resource, but to develop an optimal management capacity. This is accomplished by maintaining ecological resilience, in the specific sense proposed by Holling (1973) and described further in Holling and Meffe (1996), that allows the system to react to inevitable stresses, and by generating flexibility in institutions and stakeholders that allows managers to react when conditions change (Gunderson 1999). The result is that, rather than managing for a single, optimal state, we manage within a range of acceptable outcomes while avoiding catastrophes and irreversible negative effects."

Understanding the ecology of vernal pools and life histories of targeted LB's is crucial for selecting the parameters influencing LB occurrences and distributions which need to be monitored in the present and future.

VERNAL POOL ECOLOGY

California vernal pools are extremely diverse as are the geologic surfaces and landforms, soils, elevations, slopes, latitudes, and climatic regimes in which they occur. Regardless of their diversity, they have similarities in their hydrologic phases and invertebrate phenology, which are discussed below.

Hydrologic Phases

Vernal pools typically have five hydrologic phases (modified from Keeley and Zedler, 1998): 1) wetting phase, 2) initial inundation phase, 3) undulating phase, 4) drying phase, and 5) desiccated phase.

Wetting phase
The wetting phase occurs when water deposited in the vernal pool basin (from direct inception of rainfall, overland flow and/or seasonally high perched ground water) wets or saturates the soil surface but no inundation occurs. Generally, there are few changes in the flora and fauna composition during this phase. And yet, the moisture will stimulate the reproduction of terrestrial algae, the initiation of hatching of many resident invertebrates (e.g., eggs and cysts [embryonic eggs]), and germination of many plant propagules (e.g., seeds, spores). If the wetting phase is prolonged, then germination of some plants (Naylor, 2002) can occur as well as hatching of some semi-aquatic invertebrates (moist soil-dwelling springtails [Collembola], roundworms [Nematoda], and mites [Hydracarina]) (Szalay et al., 1999; Stork and Eggleton, 1992).

Initial inundation phase
The initial inundation phase occurs when the soil profile above the impervious layer(s) is completely saturated (all of the interstitial spaces [pores] are filled with water). The vast majority of California vernal pools fill from rainfall; however, some hydrologic inputs occur from snowmelt (e.g., in the Modoc Plateau), or traces can be derived from fog and dew (mostly coastal). The amount of rainfall needed to initiate ponding within a given vernal pool depends on various parameters including depth to impervious layers, soil texture, slope, contributing watershed (surface and subsurface flow), and surface weather conditions (air temperatures, wind speed and direction, and humidity).

Occasionally, pools can have a temporary initial inundation phase when the rate of water entering the pool basin is greater than the pool's soil absorption rate. Generally, the initial water influx hydrates the soil and detritus, providing a medium for the aquatic detritivores (discussed below under Aquatic Invertebrate Phenology). Although the vascular macrophytes have not yet established in this phase, many have initiated germination, and unicellular algae (e.g., diatoms – Bacillariophyta) and bacteria (e.g., blue-green algae – Cyanophyta) populations are establishing.

Undulating phase
The undulating phase is characterized by fluctuating water depths from sequential rainfall and evapotranspiration rates. Once the pools reach their maximum water volumes, they discharge water from their "spillways." The aquatic stages of vascular macrophytes have established and filamentous algae (e.g., *Pleurodiscus*, *Spirogyra*, and *Zygnemopsis*), attached to last year's growth of emergent macroscopic hydrophytes (e.g., *Eryngium*, *Eleocharis*) are prominent. This phase has high species richness and the greatest productivity of animals and plants.

Drying phase
The drying phase is characterized by the establishment of the terrestrial and flowering stages of vascular macro-hydrophytes, the dominance of filamentous algae, and the declining of water quality conditions (e.g., decreasing dissolved oxygen [DO] and increasing water temperatures). During this period, large clumps of filamentous algae have detached from the macrophytes and float on the surface ("flab"). Flab has a significant effect in shallow waters, causing a distinct vertical stratification of extreme temperature, pH, and DO (Hillebrand, 1983). In addition, biological oxygen demand (BOD) increases as the flab decays and settles to pool bottom as flocculent. The presence of flab is generally considered a result of eutrophication (Hillebrand, 1983).

Desiccated phase
The desiccated phase is the terrestrial phase of the pools' annual cycle and is characterized by the absence of surface soil moisture. At this point in the phase, the vascular macrophytes

have senesced, the resident active aquatic invertebrates have perished, and the winged insects have fled. However, their propagules are strewn throughout the upper sediments of the pool bottom awaiting next season's rains. Visual clues of an aquatic existence are found within the micro-depressions laden with shells of ostracods (Ostracoda), and the occasional carapaces of clam shrimp (Laevicaudata and Spinicaudata) and tadpole shrimp (Notostraca).

Aquatic Invertebrate Phenology

The appearance of aquatic invertebrate species generally coincides with food availability (Helm, 2016). The phenology of aquatic invertebrates described below is an example of a clear fresh-water vernal pool with moderate ponding duration and size that lacks high turbidity and strong alkaline/saline soils. The phenology presented below is derived from over 30 years of observations from wet-season surveys and culturing of vernal pool invertebrates, along with various other data sources concerning aquatic invertebrates (Pennak, 1989; Thorp and Covich, 1991; Williams, 1997; Eriksen and Belk, 1999; Helm, 1999, 2016).

Detritivores

Within the first 24 hours of inundation, the first group of detritivores emerges in three length classes: microscopic (< 0.5 mm l), mesoscopic (0.5-2 mm), and macroscopic (> 2 mm).

Microscopic detritivores. Protozoans consisting of amoebas (200 µm), flagellates (5 µm-2 mm), ciliates (10 µm-4 mm) and sporozoans (8-100 µm) make up the microscopic detritivores. (Note: 1,000 µm = 1 mm.) These protozoans eat detritus, bacteria, algae and other protozoans.

Mesoscopic detritivores. The mesoscopic detritivores consist of water mites (Hydracarina, 0.5-2 mm) and "tiny" seed shrimp (Ostracoda, 0.2-1 mm). The water mites are mostly free living parasites although some are carnivorous. The seed shrimp are benthic (bottom) dwelling filter feeders that scavenge dead organic material. These tiny seed shrimp have withstood desiccation between wetting and initial inundation phases by closing their tightly sealed carapaces. Some species can survive longer periods by going into a torpid state (Delorme and Donald, 1969; Horne, 1993).

Macroscopic detritivores. The macroscopic detritivores include springtails (Collembola, 0.2-6 mm) and roundworms (Nematoda, 1-2.5 mm). Springtails are neuston (surface)-dwelling and generally are herbivores, feeding on plant material, feces, and algae. However, some are carnivores feeding on nematodes, small arthropods, and even other springtails. In contrast, roundworms are benthic dwellers consuming bacteria, plants, algae, and microscopic organisms.

Crustaceans

The second group of detritivores to emerge, usually within the first week of inundation, are the crustaceans consisting of fairy shrimp (Anostraca, 5 mm-5 cm, 10 cm), water fleas (Cladocera, 0.5-6 mm), "big" Ostracods (0.2-5 mm), and copepods (Copepoda, 0.5-2 mm). These macroscopic organisms feed on detritus, bacteria, algae, and microorganisms using a variety of creative filtering mechanisms.

Flatworms

Within the second week of inundation, the scavenging flatworms (Microturbularia, < 5 mm) are present. Although related to tapeworms (Cestoidea) and flukes (Trematoda), most turbellarians are free-living (not parasitic), eating bacteria, algae, protozoans, and small invertebrates. However, most of the time they scavenge on dead and dying invertebrates instead of hunting prey.

Insects

Generally before the third week of inundation, the invading insects arrive, consisting of water scavenger beetles (Hydrophilidae, 4-60 mm), predaceous diving beetles (Dytiscidae, 1-35 mm), backswimmers (Notonectidae, 5-15 mm), crawling water beetles (Haliplidae, 1.5-5 mm), and water boatmen (Corixidae, 3-11 mm). These flighted adult insects have left their perennial habitats to plunder the vernal pools' concentrated food sources. Although water scavenger beetles do scavenge dead animals, they are mostly predators of small invertebrates. Predacious diving beetles prey on aquatic insects and occasionally amphibians and small fish, if present. Piercing their prey's exoskeletons with their "beak" (piercing and sucking mouthparts), oarsmen are raptorial predators of invertebrates only. Crawling water beetles are omnivorous, consuming algae and small aquatic invertebrates. In contrast to the other insects, the water boatmen are largely herbivores, eating algae and detritus.

Second group of LB's and insects

By this time, a second wave of LB's and insects have hatched and reached macroscopic sizes. The LB's consist of the filter-feeding clam shrimp (Laevicaudata and Spinicaudata, 2-15 mm) and tadpole shrimp (Notostraca, 10-80 mm). Tadpole shrimp are omnivorous, bioturbating the benthic sediments (Croel and Kneitel, 2011b). Although tadpole shrimp have started to hatch during the first or second week of inundation, they may stay in their opened eggshells for several days prior to emerging.

The larvae of the predaceous diving beetles (Dytiscidae, 1-50 mm) and water scavenger beetles (Hydrophilidae, 1-48 mm) prey on aquatic invertebrates and small amphibians using their claw-like mandibles to pierce their prey's flesh. By the second to third week of ponding, most of the significant invertebrate players are present, albeit many in juvenile form.

Marsh species

If inundation lasts greater than four months, some vernal pools will support invertebrates generally associated with emergent marsh habitats. Mayfly larvae (Ephemeroptera, 1-30 mm) are typically detritivores or herbivores, although occasionally carnivorous. However, the adults do not feed. They emerge, reproduce and die. Damselfly larvae (Zygoptera, 15-30 mm) and dragonfly larvae (Anisoptera, 25-50 mm) consume aquatic invertebrates and intermittently tadpoles and small fish, if present. In contrast to mayfly adults, adult damselflies and dragonflies consume small soft-bodied flying insects (e.g., flies, aphids, mosquitoes, gnats). Other occasional occupants include leeches (Hirudinea) and snails (Gastropoda) consisting of orb snails (Planorbidae), pond snails (Lymnaedidae), and bladder snails (Physidae).

Flies

The flies (Dipterans) are usually the last of the insects to invade. Midge larvae (Chironomidae, 2-30 mm) are omnivores, feeding on diatoms, detritus, and small plants and anim als. Adults feed on nectar, pollen, and plant juices. Mosquito larvae (Culicidae, 2-12 mm) feed on algae, bacteria, and other microbes. Adults typically feed on nectar and plant juices but in many species, the female can feed on the blood of animals.

Most of the aquatic vernal pool invertebrates are univotine (producing one brood per ponding event), with the notable exceptions of the water fleas and copepods. As such, huge population oscillations in these species are known to occur within a single inundation period. Some pools repeat the inundation/desiccation cycle more than once during the wet season, which may allow for additional generations of aquatic invertebrates to hatch. With the general exception of the fairy shrimp, all representatives of the macroscopic aquatic invertebrate assemblages are generally present until desiccation of the pool.

Aquatic Invertebrate Diversity

Species richness in vernal pools, whether it be plants or animals, generally increases with pool size and ponding duration (Zedler and Ebert, 1979; Simovich, 1998; Helm, 1999). Analogous to the temporal differences in vernal pool aquatic invertebrate diversity, spatial diversity is great, especially of the pico-nano ($1,000^{-4}$ to $1,000^{-3}$ m in length) and microscopic (0.2-200 µm in length) organisms (Helm, 1999). The unvegetated portions of the water column generally support ciliates, bacteria (coating detrital surfaces), and diatoms (Bacillariophyta, mostly associated with the benthos). Portions of the water column with filamentous algae support bacteria, amoebas, ciliates, flagellates, rotifers, gastrotrichs, microscopic ostracods, copepods, and cladocerans.

For additional information concerning vernal pool macroscopic aquatic invertebrate assemblages, refer to (Balko and Ebert, 1984; Dehonet and LaVigne, 1984; Zedler, 1987; King et al., 1993; King et al., 1996; Helm and Fields, 1998; Helm, 1999), phenology (Dubbs, 1987; Wright, 1990; Helm, 2016), and taxonomic keys (Usinger, 1956; Menke, 1979, Merritt and Cummins, 1988; Pennak, 1989; Thorp and Covich, 1991; Eriksen and Belk, 1999; Burres et al., 2014).

Large Branchiopod Life Histories

Brief Overview

With the exception of the omnivorous vernal pool tadpole shrimp, California endemic LB's are filter-feeding detritivores. Although these endemics can be found in a variety of habitats, they are generally confined to seasonally inundated habitats within vernal pool terrains (Pennak, 1989; Hathaway and Simovich, 1996; Helm, 1998; Eriksen and Belk, 1999). Vernal pools are harsh environments and the only way LB's progress from one generation to the next is by their cysts (embryonic eggs). These diapause (dormant) cysts can withstand extreme temperatures and dehydration, and reactivate in response to essential stimuli (e.g., water temperature, DO, pH, barometric pressure, salinity, etc.) during inundation (Lanway, 1974; Ahl, 1991; Helm, 1998). The exact parameters for "breaking" the cyst's dormancy are largely unknown (Eriksen and Belk, 1999; Alekeev et al., 2006). Some may need several wetting and drying cycles while others may need a "cold snap" or freeze (Lanway, 1974; Ahl, 1991; Eriksen and Belk, 1999). However, only a fraction of the cysts within a given pool hatch during a season. This "bet hedging" minimizes the chance of extirpation within a pool from inappropriate conditions to complete their life cycle (Simovich and Hathaway, 1997). The hatchling naupli or metanaupli (larvae) emerge and quickly go through several molts (instars – the developmental stages between molts), growing larger and gaining more phyllopods ("gilled feet") until reaching maturity (Health, 1924; Pennak, 1989). While avoiding predators and parasites, these endemic LB's need to cope with extreme diurnal (daily) and seasonal fluctuations in water parameters (e.g., temperature, depth, volume, DO, pH, turbidity, etc.) while striving to complete their life cycle before the aquatic period of their habitat ends.

Targeted Species

Life history data consisting of maturation, reproduction and life-expectancy rates of the majority of California endemic LBs are provided in Tables 1 (Helm, 1998) and 2 (Hathaway and Simovich, 1996). Habitat "preferences" of California endemic LBs are shown in Table 3 and highlights of their life histories are briefly discussed below.

San Diego fairy shrimp
This species is restricted to the southern border of California, almost exclusively west of the coast range. It has the shortest maturation rate

TABLE 1. Life history characteristics* of California Central Valley endemic large branchiopods (from Helm, 1998).

Large Branchiopods	Sample Size[1]	Days to Mature[2]			Days to Reproduce[3]			Population Longevity[4]		
		Min	Mean	SE[5]	Min	Mean	SE[5]	Max	Mean	SE[5]
Vernal Pool fairy shrimp	24	12	18.0	1.3	18	39.7	1.9	139	90.6	5.0
Midvalley fairy shrimp	8	8	26.3	4.5	16	42.6	5.6	143	110.5	12.7
California fairy shrimp	25	16	32.9	2.4	31	42.8	1.3	168	138.7	3.2
Conservancy fairy shrimp	10	14	36.5	4.9	19	46.2	4.2	154	113.9	10.9
Longhorn fairy shrimp	3	16	22.4	6.1	23	43.0	5.9	147	114.0	18.0
Vernal pool tadpole shrimp	20	25	38.1	2.7	41	54.1	1.5	168	143.6	3.2
California clam shrimp	6	38	49.4	3.1	43	57.7	4.4	177	155.7	5.9

* Obtained from plastic pool cultures between 1990-1996.
[1] Number of populations (one population per pool) observed during a complete ponding event.
[2] First observation of maturity: at least one individual in the population has apparently functioning sex organs (i.e., ova observed in oviduct).
[3] First observation of reproduction: at least one individual female in the population has two shelled cysts (the minimum number of cysts to complete the life cycle, assuming each cyst is a different sex, thereby replacing its parents)
[4] The last individual in the population is dead.
[5] Standard Error.

and life span of all of the endemic LB's. It can mature in as little as 7 days, with a maximum longevity (birth to death of an individual) of 42 days (Hathaway and Simovich, 1996). It inhabits the shortest-lived pools as well as deep turbid road ruts. It is difficult to distinguish it morphologically from the versatile fairy shrimp (*B. lindahli*), especially if female or immature, with which it often co-occurs and on occasion hybridizes (Eriksen and Belk, 1999).

Midvalley fairy shrimp

With the exception of the San Diego fairy shrimp, this species occupies the most ephemeral of habitats. These habitats are generally transitional between upland annual grasslands and vernal pools and are often dominated by grasses (e.g., Mediterranean barley [*Hordeum marinum* ssp. *gussoneanum*], Italian ryegrass [*Festuca perennis*], and annual hairgrass [*Deschampsia danthonioides*]). Although it can mature in as little as 8 days, this shrimp has a fairly high maximum population longevity, from the appearance of the first individual to the disappearance of the last – 143 days (Helm, 1998). It is often overlooked or assumed to be the vernal pool fairy shrimp, especially the females. In contrast, male midvalley fairy shrimp superficially resemble males of the Conservancy fairy shrimp.

TABLE 2. Some life history characteristics of San Diego fairy shrimp and Riverside fairy shrimp (From Hathaway and Simovich,1996*).

Life History Parameter	Aquaria		Field	
	San Diego Fairy Shrimp	Riverside Fairy Shrimp	San Diego Fairy Shrimp	Riverside Fairy Shrimp
Maturity	7-10 days	7 weeks	10-20 days	8 weeks
Longevity	28-30 days	2.5 months	4-6 weeks	4 months[1]

*A comparison of approximate developmental rates of San Diego fairy shrimp and Riverside fairy shrimp based on laboratory mass-rearing in aquaria (Approx. 20-22°C) and field observation.
[1] Still present as the pool dried.

Vernal pool fairy shrimp
The vernal pool fairy shrimp is perhaps the most adaptable of all of the endemic LB's. It co-occurs with more other LB species than any other endemic LB (Eriksen and Belk, 1999), has the widest range of occurrences, and occupies the most diverse habitats and elevations of all of the endemics (Helm, 1988; Helm and Noyes, 2016). The vernal pool fairy shrimp can be found co-occurring in flashy pools with midvalley fairy shrimp; medium-sized pools with California fairy shrimp; large turbid pools with the Conservancy fairy shrimp, longhorn fairy shrimp, and vernal pool tadpole shrimp; and even highly alkaline pools with versatile fairy shrimp (*Branchinecta lindahli*). However, its population densities when co-occurring with other LB's are generally among the lowest of the endemics (Helm, 1998; Eriksen and Belk, 1999). The vernal pool fairy shrimp "prefers" habitats that are inundated for shorter periods to minimize competition and predation. It obtains high populations in clear-water pools with low-growing vegetation, with highly fluctuating hydroperiods and the absence of other LB's. Although this species is the second fastest-maturing endemic, it takes nearly twice as long to mature (12 days) than the San Diego fairy shrimp. Its maximum population longevity, however, is fairly high (139 days, Helm, 1998). Albeit adaptable, it seems to be susceptible to low DO and warm water conditions, especially when immature.

Longhorn fairy shrimp
This species is probably the least understood of all of the endemic LB's. It has a moderate maturing time of 16 days and a maximum longevity of 147 days (Helm, 1998). It occupies two distinct habitats: tiny rock outcrop pools and small to large alkaline playa pools. The species' population densities within a pool are highest in rock outcrop pools with no other LB's. In contrast, its densities are low in turbid playa pools. Like Conservancy fairy shrimp, the active longhorn fairy shrimp generally disappears long before its large turbid playa habitat dries. Yet, it can be found in drying rock outcrop pools. Some recent evidence suggests that this species may be susceptible to cooler water temperatures. If true, this may help explain its distributions in the San Joaquin Valley and Bay Area, but its absence in the Sacramento Valley.

California fairy shrimp
This species has a moderate maturing time of 21 days and a fairly high maximum population longevity of 168 days (Helm, 1998). It can tolerate high water temperatures, low DO for a fairy shrimp, and thatch buildup. However, population densities are significantly decreased in ungrazed pools and pools with large amounts of filamentous algae. The California fairy shrimp is a strict mid-water column filter feeder and tends to "hover" in non-vegetated areas (Helm, 1999). It will seek lower water temperatures in the bottom of hoof prints or

TABLE 3. California endemic large branchiopod habitat "preferences"[1].

Large Branchiopod Species	Ponding																	
	Maximum Depth (in inches)						Duration (consecutive days)						Area (in acres)					
	1-2	2-4	4-6	6-8	8-10	>12	<14	14-30	30-60	60-90	90-120	> 120	<0.0005	0.0005 - 0.005	0.005 - 0.05	0.05 - 0.5	0.5 - 5.0	> 5.0
San Diego Fairy Shrimp																		
Midvalley Fairy Shrimp																		
Vernal Pool Fairy Shrimp						X					X						X	
Longhorn Fairy Shrimp[2]																		
California Fairy Shrimp													X					
Santa Rosa Plateau Fairy Shrimp																		
Vernal Pool Tadpole Shrimp		X						X						X				
California Clam Shrimp																		
Conservancy Fairy Shrimp																		
Riverside Fairy Shrimp																		

[1] Data from Helm (1998), Helm and Noyes (2016), and unpublished data from Brent Helm's files
[2] Diagonal lines represents data from rock outcrop pools

Categories of Relative Proportions of Species Occurrences*

- Very Common (> 50% of occurrences)
- Common (< 1/4 of occurrences)
- Not Common (<1/8 of occurrences)
- Rare (< 1/20 of occurrences)
- X Anomalies – mostly explainable

* Adjacent columns with same shade are considered one unit for the purposes of the proportion of occurrences

ruts during warm days. Mating instinct in males is strong, and they remain "clasped" to females for extended periods even when removed from water. Of the endemics, the California fairy shrimp is known to occur in the lowest-pH waters (pH 6.5, Eriksen pers. comm.). It is not uncommon to see California fairy shrimp swimming in pools with large quantities of leaves, especially oak leaves which produce tannic acid, reducing the pH.

Santa Rosa Plateau fairy shrimp

As its name implies, the Santa Rosa Plateau fairy shrimp is restricted to southern basalt flow vernal pools on the Santa Rosa Plateau, in western Riverside County (Thiéry and Fugate, 1994; Eriksen and Belk, 1999). This species is very similar to the California fairy shrimp in morphology, and (based on the limited data) has a similar life history. According to Chester (2008), the Santa Rosa Plateau fairy shrimp takes roughly 30 days to mature and has a life expectancy of around 50 days.

Vernal pool tadpole shrimp

This species is slow-maturing, at a minimum of 25 days with a maximum population longevity similar to the California fairy shrimp at 168 days (Helm, 1998). The vernal pool tadpole shrimp can tolerate high levels of livestock trampling (which creates hoof prints [punch/pockmarks]), low DO, and warm water. The species' greatest population densities occur in turbid pools with low plant densities. Its bioturbating activities can uproot young plants and help entrain sediments in the water column, thereby perpetuating a turbid habitat (Croel and Kneitel, 2011b). Because the vernal pool tadpole shrimp is a bottom dweller – filter-feeding the sediments – it may be more susceptible to the accumulation of soil pollutants. Due to their hydrodynamic shape, large adults can move upstream within slow-flowing swales and drainages, but the young are poor swimmers and are swept downstream.

California clam shrimp

This species prefers long-inundated vernal pools, ponds, and other semi-permanent and sometimes permanent habitats. The California clam shrimp can reach maturity in 38 days (Helm, 1998) and can live as long as six months, or as long as the pool contains water. It can tolerate high levels of cattle trampling, high water temperatures, and the lowest DO (Eriksen and Brown, 1980) when compared to other endemic LB's. However, California clam shrimp are not as tolerant of cool water conditions (50º F [10º C] or below) as the other endemic LB's (Thorp and Covich, 1991). Similar to the vernal pool tadpole shrimp, this species' greatest population densities occur in turbid pools. Yet the species can tolerate ungrazed pools since they can swim vertically in the water column between clumps of thatch.

Conservancy fairy shrimp

In general, this species occurs in large playa-type pools with high turbidity. Although a few occurrences have been in small clear pools (Helm, 1998), upon further evaluation these occurences are presumed to to have been the result of "wash downs" from turbid source-pools upslope. The turbidity generally is derived from clay sediments that are entrained in the water column from the action of wind across the large fetch of the pool and repulsion properties of indiviual clay particles (Barclay and Knight, 1981). Although the Conservancy fairy shrimp has a relatively short maturation time of 14 days, its maximum population longevity is 154 days (Helm, 1998). Although moderately tolerant to DO and warm water, the species generally disappears long before its turbid habitat dries. The Conservancy fairy shrimp generally co-occurs with vernal pool tadpole shrimp. With the exception of the Riverside fairy shrimp, this species is the largest of the endemic fairy shrimp. However, it is quite fragile (soft) until mature.

Riverside fairy shrimp

This species is very slow maturing, taking 49-56 days (7-8 weeks, Hathaway and Simovich, 1996). Yet its maximum longevity is only 120 days (Hathaway and Simovich, 1996). The Riverside fairy shrimp needs warm water to hatch and occupies deep pools. The pool size is not as important as depth, so long as ponding duration is adequate to complete its life cycle. It is the fastest swimmer and perhaps the most tolerant of high water temperatures and low DO of all the endemic fairy shrimp. It is not uncommon to see the species coming to surface for oxygen ("porpoising").

For additional information concerning the life histories of California endemic LB's, review the following: Heath (1924), Gordon (1948), Patton (1984), Ahl (1991), Hathaway and Simovich (1996), Simovich and Hathaway (1997), Helm (1998, 1999), and Eriksen and Belk (1999).

MONITORING

For the land steward, there are perhaps more questions regarding monitoring than there are for any other topic in this paper. How many pools should a land manager sample, and which ones? What is the sampling frequency per season? Does the land manager monitor the same pools each time? What sampling methods are best to use?

The answers to all these questions depend on various factors: the number of onsite pools, the level of precision targeted, the level of confidence required, the degree of variability in the sample parameter, the statistical method used, and the cost effectiveness desired/needed.

Sample Size – Number of Pools

Sampling 10% of the population is often given as an ideal sample size when conducting field studies in the biological sciences. However, according to Table 4, to obtain a 95% confidence interval with a 5% margin of error, sampling 10 percent of a population (in this case, number of pools) would entail sampling 346 pools out of a total of 3,460 pools. Fortunately, the percent of the population that will need to be sampled decreases disproportionally with an increase in population size (Table 4). Hence, unless the population is large (> 3,400 pools) it is impossible to sample only 10% of the population with a high confidence and low margin of error. Furthermore, required sample size increases considerably with the increase of the confidence interval and the reduction of the margin of error. Therefore, if there are 100 pools or less onsite, it is suggested to sample them all. If there is an indication of the number of pools that can be sampled in a given season due to financial and workload constraints, then the margin of error under differing confidence intervals to understand the statistical constraints of the decision can be calculated. Sample size calculators can be obtained for free online, or within statistical software (JMP, XLSTAT, SAS, SPSS, R, Stata, Minitab, etc.).

TABLE 4. Ideal sample size[1].

Population Size	Sample Size[2]	Percent of Population
50	45	90
100	80	80
200	132	66
400	196	49
800	296	32.5
1,600	310	19.3
3,200	343	10.7
3,460	346	10

[1] based on 95% confidence interval* and 5% margin of error**.
*a range of values so defined that there is a specified probability that the value of a parameter lies within it.
**an amount (usually small) that is allowed for in case of miscalculation or change of circumstances.
[2] determined from online sample size calculator (http://www./qualtric.com/ calculating-sample-size

Pools to Select for Sampling

If the site has never been sampled for LB's, using the dry-season method first to sample as many pools as possible is suggested. Then, target those pools with positive evidence of LB's (LB cysts or carapaces [shells] of tadpole shrimp) during an upcoming wet season to confirm occupancy (see "Monitoring Parameters and Techniques" section below for a discussion on wet-sampling and dry-sampling methods). If the site has known LB occurrences, sample those pools first. Sampling pools absent of LB's will answer few questions. If it is not possible to sample all of the pools with known occurrences of LB's, then stratify them according to geologic surfaces/soil types, pastures (paddocks), pool sizes, pool depths, and LB species presence. Priority should be on those pools that generally inundate during below-average annual rainfall years. This will help ensure obtaining positive data in most years.

Frequency and Timing of Sampling/Surveys

Although the number of wet-season survey events in a given monitoring year is dependent upon time, fiscal, and personnel constraints, generally more survey events will result in more accurate data concerning the abundance of LB's and the factors influencing them (see USFWS [2017] Survey Guidelines for the Listed Large Branchiopods for full protocol-level wet-season sampling requirements). However, in a given monitoring year it is suggested here that a minimum of three survey events should be conducted during the wet-season, timed to coincide with the peak occurrence and identification times of target species (e.g., federally-listed LB's).

Ideal timing of these survey events will vary greatly on an annual basis depending mostly upon local climatic conditions (e.g., amount and timing of rainfall, air temperatures). Therefore, it is more reliable to time survey events based upon the number of days since the inundation of that habitat (e.g., vernal pool) than to assign survey events to a specific time window (e.g., early January). This will require hydrological monitoring (e.g., staff gauges, camera monitoring, visual observations) of the LB habitats to ensure surveys are initiated at the appropriate times. Table 5 provides an example of ideal wet-season survey timing for maximizing detections of federally-listed large branchiopods during an average climatic year for each of the respective Survey Zones (Figure 1).

The timing of surveys presented in Table 5 are intended to maximize the detection of federally-listed LB's based upon known life history data (see Tables 1 and 2). However, vernal pool fairy shrimp within Survey Zone C, Conservancy fairy shrimp, and longhorn fairy shrimp are not presented in Table 5 due to their restricted habitat types and distributions. Additionally, this table does not include dates for maximizing detections of non-listed species. However, these species will most likely be detected concurrently with the federally-listed species due to their similar life histories (e.g., California fairy shrimp detected at the same time as vernal pool fairy shrimp, California clam shrimp detected at the same time as vernal pool tadpole shrimp).

FIGURE 1. California Survey Zones for listed large branchiopods (USFWS, 2017).

Monitoring Parameters and Techniques

Vernal pool ecosystems are complex and made up of biological, physical, and chemical components in a constant state of action and interaction. Given these factors, monitors/land stewards must be able to separate true long-term changes from diurnal, seasonal, and annual fluctuations. This may require years of reliable observations and data collection and analysis on a consistent and regular basis. Several parameters to monitor and their importance to LB's, and other macroscopic aquatic invertebrates, are described below. Although comparisons among pools could be useful at times, due to the uniqueness of individual pools, the majority of the sampling parameters described below are proposed for trend analysis of discrete pools.

Large branchiopod abundance
LB's are often indicative of high-ecological-functioning habitats (e.g., with high levels of nutrient cycling, species diversity, and water

TABLE 5. Ideal wet-season survey timing for federally-listed large branchiopods.

	Survey Zone*					
	A		B		C	
Survey Round	Days since habitat inundation	Target Species	Days since habitat inundation	Target Species	Days since habitat inundation	Target Species
1	14	Immature *B. lynchi*	10	Immature *B. lynchi*	7	Immature *B. sandiegonensis*
2	28	Mature *B. lynchi*	20	Mature *B. lynchi*	14	Mature *B. sandiegonensis*
3	42	Mature *L. packardi*	40	Mature *L. packardi*	50	Mature *S. woottoni*

*Survey zone as defined by USFWS (2017) Survey Guidelines for the Listed Large Branchiopods.

quality) (Davenport, 2011). Reductions in LB abundances (population sizes or detection rates) over time could be an indication that these habitats are no longer able to provide ecological services to the same degree that they did historically.

There are three sampling techniques to monitor LB abundance: wet-season sampling, dry-season sampling, and environmental DNA (eDNA) sampling. The eDNA method is eloquently described by Shannon Kieran and co-authors within in this symposium proceedings (Kieran et al., 2019) and therefore will not be discussed further here. As their names imply, wet-season sampling occurs when the pools are inundated and dry-season sampling occurs when the pools are dry. Wet-season sampling entails sampling habitats that can potentially support LB's (potential habitat), with the aid of a dip-net (or seine), for active individuals. Dry-season sampling involves the collection of soil from the dry basins of potential habitat and analyzing the collected soils for the presence of LB cysts. [Editor's note: both wet- and dry-season sampling require collection permits from USFWS.]

Dry-season sampling. Dry-season sampling is generally not a preferred method for continuous long-term monitoring because it can only detect the presence, or presumed absence, of cysts and not whether the species is completing its life cycle (hatching, maturing and reproducing). This is especially apparent if the pools being monitored were constructed and inoculated with LB cysts. Most of the species from the genus *Branchinecta* cannot be differentiated by the external morphology of their cysts (Moorad et al., 2001; Helm and Noyes, 2016) and therefore the cysts would need to be cultured (hatched and hatchlings reared to maturity) or genetically analyzed to determine species. Hence, dry-season sampling is mainly a useful baseline monitoring tool to determine if there *may* be active populations. Dry-season sampling can be performed as a qualitative, semi-quantitative or quantitative method. The qualitative method is to consolidate the sub-samples of soil to determine presence or presumed absent of cysts. The semi-quantitative method is used to measure the volume of a consolidated sample or the individual sub-samples and perform counts of cysts to obtain concentrations (number of cysts per unit of soil volume). A small garden spade is generally used to collect soil using the first two methods. In contrast, a soil core sampler is needed to obtain a truly quantitative sample.

Wet-season sampling. Wet-season sampling is preferred to dry-season sampling for long-term monitoring (Helm and Noyes, 2016). There are also a greater number of parameters available to monitor during the wet season in comparison to the dry season. In contrast to dry-season sampling, species detections during the wet season are determined by seasonal environmental conditions (rainfall patterns and other environmental cues to break cyst dormancies [e.g., water temperature, barometric pressure, salinity, etc.]) and the monitors' experience (timing, LB identification and netting skills). Therefore wet-season sampling provides an opportunity for "boots on the ground time" that is essential to understanding the ecosystem processes and how management actions influence *in situ* conditions.

Similar to dry-season sampling, wet-season sampling can be qualitative, semi-quantitative or quantitative. The first two methods can be obtained using a dip net or seine. The qualitative method is used to determine presence or absence and cannot be utilized to estimate LB concentrations (number of individuals per unit volume, sometimes referred to as density) or used for extrapolations of water volumes to estimate population sizes. A semi-quantitative sample is obtained by measuring the net aperture and multiplying it by the distance the net is pulled through the water column to get an estimate of water volume sampled. The species collected can then be counted individually or their abundances estimated using categories. A vertical tube sampler (Helm, 1999) can be used to obtain a truly quantitative sample. Horizontal tube samplers collect only portions of the water column, whereas vertical tube samplers collect the entire water column and the benthos. However, many vertical tube samplers are disruptive to the pool bottoms.

At the very least, monitors should sample for the presence or absence of LB's. For most land stewards' needs, data obtained from semi-quantitative methods should be adequate. Described below is an example of a semi-quantitative wet-season sampling method that has been successfully utilized for more than 20 years by the author, and adopted by numerous large branchiopod biologists, including USFWS biologists (Helm Biological Consulting, 2002).

Example of successful semi-quantitative wet-season sampling. Each habitat chosen for sampling is first viewed for active LB's prior to entering the water. Any LB's observed should be quickly netted, viewed with the aid of a hand lens to determine species, and released unharmed back into the environment from which they were obtained. If a positive species identification cannot be performed in the field, then voucher specimens should be taken.

A semi-quantitative sample is then taken to determine the relative abundance of LB's and other macroscopic (> 2 mm) aquatic invertebrates (described below under *"Other Macroscopic Aquatic Invertebrates"*) as follows. A dip net is lowered vertically into the deepest portion of the inundated pool (usually the center) and rested on the bottom. The dip net is then moved in the direction of the longest axis of the pool for approximately one meter. In instances where half of the pool length is less than one meter in length, the dip net should be repositioned in the deepest portion of the pool and moved in the opposite direction for the remainder of the one-meter sample. If the water column is shallower than the dip-net aperture height, the volume of water per sweep should be calculated by the horizontal distance the net is moved multiplied by the width of the dip net, multiplied by the depth of water. After the completion of each sample sweep, the contents of the net are examined for LB's. LB's captured are identified to species with the use of a hand lens, and their relative abundances are recorded in one of five categories: rare (R, ≤ 2 individuals), not common (NC, 3-10 individuals), common (C, 11-50 individuals), very common

(VC, 51-100 individuals), and abundant (A, > 100 individuals) on standardized data sheets (Appendix A). These abundance categories are generally sufficient to detect long-term population trends because LB population densities within a given pool or conservation/preserve site can differ greatly on an annual basis. These annual population shifts are due mainly to climatic factors (surface weather) but can also occur from predation or human disturbances (e.g., pollution, disking, pesticides, etc.).

After the taxonomic identifications and enumerations are quickly completed, the contents of the net are returned back into the habitat from which they were collected. If LB's are not detected during the semi-quantitative sampling method, then the entire habitat is sampled as follows. Starting at one end of the habitat, the net is moved from one side of the pool to the other in a zigzag fashion, until the opposite end of the pool is reached. During this procedure, the net should be bounced along the pool bottom to encourage LB's to move up into the water column from hiding places for easier capture and viewed often for evidence of LB's. If still no LB's are captured, then additional netting should take place in specific locations within the pool that may have not been sampled during prior efforts. LB's detected using this alternative, non-quantitative method are noted as present by an "X" on the standardized field data sheet (Appendix A).

Pool depths (maximum and average) and size (or percent of surface area inundation if maximum pool size is known) are also estimated (see below under "*Hydroperiod*") which allows for concentration estimates of LB's and other macroscopic aquatic invertebrates to be calculated as number of individuals per liter of water (= number of individuals/net aperture area x length of sweep). These semi-quantitative data permit the relative abundances and richness of LB's and other macroscopic aquatic invertebrates to be compared between and among pools over time. Nonetheless, because of the various parameters that affect population abundances of LB's and other aquatic invertebrates within a given pool, caution should be used when comparing population densities or population estimates among differing pools. For additional details on this method, refer to Helm and Noyes (2016).

In addition to LB abundances, other ecological data that can be collected during wet-season surveys include micro/macro aquatic invertebrate and plant diversity (biological); habitat size, depth and ponding durations, and edge effects (physical); and water quality metrics (chemical). Such valuable data are often underutilized in determining the health of an ecosystem.

Other macroscopic aquatic invertebrates

The presence or absence and densities of several macroscopic aquatic invertebrates may allow land stewards to assess changes in a given habitat. For instances, increases in Dipterans such as midge fly larvae (Chironomidae) and mosquitoes (Culicidae) could be indicators of an altered habitat, such as increased ponding duration from artificial water sources or nutrient loading (fertilizers, detergents) from adjacent parcels of land.

LB competitors and predators

The presence and abundance of potential LB predators including macroscopic aquatic invertebrates, amphibians, and birds can be performed concurrently with LB wet-season sampling. However, seines may be needed in lieu of dip nets to quantify amphibian uses; general counts of avian use will suffice for bird abundances. Monitoring macroscopic aquatic invertebrate occurrences and abundances may distinguish LB population decreases caused by predation from those caused by other factors. For example, huge population losses of active LB's and other macroscopic aquatic invertebrates can occur within short periods from for-

TABLE 6. Most common predators of large branchiopods.

	Common Name	Scientific Name
Arthropods	crayfish	*Procambarus* sp.
	oarsmen	Notonectidae
	predacious diving beetle	Dytiscidae
	Red swamp crayfish	*Procambarus clarkii*
	vernal pool tadpole shrimp	*Lepidurus packardi*
	water scavenger beetle	Hydrophilidae
Amphibians	American bullfrog	*Lithobates catesbeianus*
	California tiger salamander	*Ambystoma californiense*
	Sierran tree frog	*Pseudacris sierra*
	western spadefoot	*Spea hammondii*
Predatory Fish	green sunfish	*Lepomis cyanellus*
	inland threespine stickleback	*Gasterosteus aculeatus microcephalus*
	prickly sculpin	*Cottus asper*
	striped bass	*Morone saxatilis*
	western mosquitofish	*Gambusia affinis*
Birds	American avocet	*Recurvirostra americana*
	American widgeon	*Mareca americana*
	black-necked silts	*Himantopus mexicanus*
	cattle egret	*Bubulcus ibis*
	cinnamon teal	*Anas cyanoptera*
	great blue heron	*Ardea herodias*
	great egret	*Ardea alba*
	greater yellow legs	*Tringa melanoleuca*
	killdeer	*Charadrius vociferous*
	mallard	*Anas platyrhynchos*
	snowy egret	*Egretta thula*

aging waterfowl, such as the American widgeon (*Mareca americana*).

There is little information regarding the impact of competition among the differing LB's (Belk, 1991; Hathaway and Simovich, 1996). Because the great majority of invertebrates occurring in vernal pools are native (or assumed to be), any competitive forces have likely "played out." That is, co-occurring LB species and other filter-feeding crustaceans (ostracods, copepods, and cladocerans) have probably achieved niche-partitioning. However, there are numerous predators of LB's; the most common are listed in Table 6.

Macroscopic plant community

<u>Problems caused by certain plants and thatch</u>. Although vernal pools vary in vascular macroscopic plant communities on a seasonal and annual basis, deviations in the ratio of nonnatives to natives may be an indicator of more long-term changes. For example, most vernal pools support Italian ryegrass and/or Mediterranean barley along their edges, but an increase in the relative cover of these nonnative grasses may be an indication of reduced ponding depth or duration.

Other nonnative species have the ability to significantly change the vernal pool habitat they occupy (DiTomaso et al., 2013). For example, waxy manna grass (*Glyceria declinata*), though perennial, grows as a facultative annual in vernal pools. It is caespitose (growing in clusters or tufts), with long surface- floating leaves up to 6.75 inches (17 cm) in length. At high densities, its leaves can cover most of the pool's water surface area, which reduces the effects of wind in mixing the pool's water. This effect can in turn reduce the amount of DO and decrease turbidity. Its fine root masses growing at or just below the soil surface can change nutrient cycling, and its biomass can increase transpiration rates and effect BOD. Low DO concentrations are inappropriate for many vernal pool endemic invertebrates, such as the vernal pool fairy shrimp, and can create habitat for mosquitoes and other low DO-tolerant invertebrate species. In short, waxy manna grass not only competes with native vernal pool grass species (for sprouting sites, nutrients, and light sources) but has dominating effects (increase biomass, shading water surface, nutrient cycling) that can compromise the integrity of vernal pools. Waxy manna grass generally invades recently disturbed areas (bare ground) in pools with moderate depths but with minimum surface areas that are not directly exposed to winds.

In the northern half of the Sacramento Valley, the native common spikerush (*Eleocharis macrostachya*) can become a problem in vernal pools. This perennial rhizomatous species can dominate pool basins, squeezing out endemic plants. Dense growths of this emergent species serve as attachment for filamentous algae, and both obstruct LB movements.

Dead and dried vegetation (thatch) in grasslands adjacent to vernal pools can restrict overland flows to vernal pools and can act as a sponge, absorbing water (Barry, 1998). Hence, upland thatch can reduce surface runoff, thereby decreasing pool hydrologic inputs as well as increasing eutrophication via decomposition and nutrient release (Bartolome et al., 2001).

Some upland plants that can cause thatch buildup include ripgut brome (*Bromus diandrus*), medusa head (*Elymus caput-medusae*), and yellow star thistle (*Centaurea solstitialis*). Yellow star thistle has been shown to deplete soil moisture and alter hydrologic cycles in annual grasslands (Enloe et al., 2005). According to Jetter et al. (2003) the depletion of soil moisture by yellow star thistle compared to annual grasslands is equivalent to a loss of 15-25% of mean annual precipitation. Thus, yellow star thistle infestations can actually create drier than normal conditions in a vernal pool landscape, even in subsequent years with average rainfall (Gerlach et al., 1998).

One of the most germane studies regarding the effects of thatch in vernal pools, as well as hydroperiods and nutrients, on California endemic LB's is the work by Jamie Kneitel and colleagues (2017). They studied the vernal pool fairy shrimp, vernal pool tadpole shrimp, California clam shrimp, and California fairy shrimp in mesocosms (outdoor experimental systems that allow the natural environment to be examined under controlled conditions – in plastic wading pools). Kneitel et al. (2017) found that thatch treatments (where thatch was added to the pools) significantly reduced DO and that densities of vernal pool fairy shrimp, California fairy shrimp and vernal pool tadpole shrimp significantly decreased with thatch treatment (native or nonnative plants). However, the California clam shrimp was unresponsive to thatch. This should not be surprising, since the California clam shrimp is the most tolerant of all the endemic LB's, to low DO. Lis and Eggenam (2000) at the Dales Lake Ecological Reserve found that an increase in decaying vegetation in pools decreased densities of California fairy shrimp.

Techniques for monitoring vernal pool plant cover. The composition of macroscopic vegetation in vernal pools, as well as uplands, can be monitored in numerous ways (e.g., Mueller-Dombois and Ellenberg, 1974; Barbour et. al., 1987; Thomas et al., 1998). The common methods are relevé, permanent transects, and visual residual dry matter (RDM) estimates. The use of the first two methods requires the surveyor to be experienced with the flora of vernal pool terrains.

Relevé method. The relevé method (Mueller-Dombois and Ellenberg, 1974) can rapidly yield information on species composition, species dominance, and cover as follows:

- all plant species present (species richness),
- relative cover of all plant species using the Daubenmire or Braun-Blanquet cover class scales (Barbour et al., 1987) or ocular estimates, and
- absolute vegetative cover.

Permanent transects. The focus of permanent transect monitoring is to reveal trends in vegetation structure and species composition over time in the area between two points. This sampling method entails collecting point intercept data at regular intervals (e.g., a 1-foot interval) on a measuring tape along a permanent transect (e.g., a 100-foot tape), resulting in 100 data points per transect. The point intercept data rapidly yields relative percent-cover data for each species as well as absolute cover for the entire transect. This method is considered one of the most objective ways to sample cover (Bonham, 2013). The observer needs to decide only whether a point intercepts a plant species, plant litter, rock, or bare ground. No cover estimates are required.

Residual Dry Matter (RDM) estimates. RDM is a standard method to assess the level of grazing use on annual grass rangelands, which can be applied to vernal pool vegetation in some situations. Residual dry matter is the senescent plant material left standing or on the ground at the end of the growing and grazing season. It indicates the combined effects of the season's biomass production and its consumption by all grazing animals. RDM estimates are generally recorded in pounds per acre and can be obtained by clipping, drying, and weighing (Clawson et al., 1982) or less invasively by a visual estimate (Guenther and Hayes, 2008).

The locations of different plant species patches, in particular invasive species, can be mapped with a GPS unit or sketched on aerial photographs.

Microscopic plant community

With the exception of the marine assemblages (Haptophyta, Phaeophyta, Rhodophyta), most of the algae groups (Bacillariophyta [diatoms], Charophyta [stoneworts], Chlorophyta [green algae], Chrysophyta [golden-brown algae], Cryptophyta, Dinophyta [dinoflagellates], Euglenophyta, Xanthophyta. [yellow-green algae]) are present in vernal pools.

In general, a significant increase in many of the above-mentioned algae groups may be an indicator of eutrophication. Chlorophyll-a, a component of the cells of most plants, can be used to measure the concentration of algae in water. It is interesting that Kneitel et al. (2017) found chlorophyll-a positively correlated with the four CV endemic LB's. Other water quality parameters, especially nitrate and phosphate, may be sampled as indicators of eutrophication.

Water quality

Since the majority of water quality parameters are interrelated, it is advisable to standardize timing of water quality parameter collections to minimize diurnal effects. For example, pH decreases with an increase in temperature, and increases from morning to midday due to photosynthesis. Similar to pH, DO decreases with an increase in temperature. In contrast, conductivity increases with increased water temperatures. Furthermore, since the properties of water are well understood, modeling diurnal changes in water temperature, DO, pH, and several other water quality parameters in a given pool can be accomplished when water volume is estimated and surface weather observations (e.g., air temperature, wind speed and direction, humidity) are known. Relative to LBs, some of the more important water quality parameters to monitor are discussed below.

Turbidity and DO. High levels of total suspended solids will increase water temperatures and decrease DO levels because suspended particles absorb more heat from solar radiation than water molecules will (Davies-Colley and Smith, 2007). DO levels will drop because warm water holds less DO than cold water (Environmental Protection Agency [EPA], 2012b). Turbidity can also inhibit photosynthesis by blocking sunlight. The higher the turbidity levels, the less light that can reach the lower levels of water. Reduced photosynthesis decreases plant survival and thus DO output. Turbidity can result from several factors including suspended clay particles, bacteria and plankton, particulate organic matter, and the pigments caused by the decomposition of organic matter (Bhatnagar and Devi, 2013).

Yet, several of the endemic LBs – including the Conservancy fairy shrimp, vernal pool tadpole shrimp, and California clam shrimp – flourish in highly turbid pools. The high concentrations of these LB's is supported from an increase in food availability, organic matter attached to clay particles (Barclay and Knight, 1981; Mizutani, 1982; Thiéry, 1991), and a decreased effectiveness of visual predators. Kneitel et al. (2017) found turbidity positively correlated with vernal pool tadpole shrimp and California clam shrimp.

In regard to DO, Kneitel et al. (2017) found it positively correlated with vernal pool fairy shrimp but negatively with vernal pool tadpole shrimp and California clam shrimp. This is not surprising, since fairy shrimp, in general, have lower tolerances to low DO than clam shrimp and tadpole shrimp. Evolutionarily, fairy shrimp may have lost their carapace to allow them to swim upside down in order to reach the surface water, exposed to the atmosphere, when DO concentrations are low.

Temperature. Water temperature is important in numerous ways. It plays a role in cysts hatching (Lanway, 1974; Ahl, 1991; Eriksen and Belk, 1999), oxygen uptake, pH, metabolism, and mobility. For every increase of 10^{o} C in water temperature, the metabolic rates of LB's as well as other aquatic ectotherms (animals dependent on external sources of body heat) will generally double (Eriksen and Brown, 1980). Plant respiration and photosynthesis generally increases with warm water temperatures, especially for algae (Wetzel, 2001). The solubility of oxygen and other gases will decrease as temperature increases (Perlman, 2013). The warmer the water, the less oxygen it can hold. High water temperatures can increase the solubility and thus toxicity of certain compounds (Washington State Department of Ecology, 1991), including heavy metals such as cadmium, zinc and lead as well as compounds like ammonia (Bhadja and Vaghela, 2013; Wurts, 2012).

Conductivity. Conductivity is a measure of water's capability to pass electrical current. A sudden increase or decrease in conductivity in a pool can indicate pollution. Agricultural/urban runoff or sewage will increase conductivity due to the added chloride, phosphate and nitrate ions (EPA, 2012a). Other organic compounds, including petroleum products, would decrease conductivity as these elements do not break down into ions (LCRA, 2014). In both cases, the additional dissolved solids will have a negative impact on water quality. Conductivity is dependent on water temperature, salinity, and total dissolved solids (Talley, 2000). For every

1° C increase in temperature, conductivity values can increase 2-4% (Miller et al., 1988). An increase in salinity (which is one component of conductivity) can decrease DO (Fondriest Environmental, 2014a). In general, LB's are euryhaline (can adapt to a range of salinities); however, high conductivity was found to be negatively correlated with vernal pool fairy shrimp and California fairy shrimp (Kneitel et al., 2017).

pH. LB's occur mostly in neutral to alkaline water habitats: no California endemic LB's have been recorded inhabiting waters with a pH of six or less (Eriksen and Belk, 1999). Of the California endemic LB's, California fairy shrimp is known to occur in the lowest pH waters (pH of 6.5, Eriksen, personal communication). Given this constraint, extirpations of LB's may occur in occupied habitats that are increasingly acidic.

Hydroperiod

Perhaps the most important factor dictating the presence or absence of LB's is hydroperiod (Helm, 1998). A wetland's hydroperiod is the seasonal pattern of inundation and water levels. What is most important for LB survival is the minimal duration of inundation ("ponding duration"), or more specifically, the minimum number of consecutive days that a pool is inundated during any given season. Depth of ponding is also important because shallow pools, regardless of their ponding durations, can be inhospitable to certain LB species due to temperature extremes (especially ice), reduced escape cover, increased probability for desiccation, and reduced respiration (insufficient amount of water for phyllopods, or "gilled feet").

Using pool depths and ponding areas as a surrogate for hydroperiod, Helm (1998) found significant correlations between hydroperiod and the life history parameters (e.g., maturation and reproduction rates) of endemic LB's. That is, fast-maturing species usually occurred in the more ephemeral habitats, and slower-maturing species occurred in habitats with long ponding durations.

Kneitel et al. (2017) found that CV endemic species densities differed according to hydroperiod treatments. Stable-hydroperiod treatment consisted of a continuous 20-week inundation. The unstable-hydroperiod treatment consisted of continuous inundation for nine weeks followed by desiccation for two weeks, and then another nine-week period of continuous inundation. Although California fairy shrimp densities were not affected by hydroperiod treatment, vernal pool fairy shrimp densities increased with the unstable-hydroperiod treatment, and California clam shrimp and vernal pool tadpole shrimp densities decreased with an unstable-hydroperiod treatment. Why? Life cycles! The vernal pool fairy shrimp is fast maturing. On the other hand, the California fairy shrimp has a moderate maturation rate, and both the vernal pool tadpole shrimp and the California clam shrimp are slow maturing (Helm, 1998). The desiccation and re-inundation of the unstable hydroperiod allowed a second cohort of vernal pool fairy shrimp to emerge (Kneitel et al., 2017). The California fairy shrimp generally has only one cohort per inundation cycle and one cohort per wet season, and therefore densities were unaffected by hydroperiod treatments.

Measurements of maximum depth, average depth, and surface area when inundated are important parameters for determining the hydroperiods of pools for a particular wet season. However, because of fluctuating seasonal rainfall, especially in Southern California, measuring the *maximum potential* of these parameters (potential maximum depth, potential average depth, and potential maximum surface area) may help explain why LB species occur or why they do not. Potential maximum ponding depth is the maximum depth that a vernal pool can potentially pond water. It is measured by the

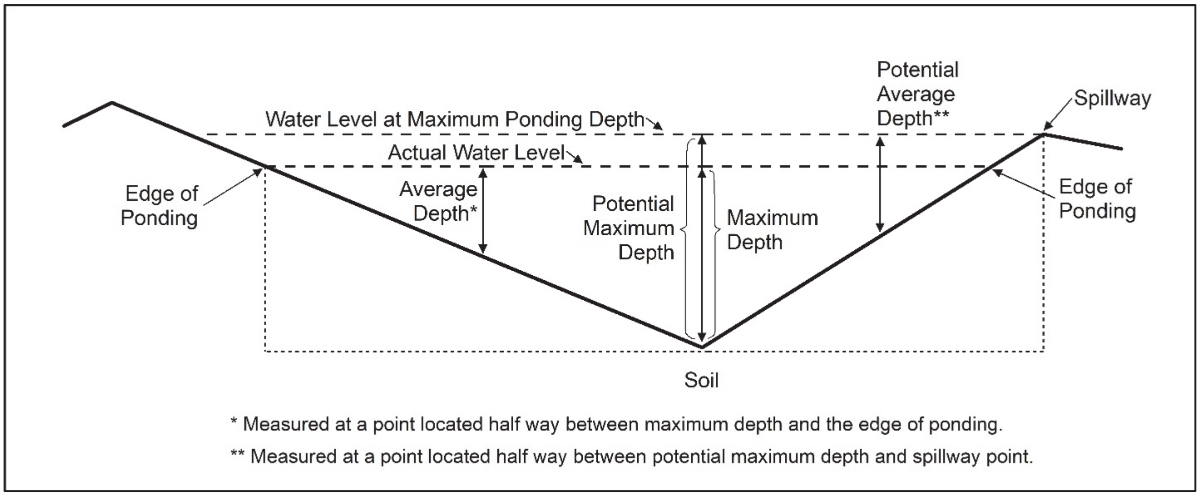

FIGURE 2. Cross-sectional graphic of a hypothetical vernal pool and various ponding measurements.

vertical distance from outlet (spillway) elevation to the deepest point in a pool (Figure 2). Potential average ponding depth is the average depth that a pool can potentially pond water. It can be calculated by taking several depth measurements (i.e., vertical distance from the spillway elevation to its substrate) across the pool, and dividing their sums by the number of sample measurements. A quicker method is measuring the depth halfway between the spillway elevation and the lowest point in the basin (Figure 2). Potential maximum ponding surface area is the area of water contained by a pool before exiting through the spillway. An estimate of potential maximum ponding volume can be obtained by multiplying potential average ponding depth by potential maximum ponding surface area. In specific cases the potential maximum inundation area and depths are unattainable due to topography and rainfall. Therefore, these estimates may be obtained from extrapolations of maximum rainfall events from the evidence of an ordinary high-water mark (e.g., hydrophytic vegetation, water-lines, drift marks, etc.).

Edge effects and other disturbances For vernal pool terrains, edge effects include access points – off-site contributory water sources whose waters enter during the dry season or are polluted; vandalism; foot trespass; vehicle ruts; excessive cattle tramping/punch and excretions; litter and other pollution; and exotic species introductions. Monitoring for such disturbances can play an important role in understanding changes in habitat quality and LB species population declines. Since most of these will occur along the periphery, monitoring a buffer within the outside fence line as well as areas around troughs, mineral licks and trails (if grazed) is suggested. Edge-effect monitoring should occur during both the wet and the dry seasons.

Hydrological inputs into vernal pools from urban or agricultural runoff, especially during the dry season, can cause “false starts” for LB’s. “False starts” describes the hatching of LB’s when the conditions of the pool (e.g., depth, duration and or water temperatures) are not conducive to completing their life cycle. Although false starts can occur naturally from too-early or too-late isolated rain events, they generally are more severe when they are anthropogenic, such as irrigation water entering vernal pools during the non-rainy season.

MANAGEMENT

It is rare that a land steward can focus on a single species or suite of species, such as endemic LB’s. More than not, land stewards are challenged with maintaining if not increasing overall species diversity on a parcel. According to the Intermediate Disturbance Hypothesis (Wilkinson, 1999), the highest levels of biotic diversity are maintained at intermediate levels of disturbance. In vernal pool terrains, generally vegetation management, including the removal of phytomass (plant biomass), especially thatch and invasive weeds, can help achieve this goal.

Most vernal pool terrains within the Sacramento Valley, especially preserved lands, have too much phytomass. Much of this has resulted from parcel splits that are too small to graze or burn, or large mitigation and preserve lands in which regulatory agencies demand high residual dry matter (RDM) rates. A few examples of LB extirpations suspected from lack of vegetation management follow:

- vernal pool tadpole shrimp and vernal pool fairy shrimp from pools surrounding the Redding Airport, Shasta County,
- vernal pool fairy shrimp from pools surrounding the Lincoln Airport, Placer County,
- vernal pool tadpole shrimp from the Beale Air Force Base Lincoln Receiver Site, Placer County, and
- vernal pool fairy shrimp and vernal pool tadpole shrimp from east of Mather Lake, old Mather Air Force Base, Sacramento County.

Vegetation Management

A successful long-term management program for a vernal pool landscape should be designed to include combinations of control techniques which have been tailored to the site, since a single method rarely gives sustainable control of vegetation (weeds, thatch, and desired plants). Livestock grazing, prescribed fire, mechanical and hand removal, herbicide applications, and revegetation are common vegetation management tools that are described below. These techniques can be applied to grasslands surrounding vernal pools, and in some cases to the pools themselves.

Livestock grazing

With the exception of fire, livestock grazing is one of the oldest management tools for maintaining species diversity in vernal pool terrains. Historically, poorly managed livestock grazing has contributed to the reduction in biodiversity and decline and extirpation of numerous California species (Fleischner, 1994; USFWS, 1994; HilleRisLambers et al., 2010). Recently, an ever-increasing number of articles have been published concerning the ecological benefits of grazing (Germano et al., 2001; Barry, 2011; Germano et al., 2012; Gennet et al., 2017; Bartolome et al., 2014; Larson, 2016). Moderate livestock grazing can play an essential role in assuring the long-term health of the habitats and resources within vernal pool terrains. Proper livestock grazing is effective in the reduction of herbaceous thatch layers, control of some invasive species, increased rate of nutrient cycling, and the promotion of overall resource health in grassland-based community types. Livestock grazing can be a more effective management tool than prescribed fire in areas when fire-sensitive resources are present or when conditions necessary for proper fire management do not correspond with appropriate burning conditions (e.g., yellow star thistle fire-based control in mid-summer).

The greatest benefit of livestock grazing to LB's is the maintenance or improvement of vernal pool hydroperiods. Grazing reduces thatch buildup in uplands as well as vernal pools. Grazing during the wet season can also increase soil compaction (Gifford and Hawkins, 1978), resulting in increased hydrologic inputs to pools from adjacent uplands (Liacos, 1962). Bauder (1987) found hydroperiod maintenance was paramount for maintaining native vernal-pool plant species; when ponding duration decreased, exotic plants increased. Thatch reduction within a pool increases the pool's ponding duration and depths (Marty, 2005), and reduces both organic build-up and BOD. Hoof prints within vernal pools increase micro-topography, increasing plant species richness and decreasing overall plant cover (Barry, 1998; Robins and Vollmar, 2002). This allows open-water areas needed for vernal tadpole shrimp and California fairy shrimp. However, too much grazing can have a detrimental impact to CV endemic LB's caused by increased fertilization and sedimentation (Croel and Kneitel, 2011a).

Common spike-rush can be managed by grazing since it is quite palatable and nutritious to livestock and other grazers, including water fowl. There is some evidence that waxy manna grass may be targeted by cattle prior to establishment of its aquatic leaves (Rozumowicz-Kodsuntie personal communication). See Appendix B for details on efficient grazing regimes and comparisons of livestock groups.

Prescribed fire

Fire has long been an integral component of healthy grassland communities. When appropriately planned and executed, prescribed fire is effective in the reduction of herbaceous thatch layers, control of some invasive plant species, increased rate of nutrient cycling, and the promotion of overall resource health in grasslands. Prescribed fire is often a more effective management tool than grazing for control of invasive species that are not palatable to livestock during the phenological stage in which they can be most effectively controlled (e.g., medusahead grass when "setting" seed [seed developing, but prior to shatter]). Because vernal pools are generally located in low micro-topography and support lower frequency and less phytomass in comparison to the adjacent annual grasslands, fires generally burn around them.

The three most negative factors of prescribed fire are air quality problems near populated areas, liability issues if the fire becomes uncontrolled, and the amount of time and effort spent in coordination with local resource and fire agencies (e.g., California Department of Forestry and Fire Protection). Prescribed fire should be planned and conducted only by trained personnel familiar with fire ecology and behavior, and in close cooperation with resource and fire agencies.

Mechanical and hand removal of vegetation

Large-scale removal of vegetation by mechanical means usually involves tillage or mowing.

<u>Tillage</u>. Tillage – whether it be plowing, disking or harrowing for the removal of vegetation, even for fire breaks – is not advisable in a vernal pool landscape because it opens up the soil for invasive plant infestations and erosion. Tillage also tends to "smear" vernal pools and swales across the landscape, thus changing the habitat for LB's as follows:

- Increases the ponding surface area while decreasing the ponding depth and duration of vernal pools and other seasonally-inundated habitats. This negatively effects LB's in several ways:
  a) Reduced ponding depth tends to increase the amount of rooted emergent vegetation, which can be used by LB predators (e.g., predaceous diving beetle larvae) for ambush cover.
  b) Increased rooted vegetation also increases attachment for filamentous algae. Fairy shrimp have been known to get entangled in filamentous algae and die. The California fairy shrimp avoid filamentous algae (Helm, 1999).
  c) Reducing water depth allows for increased water temperature fluctuations. Given two pools with the same water volume, depth to impervious layer, and climatic condition, the pool having the larger surface area and shallower depth will undergo more extreme diurnal temperature fluctuations in comparison to the pool with a smaller surface area and

greater depth. Increasing the upper water temperatures and decreasing the lower water temperature of a pool adds more strain to LB's in an already stressful environment. The young of vernal pool fairy shrimp die with the onset of warm water temperatures (Helm, 1998).

d) LB's may not be able to complete their life cycle (hatch, mature, mate, and lay eggs) in a basin that has had its ponding duration decreased.

- Reduces the proportion of cysts in a cyst-bank available to hatch, by turning over the soil profile (i.e., top goes to bottom and vice versa).
- Decreases soil compaction, therefore increasing the soil's ability to absorb water, thus delaying the onset of ponding and decreasing ponding duration.

In addition, tillage has negative impacts on resident wildlife utilizing the annual grassland habitats in vernal pool landscapes. The disks cut through burrows and can injure or bury numerous common (rodents, snakes, and California toad [*Anaxyrus boreas halophilus*]) and special-status species (e.g., American badger [*Taxidea taxus*], San Joaquin kit fox [*Vulpes macrotis mutica*], various kangaroo rats [*Dipodomys* spp.], San Joaquin antelope ground squirrel [*Ammospermophilus nelsoni*], western spadefoot [*Spea hammondii*], California tiger salamander, blunt-nosed leopard lizard [*Gambelia sila*], and western burrowing owl (*Athene cunicularia hypogaea*]). Therefore, tillage even for firebreaks is not recommended.

Mowing. Mowing is preferred for large-scale vegetation removal. However, when the cut biomass is not removed, this cut vegetation must break down through the decomposition cycle; mulching hastens the process (Bartolome et al., 1980). Mowing has proven useful in the control of several exotic plants (DiTomaso et al., 2013). Properly timed mowing has been demonstrated to be a successful tool for the control of yellow star thistle (Benefield et al., 1999). Kyser et al. (2007) found that removing the thatch in the fall can provide better than 50% reduction in medusahead the following year. The optimum time for mowing most annual species is in the flowering stage before seed development. It should be realized that continuous mowing prior to seed-set can select for lower-statured forms (due to phenotypic plasticity) of the same species, or for different species. Also, caution is needed when using mowing to manage vegetation, since mowing can create a fire hazard because of sparks generated by contact of the equipment with rocks.

Hand Removal. Although labor intensive, hand removal can be effective in removing unwanted vegetation. Its effectiveness can be increased with the aid of hand tools (spade, trowel, pick, etc.) and its costs can be reduced by volunteers (weed-pulling days). A benefit of hand removal is that desirable species, if present, can be left in place. Hand removal has been fairly successful in reducing waxy manna grass infestations from targeted vernal pools (Noyes, personal communication).

Herbicides

With careful application, herbicides are important management tools for the control of invasive plant species. Herbicides can be applied to grasslands in vernal pool landscapes by a number of methods, including fixed-wing aircraft, helicopters, tractors, ATV/UTVs, ground applicators, backpack sprayers, and rope-wick applicators (Manning and Miller, 2011). While prescribed fire and livestock grazing are generally preferred management tools for invasive species management, herbicide application may be used in situations where prescribed fire and livestock grazing are not practical or prove to be ineffective in invasive species control. Herbicides should only be used when the benefit to the resources outweighs any potential ecological costs. Special regulations may apply if rare, threatened, or endangered species are present in the management area.

Revegetation
Using desirable and competitive native plants, revegetation is another approach to achieving long-term, sustainable suppression of weed population growth in vernal pool landscapes, while providing high forage production and plant diversity (Borman et al., 1991; Lym and Tober, 1997). The choice of species used in a revegetation effort is critical to its success. These species need to be adapted to the specific biotic and abiotic conditions (soil, elevation, climate, and precipitation) of the site (Rinella et al., 2012).

Biological control
*"*Biocontrol" is the method of introducing and releasing exotic species to control another exotic species (McFadyen, 1998). Unfortunately, there are few biological control agents, since they are generally specific to a species or to suites of species and the process of approval is extremely long. According to DiTomaso et al. (2013) there are no or active biological control agents for the problem weedy grasses in vernal pool terrains (e.g., waxy manna grass, Mediterranean barley, Italian rye grass, hare barley [*Hordeum murinum* ssp. *leporinum*), rip-gut brome [*Bromus rigidus*]). However, there are some biocontrols for several weedy forbs. Randall et al. (2017) reports varying success on yellow star thistle control from various invertebrate species.

Information sources
Refer to Engler and Chapin (1995), Pollack and Kan (1998), D'Antonio et al. (2002), Borgias (2004), DiTomaso and Johnson (2006) for details on vegetation management using prescribed fire; Thomsen et al. (1996), Olson (1999), Frost and Lauchbaugh (2003), Marty (2005), Barry (2006), and Jackson and Bartolome (2007), for details on grazing; Tu et al. (2001) and DiTomaso, et al. (2007) for exotic plant management; McFadyen (1998), Flint and et al. (1998), DiTomaso et al. (2013), and Randal et al. (2017) for biological control of weeds; and Clark et al. (1998) and Vollmar and Platenkamp (2009) for management and effectiveness of small vernal pool preserves, respectively.

Invasive Wildlife Species Control

As mentioned above under Monitoring, there are numerous predators of LB's. Most of these are considered native, and generally not considered to be a limiting factor in LB population declines. Therefore, management efforts should concentrate on removal or reducing the populations of exotic predators of LB's, especially American bullfrogs (*Lithobates catesbeianus*), crayfish, and predatory fish. Repeated seining during the wet season with a mesh size sufficient to entrap these exotic predators, yet large enough to allow the majority of macroscopic aquatic invertebrates to pass unharmed may be effective in reducing predation on LB's during a given ponding season. However, without removing the source population, this method would need to be repeated seasonally, when occupied.

American bullfrogs
American bullfrogs require a perennial wetland habitat for their larvae to complete metamorphosis. Although they can complete metamorphosis rather quickly at 10-40 days, they usually do not start the process until at least nine months of age (Bruening, 2002). So breeding within vernal pools is usually not an issue. However, young bullfrogs will disperse over great distances, and their diet consists primarily of invertebrates (Taylor and Michael, 1971). Eradication efforts should first be prioritized within perennial onsite breeding habitats that are occupied by American bullfrogs. If possible, offsite sources should be targeted as well. Air rifles have been successfully utilized for

American bullfrog removal (Alverez, personal communication).

Fish

Although fish will occasional occupy a few vernal pools, their occurrence year after year should be of concern. Prior to any eradication effects, positive fish species identifications should be performed because not all fish occupying vernal pools are predators (e.g., California roach [*Hesperoleucus symmetricus*], fathead minnow (*Pimephales promelas*], and golden shiner [*Notemigonus crysoleucas*]). Generally, fish enter vernal pools from waterways (ephemeral drainages and swales) that hydrologically connect to perennial habitats during flood conditions. An understanding of these fish species' life histories and when, where, and how they are entering the target vernal pool site will prove helpful in their management.

Crayfish

There are several exotic species of crayfish that have been introduced into California: red swamp crayfish (*Procambarus clarkia*); signal crayfish (*Pacifastacus leniusculus*) and associated cryptic species (Larson et al., 2012); virile crayfish (*Orconectes virilis*); white river crayfish (*Procambarus acutus*); and Florida crayfish (*Procambarus alleni*). The red swamp crayfish can be a transient visitor to vernal pools. It has the ability to tolerate various water conditions, travel short distances over land, and wander long distances during breeding (Nagy et al., 2017). However, due to summer desiccation, they are highly unlikely to successfully breed in vernal pools.

Edge Effects and Other Disturbances

One of the greatest challenges of land management is minimizing impacts from adjacent offsite sources. Hopefully these disturbances are brought to managers' attention from the monitoring program. Obviously, management is dependent on the type and severity of the disturbance. To minimize edge effects, pursue opportunities to expand the managed lands through fee title, easements, or other means. In general, the smaller a parcel and the longer it is isolated, the less likely it will continue to support LB's, regardless of whether the habitats onsite appear to be suitable for their presence (Helm, in preparation). The absence of LB's from suitable habitat (e.g., adequate inundation depth and duration) is most likely attributed to chance extirpations from disturbance regimes without recolonization from dispersal agents. Many of the dispersal agents (e.g., wading birds, shorebirds, and waterfowl) (Proctor et al., 1967) for LB's avoid human disturbances. Smaller sites have less of a buffer from human presence. In addition, smaller parcels generally have fewer types of wetlands, such as perennial stock ponds or reservoirs, which can attract migrating waterfowl from high-elevation flights. Waterfowl are important dispersers of vernal pool seeds, cysts (Proctor et al., 1967), and other propagules. Therefore, increasing the size of an existing protected vernal pool parcel can have positive benefits to LB's as well as other species occurring onsite.

Financial Management

Land stewards often have to wear several hats, and sometimes the financial cap is a tighter fit for the biologist. However, without good financial management, monitoring and management of natural resources becomes limited. Adequate funding for long-term monitoring, maintenance, and management is crucial. If not in already in place, land stewards should seek funding to establish a perpetual stewardship endowment (i.e., the principle amount is sufficient to generate annual interest to cover monitoring, maintenance, and management costs).

Financial management can be as simple as skipping or deferring monitoring, or portions thereof, in drought years or reducing the number of site visits (survey rounds) or the number of pools sampled. It is not uncommon for land stewards to sample only 10% of the pools each year, such that after 10 years all of the pools onsite have been sampled (Noyes, personal communication). Obviously, the amount and quality of data collected must be weighed against reduced costs. Yet, financial management is not just about being thrifty; it is also about planning for the future – gaining greater know-ledge, finding more funds and assistance, and acquiring more land. A good financial manager should not rely on interest rates to meet the rates of inflation, nor the monitoring and management methods of today to support the conservation issues for tomorrow. This financial side of management includes coordination of fund raisers and volunteers and hiring expert consultants for monitoring or staff training.

DISCUSSION

There is no one recipe for monitoring or managing LB's. Every tract of land is unique. Land stewards must understand the ecology of the habitats that they are entrusted to manage. Although depth and breadth of this ecological understanding is imperative, the most essential information to have involves the life histories of the target species and the hydroperiods of the vernal pools which they inhabit. This information may prove critical in light of the anthropogenic global climatic changes discussed below.

Climate Change Impacts to LB Vernal Pool Habitats

According to climatic change models in California (Pierce et al., 2018), the state will continue to experience increases in mean annual air temperature (global warming). In the Sacramento Area, a 4°F increase in temperature is expected to occur from the historic period of 1966-1999 to the future period of 2050-2070. During this same time period, annual precipitation is expected to decrease from 18.7 inches to 16.3 inches. The severity of drought is also expected to increase through time with precipitation events increasingly inconsistent in timing, duration, frequency and amounts. Similar patterns of increased temperatures, decreasing rainfall and drought are expected to occur throughout California. If these climate models are accurate, within the next 30-50 years California's vernal pools will suffer a decrease in ponding depth and duration, will be inundated less frequently and when they do inundate, water temperatures will be warmer than at present.

Impacts of Altered VP Habitats on Target LB Species

Bimodal Rainfall Events

In recent years, California has experienced bimodal rainfall patterns with two large portions of the annual rainfall at the beginning of the rainy season and again at the end of the season. If this pattern continues, many large branchiopod species populations could be negatively affected.

Frequency and abundance of upland ruderal species increase in vernal pools during drought conditions (Holland and Jain, 1984). Currently, a number of weeds are increasing in vernal pool terrains. The majority of our native plants have C3 photosynthetic pathways which are adapted to temperate climates with winter precipitation. It is interesting that the majority of the invading species have a C4 metabolism, which is adapted to tropical environments with fall/summer precipitation. Some C4 weeds that seem to be on the increase in vernal pool terrains include Bermuda grass (*Cynodon dactylon*), common purslane (*Portulaca oleracea*), crabgrass (*Digitaria sanguinalis*), green carpetweed (*Mollugo verticillata*), several euphorbia species (*Euphorbia* spp.), and several species of pigweeds (*Amaranthus* spp.). Even the nasty puncture vine (*Tribulus terrestris*) has been invading pools during the drying cycle in Madera County. If this bimodal rainfall pattern continues, we should expect an even greater number of these C4 invaders – making vegetation management increasingly more important.

Increased temperatures

As previously discussed, temperature influences several other water parameters including metabolic rates and photosynthesis production; dissolved oxygen and other dissolved gas concentrations; conductivity and salinity; compound toxicity; oxidation-reduction potential; pH, and water density (Wilde, 2006; Bhatnagar and Devi, 2013). These parameters can alter the physical and chemical properties of water (Fondriest Environmental, 2014b) which in turn can modify species assemblages.

Slight increases of water temperatures may have profound effects on vernal pool invertebrate communities as well. According to Shin and Kneitel (2019), as little as a 2°F increase in water temperatures shifted the abundances of invertebrate communities from specialists to generalists. In addition to ambient temperature increases, the reduction in annual rainfall associated with global warming will likely decrease the depth and ponding durations of vernal pools. This decrease in water volumes will likely increase diurnal temperature fluctuations, which may further exacerbate the physical and chemical water changes in vernal pools.

Reduced hydroperiods

Decreases in pool depths and ponding durations could create "false starts" for numerous macroscopic aquatic invertebrates. It is likely that all of the LB species occurring in California would be negatively affected by climate change. Given the speed with which these changes are occurring or expected to occur, these species are not expected to migrate into more hospitable habitats. It is very likely that the fairy shrimp, being the more specialized of the LB's, will fare worse than the tadpole shrimp and clam shrimp species. The southern fairy shrimp species, especially the Riverside fairy shrimp, are expected to suffer the most. Because its habitat of deep and long-ponding pools is very limited in its current range due to geology and climate, it is easy to predict that with decreased ponding depths and durations, as well as increased water temperatures, the Riverside fairy shrimp could easily be forced into extinction. In contrast, the two rarest CV endemic fairy shrimp, the longhorn fairy shrimp and the Conservancy fairy shrimp, may be less affected. These species occupy playa pools which generally pond even during drought conditions (albeit not at full capacity) and in which water generally lasts longer than these two species' maximum population longevities. The midvalley fairy shrimp and the San Diego fairy shrimp are also anticipated to undergo huge population losses because their habitats are the most ephemeral.

Much of this section is speculative, and scientific studies which investigate the effects of climate change to LB's species are needed. Specifically, detailed examinations of endemic species' responses to increased water temperatures and decreased ponding durations and depths, as well as seasonal timing of inundations, are warranted. The implications of future adverse impacts and even population losses for LB's as a result of expected climate change lend urgency to the need for current land stewards to manage for these wetland species' conservation, since habitat loss and alteration is already challenging their survival.

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APPENDIX A. Standardized wet-season field sampling form for large branchiopods and other macroscopic aquatic organisms.

Project: **Surveyor(s):** **Weather Cond:**

Date: **Time:** **Air Temperature(°F):**

Abundance: R = Rare (≤2 individuals), NC = Not Common (3-10 individuals), C = Common (11-50 individuals), VC = Very Common (51-100 individuals), A = Abundant (>100 individuals)

Hydrology: D = dry, N/P = not ponding, M=moist, S = saturated to surface, I/P = intermittent ponding, X = Present but not observed in 1 meter sample

LB Redroductive Status: i = immature, m = mature, g = gravid (with eggs)

Habitat Condition:
UD = undisturbed, D = disturbed - tt = tire tracks, t = trash, p =plowing
UG = ungrazed, G = grazed - C = cattle, H = horse, S = sheep, l = light grazing, m = moderate grazing, h = heavy grazing

		Present Depth (inches)		Potential Depth (Inches)		Surface Area (ft²)		Crustacea										Insecta											Mollusca			Turbellaria	Acari	Collembola	Other	Herps			
									Copepods			Large Branchiopods (LB)						Coleoptera			Hemiptera			Odanota		Diptera													
Pool No.	Water Temp (°F)	Max	Ave.	Max	Ave.	Present	Pot. Max	Ostracods	Calanoida	Cyclopoda	Cladocera	*LIOC*	*BRLY*	*BRME*	*LEPA*	*LYBR*	*CYCA*	Dytiscidae	Hydrophilidae	Haliplidae	Notonectidae	Corixidae	Ephemeroptera	Zygoptera	Anisoptera	Culicidae	Chironomidae	Trichoptera	Lymnaeidae	Physidae	Planorbidae	Micro-turbularian	Hydracarini		Other Invertebrate	Pseudacris	Other	Habitat Condition	Comments

APPENDIX B. Grazing regimes and livestock groups (in two parts—text and table)

An efficient grazing regime is based on a complex combination of five factors:

1. Grazing System – three general types
    a. Rotational grazing (rest and deferred)
    b. Continuous grazing
    c. First-last grazing
2. Timing – time of year and frequency of grazing
3. Intensity – stocking rates
4. Targeting – which areas to graze
    - Configurations - the location of water troughs and mineral licks can be used to influence where grazing occurs
5. Stock - type of animal to be used (Frost and Mosley, 2019)

Continuous grazing is a method of grazing livestock in which the animals have uninterrupted and unrestricted access to the rangeland throughout the year (known as year-long continuous grazing), or for a period of the year (referred to as growing-season continuous grazing) (Vallentine, 2001). Rotational grazing is the practice of moving grazing livestock between pastures (often called paddocks) as needed or on a regular basis. First-last grazing is designed to allow two or more groups of livestock, usually with different nutritional needs, to graze the same paddock but at different times (Walker, 1994; Coffey, 2001).

The four most common livestock groups are cattle, horses, sheep, and goats. It is important to understand the different types of grazers and how their foraging differs (see table, below). Foraging selection also differs within each of these livestock groups and is dependent on a multitude of variables including breed, age, body condition, gender, physiological stage (young, late pregnancy, lactation), individual variation and heritability, maternal role model, and personal experiences (prior illness) of livestock as well as topography and climate (Frost and Mosley, 2019).

Unlike cattle, sheep, and goats, horses have upper incisors and can bite off plant parts. In contrast, cattle use their tongues to grab plants then use head movements to pull off plant parts, often uprooting grasses and other shallow-rooted annuals. Horses have a higher level of preference for grass than cows, goats (who browse woody plants), and sheep (who prefer forbs). Cattle used forbs and shrubs much more than horses, and horses spend much more time feeding on short grass than cattle (Menard et al., 2002). Horses are a good tool for plant management because they remove more vegetation per unit body weight than cattle do; horses also use the most productive plant communities and plant species (especially graminoids) to a greater extent (Menard et al., 2002). Like sheep, horses feed closer to the ground, and maintain a mosaic of patches of short and tall grass that contributes to structural diversity at this scale. Cattle use broadleaved plants to a greater extent than horses do, and can reduce the rate of encroachment by certain woody species. Sheep use broadleaved plants more than cattle, and goats use woody vegetation more than cattle. Managers should consider the differences in how livestock graze before establishing a grazing program.

Although sheep and goats are more reluctant to enter water than horses and cattle, all of this stock will wade in vernal pool mud late in the season to protect their ankles from biting flies. Therefore, to minimize punch in vernal pools, remove the stock prior to the onset of fly season.

APPENDIX B. Comparison of livestock group grazing differences.

Components		Livestock Group			
Type	Specific	Non ruminant (one stomach compartment - monogastric)	Ruminant (four stomach compartments)		
		Horses (*Equus ferus caballas*)	Cattle (*Bos taurus*)	Sheep (*Ovis aries*)	Goats (*Capra aegagrus hircus*)
Physical Features	Life Span (Years)	25-30	18-22	10-12	15-18
	Gestation Period	11-12 months	283 days	152 days	150 days
	Size – Adult Weight (lbs)	840-2,200	Steer 1,000, Bull 2,400, and Cow 1,600	Ram 99-350 and Ewe 99-220	44-310
	Hooves vs Terrain	Larger surface area of hoof than cattle, so punch is less of a proplem. Not so good on steep slopes. Mules and donkeys are better due to narrower hooves. Best in sandy or soft soils.	Poaching (punch or pock marks) caused by trampling of wet grasslands can lead to a hard impenetrable surface when dry, where plants are unable to germinate. particularly around water troughs, mineral licks, and feeders. Not as good on steep slopes. Best on flat or rolling terrains with moderately hard surfaces.	Smaller feet than cattle or horses and are therefore less likely to cause poaching. However, prone to foot rot, so not suited to predominantly wet sites. Cope well on sites with steep slopes.	Smaller feet than cattle or horses and are therefore less likely to cause poaching. Less prone to foot rot than sheep making them more suitable for wetter sites. Are very agile and can tackle steep hills and rock edges.
	Muzzle	Broad muzzle and tongue. Lips more mobile than cows.	Broad muzzle and tongue. They don't graze too close to the ground and are therefore less likely to create bare patches than horses. Tend to wrap tongue around tall grasses and pull them out.	Have narrow muzzles and thin mobile lips allowing them to graze very close to the ground down and when circumstances allow can be selective grazers - biting off single leaves or shoots. Generally move slowly while nibbling.	Have narrow, strong mouths and a flexible upper lip allowing them to be highly selective browsers and designed for stripping individual leaves and chewing branches.
	Teeth and Effects	Baby teeth (milk teeth) are replaced by five years of age. Unlike cattle, sheep, and goats, horses have both top and botton incisors which are forward facing. They can graze very close to the ground and can leave patches of bare ground.	All adult teeth are present between 5-6 years of age. Cattle lack upper incisors, having instead a "dental pad". The wide gap that seperates the incisors (or dental pad on the maxilla) from the premolars is called the diastema.	Develop a full set of adult teeth after 3-4 years and then steadily lose them as they age, therefore young and old sheep may not graze as effectively as middle-aged sheep.	Like cows they have dental pads instead of upper incisers. Like sheep, do not develop their full set of teeth until they are five years old and lose teeth in older age.
Foraging and Behavior	Grazer/Browser	Grazer	Grazer	Browser	Browser
	Vegetion "Preference"	Grasses	Tall grasses	Forbs, then grasses	Browse (woody plants), then forbs
	Vegetation Proportions[1] – Grass	90	70	60	20
	Vegetation Proportions[1] – Forbs	4	20	30	20
	Vegetation Proportions[1] – Woody Vegetion (Browse)	6	10	10	60
	Grazing Uniformity/ Selection/ Preferences	Select sweet grasses (tall grass, grass stems, and dead/cut vegetation) but will also eat a variety of sedges and rushes.Tend not to select flowers, as sheep do.	Prefer tall grasses. Avoid grazing around dung pats which creates patches of longer vegetation; and are non-selective grazers.Foraging is less selective than horse, sheep and goats and therefore they graze more uniformly.	Tend to leave grass stems and dead vegetation (cuttings) and avoid tall and tussocky vegetation.Do not graze uniformly since selective grazers.	Prefer the newer growth and leaves of scrub, bramble and tufted grasses rather than finer grasses. Can target grass seed heads, eating them before starting to eat the leaves. Generally select forbs over grasses.Most selective grazers. Do not graze uniformily. Generally do not eat legumes. Can reach taller branches by standing on hind legs or climbing.
	Grazed Heights of Herbs	2 - 2 1/2 in (5-6 cm)	> 1 in (. 2.54 cm)	1 1/8 in (3 cm)	2 3/8 in (6 cm)
	Dung Deposition and Effects	Tend to defecate in specific areas (latrine areas) which causes a build-up of nutrients which can encourage the spread of thistles, nettles and docks. Generally latrines are not in their preferred grazing locations.	Deposit dung randomly and generally avoid vegetation next to it.	Deposit dung randomly and will graze next to it, therefore grazing swards to a uniformly low height.	Deposit dung randomly and will graze next to it.
	Overall Grazing Effects	Grazing preferences are good for maintaining the mosaic habitats.	Generally better than sheep at creating and maintaining structurally diverse grassland.	Tthe ratio to forbs to grasses may increase under continous grazing[2]	Decrease woody vegetation and thatch. Do not avoid pricky or spiny plants.[3] Spend more time exploring than other grazers. Will strip bark; they may kill shrubs and trees.
	Tolerance to Secondary Compounds	Least tolerant of secondary compounds.	Less tolerant of secondary plant compounds than sheep or goats.	Tolerant of secondary plant compounds.	Most tolerant of secondary plant compounds.
	Nutrition and Other Needs	Need more forage per animal than cattle and can easily overgraze small sites. Spend more time foraging than cattle but need less water.	Need more forage per animal and therefore might not be appropriate for very small sites. They need more water than other grazers. Need access to troughs 24/7.	Unlike goats, they need some grass and herbaceous vegetation to satisfy their nutritional needs.	They can get all their nutritional needs from scrub when available. Woody vegetation can account for 50-75% of their diet. Need some dry, sheltered ground. Needs the least amount of water.
Other Needs	Predation	Less susceptible to predation than sheep or goats.	Less susceptible to predation than sheep or goats.	Susceptible to predation.	Susceptible to predation.
	Fencing and Effects	Require stable fencing.	Require stable fencing.	Require more secure fencing than cattle (field fence rather than barbed wire). Can be trained to use temporary, portable fencing.	Require more secure fencing than cattle (field fence rather than barbed wire).Great escape artists. Can jump over or go under fences if they want to get out!
	Herding Needs	Require periodic movement, but do not require herding.	Require periodic movement, but do not require herding.	Require herding.	Require herding, or can be tethered to concentrate grazing activity.

Main sources: Frost and Launchbaugh, 2003; Schoenian 2018; Tu et al., 2001; and Coffey, 2001. [1]Schoenian, 2018 [2]Bullock et al., 1994 [3]Elias and Tischew, 2016