Final Report for
Amphibian Management and Monitoring at
Palo Corona Regional Park,
Monterey County, California
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Executive Summary

Work Conducted

Given the need to understand the entire community of pond-breeding amphibians, we conducted surveys for a second year in spring and summer 2006 to measure: A) occurrence and breeding activity of amphibians in all known stock ponds and creeks on the property; B) relative abundance of larval amphibians in each potential breeding site, C) incidence of two important diseases in both larval and adult amphibians, chytrid fungus and a trematode parasite, D) compositions of both invertebrate prey and invertebrate and vertebrate predator communities using the stock ponds.

A. Palo Corona Regional Park (PCRP) contains ten ponds and five streams that could provide appropriate habit for water-breeding amphibians. We conducted adult activity surveys and sampled for larvae and metamorphs in both ponds and streams.
B. We found California red-legged frog (CRLF) adults at seven of ten ponds, Pacific treefrog (PTF) adults using nine of ten ponds, and California newt (CN) adults using four of nine ponds. CRLFs were breeding at five of ten ponds, CTS at one of ten ponds, PTF at nine of ten ponds, and CN at four of nine ponds. No introduced American bullfrogs were detected at PCRP. We did not detect Foothill yellow-legged frogs, Western toads, or California giant salamanders on the property.
C. We found the greatest relative abundance of larval California newts and Pacific treefrogs in Salamander Pond. Dead Pig Pond had the greatest relative abundance of larval California red-legged frogs. California tiger salamander larvae were only encountered at Roadrunner pond. These inland ponds, with the greatest abundances of breeding activity, are characterized by a variety of pond depths, mosaic of open and vegetated waters, vegetated banks, and surrounding upland grassland habitat.
D. There was no evidence of infection with the trematode *Ribeiroia ondatrae*. There were positive results for infection with the chytrid *Batrachochytrium dendrobatidis* in CRLF larvae in Animas Pond and PTF larvae in Entrance pond. Adult CRLF in Dead Pig and Salamander Ponds tested positive for chytrid.
E. A wide variety of predators and prey were detected at PCRP. Exotic predators, in particular bullfrogs, are missing from PCRP.

Recommendations for Future Management and Monitoring

California red-legged frogs are currently listed as threatened at the California State and Federal levels. California tiger salamanders are listed as species of special concern at the California State level and threatened at the Federal level; they are currently being considered for listing as endangered at the California State level. The special consideration afforded these two species requires coordination by representatives of Palo Corona Regional Park with the California Department of Fish and Game and the US Fish and Wildlife Service on activities that may impact these species. As Palo Corona Regional Park maintains active grazing, there is some flexibility under Section 4(d) of the

Endangered Species Act, as the US Fish and Wildlife Service deems appropriate for ranching activities. Under the Endangered Species Act, any animals listed as endangered are automatically protected and Section 4(d) does not allow flexibility in this case. If activities are specifically related to the maintenance of grazing and involve habitat of animals that are listed as threatened or of lesser protection status, US Fish and Wildlife does not require special take permits (pers. comm. Jacob Martin, USFWS, 19 Dec. 2006). In this situation, it is advisable to discuss the activity with a USFWS contact prior to the beginning of the project to ensure they consider it to fall under the Section 4(d) rules. Any activities that impact California red-legged frog or California tiger salamander habitat that are not related specifically to maintenance of grazing for cattle require discussion with USFWS to determine the proper permitting and required personnel are on hand. California Department of Fish and Game has their own set of requirements and permits, and they should be contacted prior to any activities that may impact these amphibians, regardless of whether activities involve maintenance of cattle grazing land. Coordination with both USFWS and CDFG on activities that affect either amphibian species is required.

The data collected in 2004-2006 provided evidence of relative numbers and habitat use for breeding. However, 2004-2005 was an exceptionally wet winter season and therefore changes in amphibian numbers that have been observed in this year must be viewed in light of the normal variability in amphibian activity that is driven by rainfall patterns across years. Because of this variation and the need to account for it in establishing baseline abundance and presence/absence patterns, we recommend ongoing monitoring of amphibian use of aquatic habitats at PCRP. While some data collection can be less extensive than in the 2005 and 2006 seasons, we recommend that sampling for both larvae and adults continue to be conducted in all ponds and streams. In particular, we suggest:

A. Monitoring for CRLF and PTF between March and August to provide feedback for management activities and to allow early detection of invasive species such as bullfrogs during the summer months, which occur on lands surrounding PCRP. The most practical method of surveying for these goals is to conduct nighttime surveys for adult frog use of habitats and daytime surveys for amphibian larvae. Audio and listening surveys can be conducted without a permit, but dipnetting requires a permit. Trained personnel should conduct eradication of bullfrogs if they are detected.
B. We recommend continued monitoring of larval amphibians to continue to establish presence/absence of breeding activity. Individuals holding state and federal permits to conduct activities that involve contact with CRLF can conduct these surveys.
C. In light of positive tests for chytrid, all people at PCRP should follow decontamination methods for all equipment that comes into contact with water bodies (Appendix A).
D. Improvement should be made to upland habitats for CTS and to promote habitat connectivity, particularly removal of French broom around and between Salamander, Dead Pig, and Roadrunner ponds. These changes

should be conducted by hand within 3 meters of pond edges to avoid injury to amphibians. If mowing is used, a permitted individual should precede the mower to remove amphibians. Proper permits from government agencies should be obtained for these activities. These improvements should be conducted between September and the first rains of the fall/winter to avoid major periods of overland movement by these amphibians.

E. Creating a mosaic of vegetation and open water in the ponds will provide habitat for larval and adult amphibians. Use of cattle to open up vegetation in River and Animas Ponds is suggested; conversely, fencing off Boundary Pond to promote recovery of aquatic and surrounding vegetation is suggested. Fencing should be constructed in a manner that will allow for full control over cattle access to ponds to avoid crushing of egg masses and tadpoles during prime breeding seasons from about December to the end of August and potentially increasing the rate of spread of *Batrachochytrium dendrobatidis* and other amphibian pathogens.

F. Currently, some of the most serious threats to native amphibians in central California are absent or of minimal importance at Palo Corona: habitat loss, bullfrogs, and fish. This makes the property of considerable importance for California red-legged frogs and California tiger salamanders. However, the relative health of these populations is almost certainly due to the limited human traffic onto this property in the past, and thus calls for careful consideration of increasing human use and its attendant threats as land management evolves on the site. PCRP is unique among the MPRPD's parks and offers a rare opportunity to be a leader for public education on a variety of conservation issues from its inception. As PCRP begins to open to the public, planning to mitigate the impacts of visitors and balancing recreation and conservation interests will go a long way to maintain the suite of species on the property. We strongly recommend a program of public education about the impacts of introducing species and pathogens and about the alternative activities and protections the public can take. Workshops, brochures, and posting signage near the freshwater habitats for staff and visitors about the introductions of these species and the impacts of invasives may keep people from negatively impacting the species at PCRP and other locations.

G. Preservation of amphibian populations through conservation of habitat at places such as PCRP is essential to the persistence of threatened amphibians like California red-legged frogs and California tiger salamanders. Just as essential is collaboration with neighboring landowners on conservation strategies that focus on maintenance of connectivity of appropriate habitat for these amphibians. While PCRP has seven ponds with California red-legged frogs and two with California tiger salamanders, this does not constitute a local metapopulation that is safe from the threat of population decline from any number of threats, including stochastic factors. Maintenance of connectivity between habitat sites is essential for healthy amphibian populations. In addition, cooperation and communication with neighboring land managers on control and eradication of invasive species will boost the efficacy of all of these measures at PCRP and the greater region. Invasion of bullfrogs, non-native tiger salamanders, and possibly disease from adjacent areas may be an ongoing concern at PCRP. Working with adjacent landowners to develop and implement an integrated amphibian management plan for the region would positively impact and improve all efforts at PCRP.

Introduction

Palo Corona Regional Park (PCRP) is located just south of Carmel, California on the northern end of the Santa Lucia Range. Previously under private ownership, the Palo Corona property was acquired in a collaborative effort by The Nature Conservancy and The Big Sur Land Trust in 2003, then transferred in portions to the Monterey Peninsula Regional Park District (MPRPD) and the California Department of Fish and Game (CDFG) for the purposes of preservation and conservation. The park property is about 4,300 acres, is largely undeveloped, and provides a variety of upland and aquatic habitats for many plants and animals, including amphibians. The property is valuable for open space preservation, recreational activities, and preservation of habitat for rare or threatened species.

Among the populations of species of concern on the Palo Corona property, some of the most unique and threatened are those of two native amphibians. Both the California red-legged frog and the California tiger salamander are endemic to California, with the latter endemic to the Central Coast of California, and are in decline in many areas. Palo Corona Regional Park property provides a relatively isolated and undisturbed area that may protect populations of both species from some of their most serious threats. Importantly, these threats include two other amphibian populations: the introduced bullfrog and an introduced species of tiger salamander. In addition, diseases that are shared by multiple water-breeding amphibian species are potentially a major threat to the rare native species. As a result, obtaining a picture of the current health and future protection of amphibian populations relies upon an understanding of the entire amphibian fauna of an area. The purpose of our work has been to provide information on the aquatic-breeding amphibian species found at PCRP, especially their locations and relative larval densities, and to make management recommendations for the continued maintenance and prosperity of these amphibians as the park develops.

Background

PCRP lies within the recorded range of four species of aquatic breeding frogs, one aquatic breeding toad, and three species of aquatic-breeding salamanders (Stebbins 2003). Of these the California red-legged frogs, Pacific treefrogs, American bullfrogs, California tiger salamanders, and California newts have been previously recorded on the property. It is unclear if the remaining species – Foothill yellow-legged frogs, California giant salamanders, and Western toads – ever occurred on PCRP, although PCRP has appropriate habitat and lies within the broadly recorded ranges for these species. Each of these native amphibian species is quite unique in its habits and habitat requirements and understanding them is essential in making management decisions. Therefore, a basic ecological description of each species follows.

Amphibian Species Descriptions

American Bullfrogs

American bullfrogs (*Rana catesbeiana)* were introduced to California from the southeast

United States in the late 1800's to take the place of over-harvested California red-legged frogs (Jennings 1985). Unfortunately, they appear to use the same resources as California red-legged frogs while also preying on them. Larger in size, bullfrogs generally out-compete and predate California red-legged frogs where they cohabitate (Lawler 1999; Doubledee 2003). Unlike California red-legged frogs, which typically metamorphose in one year, bullfrog tadpoles commonly require more than one year to metamorphose (Casper and Hendricks 2005). This is important to management of ponds for California red-legged frogs, as allowing ponds to dry after September every few years is a recommended strategy for bullfrog population control (Scott and Rathburn 2002). Bullfrogs are also suspected to be a vector for serious amphibian fungal disease *Batrachochytrium dendrobatidis*, carrying the infection but not developing chytridiomycosis (Daszak P 2004; Hanselmann 2004; Garner, Perkins et al. 2006).

California Giant Salamanders
Palo Corona Regional Park is 50 kilometers south of the reported range limit for California giant salamanders (*Dicamptodon ensatus*), although there was a disjunct population reported in Monterey County (Bury 2005). California giant salamanders primarily live and breed in streams and creeks, but have also been observed foraging on the forest floor and in subterranean burrows (Bury 2005).

California Newts
California newts (*Taricha torosa*) use an array of breeding and terrestrial habitats, including vernal and perennial ponds, streams and creeks, woodland, chaparral, and grassland (Kuchta 2005). Adults aestivate during dry summer months in leaf litter and animal burrows. They are capable of long migrations of up to 3200 meters from their breeding sites (Kuchta 2005). California newts mainly feed on a variety of invertebrates, though they have occasionally been observed feeding on vertebrates (Kuchta 2005). As they contain potent tetrodotoxin throughout their bodies in the egg, embryo, and adult stages, they have few predators (Kuchta 2005). It appears that common garter snakes (*Thamnophis sirtalis*) have resistance to this toxin and California newts may be an important part of their diet (Kuchta 2005). During their larval stage, California newts do not possess tetrodotoxin and are more prone to predation (Kuchta 2005). A few invasive species, including bullfrogs, crayfish, and mosquitofish, are believed responsible for declines in California newt populations in southern California (Kuchta 2005).

California Red-Legged Frogs
One of the largest native frogs to the western United States, the California red-legged (*Rana draytonii*) frog has been listed under the Federal Endangered Species Act as threatened within its remaining range in California since 1996 (U.S. Fish and Wildlife Service 2002). Historically, California red-legged frogs were found in 46 counties, along the coast between Marin and northern Baja California and inland counties from Shasta County southward. California red-legged frogs currently exist in 23 of these original counties (U.S. Fish and Wildlife Service 2002). Coastal populations in central California are considered the most abundant and stable, while southern and inland species are considered most vulnerable (U.S. Fish and Wildlife Service 2002). Potential threats to the California red-legged frog include elimination or degradation of habitat, disease, and

predation by introduced species such as bullfrogs (*Rana catesbeiana*) and mosquitofish (*Gambusia affinis*) (Webb 1997; Lawler 1999; U.S. Fish and Wildlife Service 2002; Fellers 2005).

California red-legged frogs require a habitat with aquatic breeding areas, such as ponds, freshwater marshes, lagoons, and streams, surrounded by a mixture of riparian and upland habitat (Stebbins 1985; Allen 2000; Fellers 2005). They will use cattle tanks and other artificial water sources as well as natural sites. They are thought to move through surrounding uplands between aquatic sites year-round and to retreat to these upland habitats during summer rains and early winter (Bulger 2003). California red-legged frogs breed from November through April and the tadpoles typically metamorphose by September of the same year (Wright and Wright 1949). Pond permanence can have a significant impact on California red-legged frog breeding success. Plans to help rejuvenate California red-legged frog populations should include management of water levels in ponds for successful metamorphosis, balanced with periods of dry-down of ponds to reduce the presence of exotic predators and competitors (Bulger 2003).

California Tiger Salamanders

The California tiger salamander (*Ambystoma californiense*) is endemic to California, preferring vernal pool and grassland habitats (Shaffer 2005). While it appears that they were historically widespread, much of their habitat has been lost throughout their range (Shaffer 2005). This loss of 60-85% of vernal pool habitat coupled with habitat fragmentation (Shaffer 2005) has led to the listing of the California tiger salamander as a Federally Threatened Species. It is currently being considered for listing as endangered at the State level.

Adult California tiger salamanders breed in seasonal, fishless wetlands, with adults migrating to and from breeding ponds during the rainy season between November and May (Trenham P.C. 2001). They have also been documented using constructed cattle ponds. Females stay for about ten days while males spend a little over a month at breeding ponds (Trenham P.C. 2001). Larvae emerge two to four weeks later and generally take about four to five months to metamorphose (Trenham P.C. 2001). Gape-limited larvae feed on zooplankton, tadpoles, various aquatic insects and crustaceans, and aquatic snails (Trenham P.C. 2001). When not feeding, they tend to spend their time on the bottom of the wetland and hide in vegetation when alerted (Storer 1925). Metamorphosed juvenile California tiger salamanders migrate to upland habitat during the summer, in both wet and dry conditions, primarily before their natal pond dries, though some can wait until after the pond has dried by finding cover in vegetation and cracks in the mud (Loredo 1996; Loredo 1996). When they are not at breeding ponds, adults spend their entire lives in California ground squirrel (*Spermophilis beecheyi)* and valley pocket gopher (*Thommomys bottae*) burrows, but appear to prefer ground squirrel burrows in open grasslands up to 115 meters away from breeding ponds (Loredo 1996; Trenham 2001). Some do disperse to ponds other than their natal ponds for breeding and have been observed moving up to 580 meters away (Trenham P.C. 2001).

Hybridization with introduced Eastern tiger salamanders (*Ambystoma tigrinum*) is a common problem for California tiger salamanders (Riley 2003), causing genetic contamination and potentially reduced fitness for a highly locally adapted species (Riley 2003). These invasive salamanders are often introduced through their use as fishing bait (Riley 2003).

Foothill Yellow-Legged Frogs
Foothill yellow-legged frogs (*Rana boylii*) breed in slow-moving sections of streams during the spring, depositing eggs on cobbles, pebbles, vegetation, or woody debris (Fellers 2005). Tadpoles emerge within about a month and metamorphose around four months (Fellers 2005). Adults spend most of their time along streams and creeks, dispersing along them, and they are quite cryptic (Fellers 2005). Use of upland habitat has not been described (Fellers 2005). While they have been reported historically in coastal Monterey County (Stebbins 1985; Fellers 2005), a search of the California Natural Diversity Database did not reveal any records of them in the region surrounding Palo Corona Regional Park.

Pacific Treefrogs
The third aquatic-breeding frog in this region is the Pacific treefrog (*Pseudacris regilla*), which is also by far the most abundant. As an important food source for California red-legged frogs, Pacific treefrog abundance is relevant to threatened amphibian populations (U.S. Fish and Wildlife Service 2002). Pacific treefrogs (Pseudacris regilla) utilize a variety of habitats, including woodlands, grasslands, chaparral, and farmland, but are primarily found in low foliage near water (Stebbins 2003). Pacific treefrogs (Pseudacris regilla) are susceptible to infection by the trematode parasite *Ribeiroia ondatrae* (Johnson 2002; Hemingway 2004). Their susceptibility to infection with *Batrachochytrium dendrobatidis* is not clear, but tadpoles exposed to the pathogen did not appear to be negatively affected (Blaustein, Romansic et al. 2005)

Western Toads
Western toads (*Bufo boreas*) have a wide range across the western United States (Stebbins 1985; Muths 2005). They typically breed in ponds, slowly moving streams, temporary pools, and ditches between February and July, laying their eggs in stings along the shallow substrate (Muths 2005). Adults will remain near or in these moist habitats or move to upland habitat including ground squirrel burrows and tunnels under tree roots (Muths 2005).

<u>Metapopulation dynamics for California Tiger Salamander and California Red Legged Frog</u>

While it is not clear that California red-legged frogs exhibit metapopulation dynamics according to the classical definition (i.e., extinction and recolonization of habitats), they clearly use a highly patchy habitat and move between those habitat patches (Bulger 2003; Hemingway 2004). California tiger salamanders exhibit clear metapopulation dynamics, generally have low reproductive output, and are patchily distributed across the landscape (Shaffer 1993). Thus connectivity of habitat is essential for persistence of these amphibians. If they are eliminated from one site, amphibians migrating from other areas can repopulate. Unfortunately, continued human encroachment on amphibian habitat and fragmentation through construction of roads, urbanization, invasive plant species, and agriculture decreases the ability for amphibians to repopulate sites and exacerbates threats to existing populations (U.S. Fish and Wildlife Service 2002).

Pathogen Descriptions

Trematodes

Trematodes, and specifically those of the family Digenea, have complex life cycles that involve several different hosts. The trematode *Ribeiroia ondatrae* requires three hosts, a freshwater snail, amphibian larvae, and large wading birds. It reproduces asexually in *Planorbella sp.* freshwater snails, releasing a motile stage that encysts in the developing limb buds of larval amphibians. These encysted trematodes cause limb malformations as the amphibian metamorphoses (Sessions 1990; Johnson 1999; Stopper 2002; Johnson 2003). Several species of large estuarine birds feed on the malformed amphibians and the trematode sexually reproduces in the bird's gut, completing its life cycle when eggs that are infective to the snail host are passed back into the water in feces (Yamaguti 1975). Presence of all three hosts is required for the trematode to complete its lifecycle. The amphibian host population may be affected by the trematode through massive infection and starvation or through increased risk of predation by the next parasite host (Johnson 1999). Limb malformations are the principle symptom that is used to diagnose *R. ondatrae* infections in the field.

Chytrid Fungus

Batrachochytrium dendrobatidis, a fungal chytrid disease that is possibly introduced from South Africa, is thought responsible for mass amphibian mortalities in Australia, Panama, Ecuador, Venezuela, New Zealand, Spain, and the United States (Fellers, Green et al. 2001; Weldon 2004). *B. dendrobatidis* digests keratin in mouthparts of amphibian larvae and in the skin of adult amphibians (L. Berger, R. Speare et al. 1998). Infection with *B. dendrobatidis* may lead to mortality via a reduction in the amphibian's ability to osmoregulate and/or through production of toxic compounds that are absorbed by the amphibian (L. Berger, R. Speare et al. 1998). *B. dendrobatidis* can be lethal in all life stages of post-metamorphic amphibians. *B. dendrobatidis* appears to exacerbate predator-prey relationships in lab experiments, adding to the complexity of its impact on hosts (Parris 2004). It is worth noting that not all amphibian species experience mortality due to infection with *B. dendrobatidis,* although once an amphibian habitat has been exposed to *B. dendrobatidis,* the pathogen appears to become endemic, potentially surviving as saprobes and in reservoir species (Berger 1999; Hanselmann 2004; Weldon 2004; Blaustein, Romansic et al. 2005). Infection from *B. dendrobatidis* may be detected by changes in the pigmentation of the mouthparts of larval amphibians (a quite imperfect detection method that appears to be species-specific) or by genetic laboratory tests on tissue samples (which is expense and time-consuming), or by swabbing skin and using real-time PCR to detect the chytrid DNA (Boyle 2004).

Ranaviruses

Ranaviruses are a group of viruses that infect amphibians, reptiles, and fish (Daszak 1999). Like chytridiomycosis, ranaviral infections are implicated in large-scale amphibian deaths (Daszak 1999). While symptoms vary widely and some are cryptic, a few symptoms are simple to monitor, and samples from individuals suspected to be infected can be sent to a qualified lab for follow-up tests. Tadpole edema virus causes visible ventral edema and internal hemorrhaging in tadpoles, and death rates can be greater than 80% (Daszak 1999; Wright and Whitaker 2001). Other symptoms are caused by secondary bacterial infections that often accompany ranaviral infections, such as skin ulcers and lesions (Daszak 1999; Wright and Whitaker 2001). As death rates are often quite high, up to 40-45% of frogs and salamanders infected with these viruses (Daszak 1999), regular monitoring of populations should allow for detection of moribund individuals that can be tested for these viruses.

Pond Descriptions

There are a small number of ponds that may provide important breeding and refuge habitats for California red-legged frogs and California tiger salamanders in Palo Corona Regional Park, and we thus provide a brief description of each pond.

Entrance Pond

Entrance Pond is located along the front slope of the Palo Corona Regional Park property, adjacent to Highway 1. With a size of about 500 square meters and about 2.5 meters deep, this perennial pond is surrounded primarily by grassland, with coniferous forest further up the slope. The pond is fenced to exclude cattle and subsequently has grassland and riparian vegetation growing along its entire bank. A cluster of rush grows in the center of the pond, with a few other patches growing along the banks of the pond. In March duckweed covered about 10% of the pond, expanding to almost full coverage by mid-April. Aquatic vegetation, particularly algae, covers about 10% of the pond.

Boundary Pond

Boundary Pond is located further up the front slope, with about two-thirds of its perimeter surrounded by grassland and a third by chaparral. Although similar in size to Entrance Pond, it is shallower throughout reaching a maximum depth of about one meter. While the pond has a fence running through it, it is not fenced to protect it from cattle. The banks of the pond are eroded and lack vegetation later in the spring and summer, save a few rushes. Rushes line the fence through the center of the pond. Water in the pond is quite cloudy with sediment. Aquatic vegetation covers about 5% of the pond.

River Pond

River Pond is located adjacent to the Carmel River and is surrounded by grassland and oak woodland. It is fenced and completely covered in dense rush, cattail, willow, and other riparian vegetation. There is little open water, but a portion of the pond is somewhat accessible under the willows and along a shallow, grassy bank on the west side of the pond.

Animas Pond

Animas Pond, a perennial pond, is located behind the front slopes along the main trail. It is about 1200 square meters. It is surrounded by a combination of grassland, chaparral, and oak woodland. Animas Creek gently runs through this unfenced pond. It is completely vegetated with a combination of native and exotic plants, including rush, duckweed, willow, aquatic iris, *Rorripa nasturtium-aquaticum*, and *Oenanthe sarmentosa.* There was a section of the pond covered only with duckweed in February, but was completely covered with emergent vegetation by May. While completely covered, the plant cover is not as dense as the vegetation at River Pond, allowing amphibians to use the pond. Maximum water depth at Animas Pond in February appeared to be about 1.5 meters, but the vegetation mat made it difficult to make a clear assessment.

Roadrunner Pond

Roadrunner Pond is located along the access road past the front slopes away from the coast and is surrounded by grassland, chaparral, and French broom. One of the smaller ponds, it is about 200 square meters and ephemeral. Roadrunner pond is unfenced and about 20% of the banks are unvegetated. The remaining banks of the pond are covered by rush and grasses. Roadrunner pond does not have any submerged or floating vegetation. The water is quite cloudy with sediment.

Dead Pig Pond

Dead Pig Pond is located further along the main road, receiving runoff from the road and grasslands above. A perennial pond, it is about 1000 square meters and deeper than 2 meters. Surrounded completely by tall French broom, coast live oak trees, poison oak, and grassland, it is unfenced, though not likely accessible to cattle as the surrounding vegetation is very thick and banks are steep. French broom grows along about 70% of the banks of the pond, with the other 30% surrounded with willows and rush. A large patch of rush grows over about a third of the open water of the pond. Submerged aquatic vegetation grows sparsely in the pond. The water in this pond is particularly cloudy with sediment and a deep area of loose sediment is located where the runoff from the road enters the pond.

Salamander Pond

Salamander Pond is adjacent to the Santa Lucia Preserve property and covers a portion of the road after the winter rains. With about 2500 square meters in area, it is quite shallow along the road and much deeper (greater than 2 meters) along the opposite side. One third of the pond has French broom growing along the bank, while the remainder of the banks are covered with grasses and some rushes. There are several thickets of rush covering about 20% of the pond and about 25% of the pond has submerged aquatic vegetation. While unused in the winter and early spring, the vegetation grows thick in the road, but is occasionally mowed to maintain access. The upland beyond the pond margin is covered in French broom.

Wire Corrals Pond

Wire Corrals Pond is located above the Chavote Homestead site. It was constructed several years ago below the corral area. A small pond of about 100 square meters, it is surrounded by grassland. Its banks are completely covered in rush, poison oak, and grasses. There is a small shallow, silty area, while the majority of the pond is quite steep and greater than 2 meters deep. The pond has about 10% coverage with submerged aquatic vegetation and a very small amount of floating vegetation.

Van Winkleys Pond

Van Winkleys Pond is located along Van Winkleys Creek in the middle of mixed hardwood forest. It gets little direct sunlight and tends to be quite cool. It has no vegetation and has a substrate of deep silt. It is about 80 square meters and is about 0.5 meters.

Echo Ridge Pond

Echo Ridge Pond is better described as a seep than a pond. It is not more than 10 centimeters deep in any place and is about 50 square meters. It maintains riparian vegetation, notably rush, and is fed by a spring. It is surrounded by grassland with patches of coyote brush and hardwood forest.

Creek Descriptions

There are a total of five significant creeks on the Palo Corona property. During this project we focused more effort on the ponds, but we also surveyed the creeks. We describe their basic features here.

Van Winkleys Creek is a small stream that runs along the south east of Echo Ridge and to the north of Seneca Ridge. It traverses through redwood and mixed hardwood forest before meeting San Jose Creek. It is generally high gradient and it does not appear to have many pools.

Seneca Creek, another tributary to San Jose Creek, is a bit smaller than San Jose Creek and has a variety of habitats including riffles, pools, and runs. It has sandy and coarse gravel. Much of Seneca Creek on PCRP runs through redwood forest.

San Jose Creek is the largest of the creeks at PCRP. It includes a variety of habitats, including riffles, pools, and runs with sand, gravel, and large angular rocks. It meanders through mixed forest and redwood. It was dammed in the 1950s, but the dam blew out in 1998 and is revegetated along the stream banks.

Panoche Creek is a steep, small creek that empties into Seneca Creek near the Escobar ranch and homestead.

Animas Creek runs through Animas Pond on San Jose Ridge, joining San Jose Creek along the south slopes of the property over Highway 1, above the monastery.

Methods

Much of PCRP is remote and can be challenging to access, especially in a year with high levels of precipitation. Our first trip to the property was in February, during the breeding season for California red-legged frogs. The road to the remote section of the property leading to Wire Corrals, Van Winkleys, and Echo Ridge Ponds was impassable for much of the winter and spring, thus we did not survey these pond habitats. Surveys during 2005 indicated they would be unlikely habitats for California red-legged frogs or California tiger salamanders.

We used a variety of techniques to determine species composition, relative abundance of larval amphibians, and assess disease incidence. We also performed topical surveys to get a sense for aquatic invertebrate species composition. Here we describe the methods employed in these assessments.

Determination of Amphibian Species Composition

An inventory of amphibian species using stock ponds, seeps, and creeks at PCRP was made via several standard methods, as outlined in “Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians” (Heyer 1994). These methods include

Audio Strip Transects (AST), Visual Encounter Surveys (VES), and Seine and Dipnet Sampling (SDS). This combination of methods provided a powerful tool for the assessment of species while minimizing harassment to the animals.

Audio Strip Transects, AST, were used to identify species of calling male frogs, including California red-legged frogs (January through April), Pacific treefrogs (December through May), and bullfrogs (in June through September). Habitats were quietly approached after sunset and without the use of flashlights. Standing two to three meters from the bank, we listened for about ten minutes and noted species and approximate density calling, i.e., single individual, discernable individuals calling, or full chorus with no discernable individuals. We then moved about ten meters along the bank and repeated the process. This method provides a measure of breeding activity and a very rough estimate of relative abundance, but cannot give good estimates of true numbers.

Visual Encounter Surveys, VES, a non-invasive survey method, was used to record all individuals observed, categorized by life-stage and for adults by size-class. VES was performed both during the daytime and nighttime along the shores of ponds and creeks. Visually searching along the bank and on the surface of the water, we looked both with and without binoculars to detect frogs. During nighttime surveys, flashlights were be used to first locate amphibians by eye shine and then move closer for identification with the aid of binoculars.

Seine and Dipnet Sampling, SDS, was used during the daytime to locate larval amphibians in each pond. Seining was used to survey for California tiger salamanders, as it is effective in obtaining large samples of amphibian larvae with minimal risk of injury. The seine was operated by Ben Fitzpatrick and Bradley Shaffer under the Shaffer Lab permit from US Fish and Wildlife Service. It was pulled through the water with the bottom of the net along the pond substrate at various locations throughout each pond and larval amphibians were identified. Dipnetting was used in all ponds under permits from US Fish and Wildlife Service (TE-082546-1) and California Department of Fish and Game (SC-8471) held by Antonia D'Amore and Valentine Hemingway, and, separately by Ben Fitzpatrick and Bradley Shaffer. Dipnets were pulled along the bank and in deeper areas and larval amphibians identified and inspected.

<u>Quantitative Assessment of Larval Amphibians</u>

Basic Methodology

Pitfall traps are of ten suggested to provide the most accurate estimates of relative abundance. However, pitfall surveys are very resource intensive to conduct for multiple sites, and we therefore opted to perform a quicker assessment of relative larval densities as a way of estimating abundance patterns on the property. While this method is not expected to tell us much about adult relative abundance, combined with AST and VES, it should provide a good measure of which ponds are most important breeding sites for particular species, especially Pacific treefrogs and California red-legged frogs.

We sampled larval amphibians in ponds on sunny to lightly overcast days between April 29 and May 17. We used two "D"-shaped dipnets with a 1.8-meter handle length and a net opening of 1056 square centimeters. The dipnet was quickly swept perpendicular to the bank beginning about two meters into the pond, from deep to shallow, leveraging the dipnet to get a deeper sample along the pond substrate further into the pond and pulling upwards toward the bank, being gentle through pliable vegetation or pulling the net out over stiff vegetation. This method allows the net to gather species inhabiting both the deep and shallow portions of the water column with minimal harm to the larvae. Each larval species in the net was identified, briefly inspected, and replaced in the pond. To best characterize the animals in a pond, we took standard net samples from the entire perimeter of each pond, taking ten paces along the shore between sweeps. Based on the results of our test for sampling effort reported in the "Final Report for Amphibian Management and Monitoring at Palo Corona Regional Park, Monterey County, California" for 2005, we took a minimum of 15 sweeps in each pond.

Test for Adequacy of Basic Sampling Methodology
We undertook a comparative quantitative census of larval amphibians at Entrance Pond. Entrance is primarily open water, with a small portion of the pond covered with tall emergent vegetation. The entire margin of the pond can be accessed for dipnet surveys of larval amphibians. We used both the method described in the "Basic Methodology" secion above and a stratified sampling design described by H. Bradley Shaffer in "Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians" (Shaffer, Alford et al. 1994). In short, we divided the pond into a checkerboard with 15 sections and 4 depths, shallow (0-0.5 meters), medium (0.5-1 meters), deep (1-1.5 meters), and very deep (1.5-2 meters). We dipnetted in each of these quads separately, completing all 15 sections in one depth before moving onto the sections of the next depth, thus allowing time for the amphibians to redistribute themselves after the disturbance at the shallower depth. Mean amphibian density per dipnet estimated from data was then compared to mean amphibian density per dipnet estimated from our standard dipnetting procedure.

<u>Disease Assessment</u>

Mouthparts on Pacific treefrog and California red-legged frog tadpoles were visually inspected for loss of pigmentation indicative of infection with *Batrachochytrium dendrobatidis*. We did not perform this with California tiger salamanders or California newts as they were very rare and typically tiny. We performed these inspections with all larvae of these two species that were sufficiently large to discern pigmentation differences, but not so advanced that their mouth pigmentation was changing due to metamorphosis. Loss of pigment in larval amphibian mouthparts is seen in association with infection of the Mountain yellow-legged frog, *Rana muscosa*, with the chytrid fungus *Batrachochytrium dendrobatidis* (Fellers, Green et al. 2001; Rachowicz 2004). For any tadpoles with depigmentation, we took samples by swabbing their mouthparts gently with a sterile cotton-tipped swab and running the sample through real-time PCR to determine presence or absence of *Batrachochytrium dendrobatidis* in the sample (Boyle 2004). To sample adult amphibians, we took samples by rubbing the swab gently over their drink patch, bottom of all four feet, and inner thighs and ran the samples through real-time PCR (Boyle 2004). Samples were taken opportunistically from dead or moribund amphibians at Entrance and Animas Ponds and from adults that were hand-caught at Roadrunner, Dead Pig, and Salamander Ponds on the evening of May 12. These ponds were selected to take larger samples as they are not too heavily vegetated and have robust California red-legged frog populations, thus easing hand-catching amphibians at night for sampling for *Batrachochytrium dendrobatidis.*

To determine occurrence of malformations caused by the trematode parasite *Ribeiroia ondatrae,* all larval amphibians we encountered with developing limbs were visually inspected for healthy growth. Further, we searched aquatic habitats for the first intermediate host of the parasite, *Planorbidae* snails, and noted instances of pond use by the definitive host of the parasite, large wading birds, such as egrets and herons.

Aquatic Invertebrate Species Survey

During the larval amphibian surveys, we surveyed water bodies for aquatic invertebrates, including mayflies, midges, water striders, *Culex*, dragonfly and damselfly larvae, water boatmen, backswimmers, water scavenger beetles, predaceous diving beetles, and various crustaceans. The survey involved sweeping three sites at each water body several times each with an aquatic insect net. We then placed the contents of the net into a tray with pond water to facilitate identification of invertebrates to the best of our ability. We also recorded casual sightings of vertebrates and invertebrates in and near PCRP's aquatic systems.

Results

Species Composition

All ponds and streams at PCRP were surveyed to determine amphibian community composition. Table 1 outlines the findings from the pond surveys, which collapse adult, larval, and egg mass data into an indicator of presence or absence of each species at each pond.

As in 2005, California red-legged frogs were observed in Boundary, Roadrunner, Entrance, River, Animas, Dead Pig, and Salamander Ponds, and used the last five for breeding for breeding as well (See Figure 1 for map.) The only site where we detected the California tiger salamander in 2006 was the Roadrunner pond. Based on 2005 surveys, we suspect larval density for California tiger salamanders at Salamander pond is very low and thus very difficult to detect if they are present. Using the same methods of seining and dipnetting and similar level of search intensity as in 2005, we did not detect any California tiger salamander larvae in 2006. As in 2005, we observed California newts using Boundary, Roadrunner, Dead Pig, and Salamander Ponds for breeding. As predicted from 2005, Pacific treefrogs (Pseudacris regilla) used all ponds to breed. No American bullfrogs were detected in any of the ponds on the property using dipnet, evening listening, or nighttime eyeshine surveys, though they are known to use surrounding aquatic habitats including the Carmel River area and adjacent Santa Lucia

Preserve property. One tiny tadpole at Salamander Pond looked much like a Western toad, but might have been a very small California red-legged frog tadpole. We did not find any other tadpoles that looked like Western toads, and thus we could not confirm Western toads at any of the ponds.

Table 1: Detection of Amphibians in Ponds at Palo Corona Regional Park.

Pond	American Bullfrogs	California Newts	California Red Legged Frogs	CaliforniaTiger Salamanders	Pacific Treefrogs	Western Toads
Entrance	No	No	Yes**	No	Yes**	No
Boundary	No	Yes**	Yes	No	Yes**	No
River	No	Unknown*	Yes**	No	Yes**	No
Animas	No	Unknown*	Yes**	No	Yes**	No
Roadrunner	No	Yes**	Yes	Yes**	Yes**	No
Dead Pig	No	Yes**	Yes**	No	Yes**	No
Salamader	No	Yes**	Yes**	No	Yes**	No

*Unknown indicates that more in-depth surveys, such as pitfall trapping, need to be performed to determine presence or absence of these amphibians.
**Indicates breeding activity in the form of calling, larvae, or egg masses.

We surveyed sections of Van Winkleys, Seneca, San Jose, Animas, and Panoche creeks using VES. Earl Gonsolin, a master's student at San Jose State University who specializes in Foothill yellow-legged frogs, accompanied us on surveys of Seneca, Panoche, and San Jose Creeks. During our surveys we did not observe Foothill yellow-legged frogs and Mr. Gonsolin believes the lack of cobble and the abundant silt would make these areas inhospitable for breeding Foothill yellow-legged frogs and their tadpoles. Further, the creeks offer little appropriate habitat for adult refugia. Thus, we feel confident in our assessment that Foothill yellow-legged frogs are extremely unlikely to occur in creeks at PCRP.

Along a small tributary of Seneca Creek just across the road from the old Escobar ranch and homestead, we found Pacific treefrog tadpoles and young of the year in May. Between the Escobar ranch and homestead and the Seneca Creek crossing near the foxglove (*Digitalis* spp.) stand there is a portion of the road that is rutted and holds water into the beginning of the summer months. There we observed Pacific treefrog tadpoles and a California red-legged frog juvenile, also in May. At the San Jose Creek crossing and in the creek above the crossing we observed three California red-legged frog adults along the bank and in the water. At the Animas Creek crossing, we encountered Pacific treefrog and California red-legged frog tadpoles, young of the year, and adults. In Animas creek below the crossing we observed adult California red-legged frogs.

While it is possible that California giant salamanders (*Dicamptodon ensatus*) and Coast Range California newts (*Taricha torosa torosa*) use PCRP's creek habitats, we have not observed them in or around the creeks during our past two years of surveys. Unfortunately, the portion of PCRP most likely to harbor these animals, forest adjacent to the creeks, was inaccessible during the rains when the animals are most often found above ground moving to and from the creeks for breeding. Future surveys may reveal California giant salamanders and Coast Range California newts using of these streams and creeks.

Quantitative Assessment of Larval Amphibians

Test for Sampling Design

Analysis of sampling design data taken at Entrance Pond indicate that while use of the stratified sampling design results in more detail about the depth where amphibians are found, the unstratified sampling design provides adequate data to achieve reliable amphibian density estimates. We corrected the stratified sampling to compare to the unstratified sampling design by combining dipnet samples from all four depths for one reach of the shoreline from the stratified sampling. For the stratified sampling design, we averaged 10.7 Pacific treefrog larvae per section (the combined samples for one reach of shoreline) with a variance of 8.2. For the unstratified sampling design, we averaged 10.9 Pacific treefrog larvae per sweep (defined as a sweep of the dipnet spanning all four depths for one reach of shoreline) with a variance of 10.7. The unstratified sampling design detected two California red-legged frog tadpoles while the stratified sampling design detected one, so there were not adequate data to support a quantitative analysis of capture efficiency. Overall, these results suggest that the use of the stratified sampling design is not required to assess relative abundance rankings of amphibians at the ponds in Palo Corona. Thus we continued using the unstratified sampling design in our data collection for estimates of relative larval abundance for the balance of the ponds.

Estimated and Relative Abundance Results

We included Entrance, Boundary, Roadrunner, Dead Pig, and Salamander Ponds in our sampling for relative larval abundance. River and Animas Ponds proved to be too thickly vegetated to dipnet, so they were excluded from the analysis of estimated abundance. The remaining ponds were surveyed by the unstratified sampling design as discussed above in the "Basic Methodology" section.

Below we have presented the estimated abundance rankings with their standard error for larval amphibians from our dipnetting surveys of Entrance, Boundary, Roadrunner, Dead Pig, and Salamander Ponds (Tables 2-5). These tables also include the estimated abundance rankings with standard error for larval amphibians from these same ponds, plus Animas Pond, from 2005. When examining these estimated abundances, it is important to keep in mind several things. First, some species of amphibians are better at escaping dipnets than others. In particular, California tiger salamanders and California red-legged frogs tend to flee to deeper, vegetated water or burrow into substrate when disturbed, making it much more difficult to detect them than Pacific treefrog larvae. Additionally, comparisons of estimated abundance for particular species between years may not provide much helpful information due to fluctuations in timing of larval development. Relative abundance of amphibian larvae by ponds can provide a useful comparison with this population estimate data (see Tables 6 and 7.) Relative abundance provide a rankings of the ponds in terms of production of amphibian larvae, but presence of amphibian larvae does not necessarily translate directly into production of adults as mortality can be very high for larvae, metamorphs, and juveniles. Still, while all these caveats pertain to these data, they can still be used in estimates of relative abundance, and combined with data on other life stages to better understand habitat use by different species.

Examining solely relative abundance data, in 2006, Salamander Pond stands out as the pond with the greatest relative abundance over all the species as noted by the sum of the pond rankings. Dead Pig Pond ranks next, with Roadrunner and Entrance Ponds just below, and Boundary Pond with the lowest ranking of relative abundance. This is very similar to what we found with the 2005 data, with Salamander Pond ranking at the top, Dead Pig next, Animas and Entrance Pond following, and Boundary and Roadrunner ranking last. These results are not surprising given that Salamander and Dead Pig Ponds both have a variety of habitats, from shallow, exposed areas that warm up and speed larval maturation to mosaics of deeper, cool areas and patches of dense vegetation for refuge. They also have plenty of submerged and emergent vegetation for California red-legged frogs to attach their egg masses. Salamander Pond has more upland habitat appropriate to California tiger salamanders than Dead Pig Pond. Entrance Pond also has a mosaic of open and vegetated water, both shallow and deep. However, it is too close to the coast for California tiger salamanders, as they tend to prefer warmer, drier conditions that occur a bit further inland. Boundary Pond had the lowest relative abundances. While the pond has some shallow and deeper regions, its sparse emergent and pond-side vegetation may leave amphibians with few egg attachment sites or refuge sites from predators.

Unfortunately, these overall rankings fail to capture the importance of Roadrunner Pond for the California tiger salamander population at Palo Corona. In 2005, California tiger salamander larvae were found at only two ponds, Roadrunner and Salamander Ponds. Even with the small numbers observed, they were by far more abundant at Roadrunner Pond. In 2006, we did not detect any California tiger salamanders at Salamander pond. Thus Roadrunner was the sole location of detected breeding activity for California tiger salamanders at PCRP in 2006.

California Newt larvae remained fairly rare; they are very fast swimmers so are less likely to be caught in a dipnet. One notable change in the rankings between 2005 and 2006 is the new finding of California Newts larvae in Roadrunner Pond. According to Ben Fitzpatrick of University of Tennessee, California tiger salamanders typically do not breed well in ponds occupied by adult California newts, as adult newts tend to eat the eggs of the California tiger salamanders. However, it is important to recognize that a year-round adult newt population cannot be established at Roadrunner pond because it is ephemeral. The use of Roadrunner Pond by California newts and the very low to no California tiger salamander larvae in Salamander Pond in 2006 raises the importance of managing these two ponds for California tiger salamanders, which we discuss in the Recommendations section below.

For California red-legged frogs, Dead Pig ranked highest for relative abundance of larvae in 2006, with Salamander Pond close behind. This ranking was reversed for 2005. In 2005, we were able to dipnet in Animas Pond, but the vegetation was very thick and may have affected the relative abundance estimates. We could not dipnet in 2006 as the vegetation was too thick. It is interesting to note, though, that evening listening for adult male calls earlier in the season revealed a strong chorus of many frogs. Due to this, I believe the Animas Pond may rank higher than the relative abundance rankings yielded.

It should be noted that River Pond, not included in the larval abundance ranking, may also be an important breeding habitat for California red-legged frogs and Pacific treefrogs. In 2005 and 2006 a few tadpoles from both species were identified from the bit of accessible water for dipnetting. During evening listening we heard calling Pacific treefrogs and estimated four calling California red-legged frogs males.

Table 2: Estimated Abundance of Larval Pacific Treefrogs

	Larval Pacific Treefrogs			
	2005		2006	
Pond	Estimated Abundance*	Standard Error**	Estimated Abundance	Standard Error
Entrance	2044.80	252.80	872	221.02
Boundary	794.85	112.88	603.75	155.89
Animas	2229.65	878.75	n/a	n/a
Roadrunner	267.60	68.40	124	25.82
Dead Pig	925.30	291.65	826.5	117.74
Salamander	5664.00	1068.80	2656	424.00

* Calculated as the mean number per dipnet sweep multiplied by the circumference of the pond in net widths.
** Calculated as the standard deviation of the mean number per dipnet sweep multiplied by the circumference of the pond.

Table 3: Estimated Abundance of Larval California Red-Legged Frogs

	Larval California Red-Legged Frogs			
	2005		2006	
Pond	Estimated Abundance	Standard Error	Estimated Abundance	Standard Error
Entrance	12.80	6.40	10.40	10.33
Boundary	0.00	n/a	0.00	n/a
Animas	12.35	8.55	n/a	n/a
Roadrunner	0.00	n/a	0.00	n/a
Dead Pig	55.10	21.95	142.50	39.25
Salamander	76.80	33.60	48.00	36.00

Table 4: Estimated Abundance of Larval California Newts

	Larval California Newts			
	2005		2006	
Pond	Estimated Abundance	Standard Error	Estimated Abundance	Standard Error
Entrance	0.00	n/a	0.00	n/a
Boundary	13.13	5.78	21.00	8.13
Animas	0.00	n/a	n/a	n/a
Roadrunner	0.00	n/a	2.80	2.69
Dead Pig	79.80	26.60	6.65	6.38
Salamander	179.20	59.20	49.60	19.30

Table 5: Estimated Abundance of Larval California Tiger Salamanders

	Larval California Tiger Salamanders			
	2005		2006	
Pond	Estimated Abundance	Standard Error	Estimated Abundance	Standard Error
Entrance	0.00	n/a	0.00	n/a
Boundary	0.00	n/a	0.00	n/a
Animas	0.00	n/a	n/a	n/a
Roadrunner	5.20	3.60	2.68	2.68
Dead Pig	0.00	n/a	0.00	n/a
Salamander	6.40	6.40	0.00	n/a

Table 6: Relative Abundance of Larval Amphibians 2006

	2006								
	Pacific Treefrog		California Red-legged Frog		California Newt		California Tiger Salamander		
Pond	Relative Abundance*	Pond Ranking**	Relative Abundance	Pond Ranking	Relative Abundance	Pond Ranking	Relative Abundance	Pond Ranking	Sum Pond Rankings
Entrance	7.03	4	1.00	3	0.00	0	0.00	0	7
Boundary	4.87	2	0.00	0	7.50	4	0.00	0	6
Roadrunner	1.00	1	0.00	0	1.00	2	1.00	5	8
Dead Pig	6.67	3	13.70	5	2.38	3	0.00	0	11
Salamander	21.42	5	4.62	4	17.71	5	0.00	0	14

*Relative abundance is estimated by dividing the Estimated Abundance value by the lowest Estimated Abundance value for that species. Thus the pond with a ranking of 1 corresponds with the pond with the lowest Estimated Abundance for that year as its Estimated Abundance is divided by itself.
**The highest number corresponds to the pond with the greatest relative abundance.

Table 7: Relative Abundance of Larval Amphibians 2005

	2005								
	Pacific Treefrog		California Red-legged Frog		California Newt		California Tiger Salamander		
Pond	Relative Abundance	Pond Ranking	Relative Abundance	Pond Ranking	Relative Abundance	Pond Ranking	Relative Abundance	Pond Ranking	Sum Pond Rankings
Entrance	7.64	4	1.04	4	0.00	0	0.00	0	8
Boundary	2.97	2	0.00	0	1.00	4	0.00	0	6
Animas	8.33	5	1.00	3	0.00	0	0.00	0	8
Roadrunner	1.00	1	0.00	0	0.00	0	1.00	5	6
Dead Pig	3.46	3	4.46	5	6.08	5	0.00	0	13
Salamander	21.17	6	6.22	6	13.65	6	1.23	6	24

Disease Assessment

Loss of mouthpart pigmentation and mouthpart malformation was observed in 2 of the 984 larval Pacific treefrogs and 1 of the 61 California red-legged frog larvae we examined. We took samples by swabbing mouthparts and sent them for testing for presence of *Batrachochytrium dendrobatidis*, the organism that causes chytridiomycosis. All three larvae with malformed mouthparts were positive (see Table 8). The method for using degraded mouthparts as a proxy for infection with *Batrachochytrium dendrobatidis* has not been formally tested for either of these species and we did not include a sample of larvae to test without degraded mouthparts. From these results, we conclude that *Batrachochytrium dendrobatidis* does occur in amphibians in the Entrance and Animas Ponds.

Table 8: Larval Amphibian Mouthpart Malformations and Chytrid Test

	Pacific Treefrogs		California red-legged frogs		Chytrid status for Amphibians with malformed mouthparts
	Number of Larvae Examined	Number of Malformed Mouthparts	Number of Larvae Examined	Number of Malformed Mouthparts	
Entrance	305	2	3	0	Both positive
Boundary	225	0	0	n/a	n/a
River	7	0	4	0	n/a
Animas	11	0	26	1	Positive
Roadrunner	47	0	0	n/a	n/a
Dead Pig	124	0	23	0	n/a
Salamander	265	0	5	0	n/a

We tested adult frogs for *Batrachochytrium dendrobatidis* both opportunistically and methodically. At Entrance Pond we found four dead Pacific treefrogs in February, two of which we swabbed, and caught a third healthy individual to swab. At Animas Pond in May, we encountered a dead adult California red-legged frog, which we swabbed, and we swabbed a live juvenile that we caught. In May, we hand-caught adult California red-legged frogs in Roadrunner, Dead Pig, and Salamander Ponds for the purpose of swabbing for *Batrachochytrium dendrobatidis* (See Table 9.) None of the amphibians we found dead nor any of the live animals that we caught at the same time tested positive. In contrast, half of the frogs we caught from Salamander Pond and over a third from Dead Pig Pond tested positive for *Batrachochytrium dendrobatidis*. None of the three we tested from Roadrunner Pond were positive. We did not test animals from Boundary or River Ponds as we did not encounter then there on the night we took samples.

With the exception of Roadrunner, all ponds where we tested amphibians for *Batrachochytrium dendrobatidis* had at least one positive result. This means that Entrance, Animas, Dead Pig, and Salamander Ponds have all been confirmed to harbor animals with *Batrachochytrium dendrobatidis*. It is unclear what this means for the different species of amphibians at this time. Currently, experiments have not been conducted to determine if infection with *Batrachochytrium dendrobatidis* has any impact on the four amphibian species that use these ponds.

Table 9: Tests for *Batrachochytrium dendrobatidis* in Adult Frogs

	Pacific treefrogs		California red-legged frogs	
	Number of Adults Swabbed	Number of Positive Chytrid Results	Number of Adults Swabbed	Number of Positive Chytrid Results
Entrance	3*	0	0	n/a
Boundary	0	n/a	0	n/a
River	0	n/a	0	n/a
Animas	0	n/a	2**	0
Roadrunner	0	n/a	3	0
Dead Pig	0	n/a	13	4
Salamander	0	n/a	12	6

* Two of these frogs were found dead. ** One of these frogs was found dead.

The four dead Pacific treefrog adults found in Entrance Pond in a single day in February was unusual. It is uncommon to find multiple frogs dead; typically dead or dying frogs are eaten. Die-off events are common to chytridiomycosis and ranavirus. As the tests for *Batrachochytrium dendrobatidis* on two of these frogs was negative, it is unlikely that chytrid was the cause. It is more likely that ranavirus or some other agent we have not considered here was responsible.

No limb malformations typical of infection with *Ribeiroia ondatrae* were observed in any larval amphibian. The snail hosts and definitive host have both been observed in aquatic habitats at PCRP, including Entrance, Boundary, Animas, Roadrunner, Dead Pig, and Salamander Ponds, so it is possible malformations could occur in amphibians on the property. The parasite and its hosts are native to this region and malformations have been found in North American museum specimens dating back to the 1940s. An incidence of five percent or less is considered an acceptable background level of infection while a greater incidence may implicate another factor, such as nutrient loading, interacting in this system (Johnson 2003).

<u>Other Vertebrate and Invertebrate Species Observed</u>

The Entrance Pond is used by a variety of birds and insects. We observed red tailed hawks, barn swallows, dark-eyed juncos, turkey vultures, mallards, and red-winged blackbirds, painted ladies, flame skimmer dragonflies, and boreal bluet damselflies in and around the pond. Dipnetting of aquatic insects revealed a wide variety of aquatic invertebrates, including Diptera: chironomonidae and chaoboridae (midges); Crustacea: amphipoda (scud), ostracoda, and cladocera (water flea); collembolla (globular springtail); Hemiptera: notonectidae (backswimmer) and corixidae (water boatman); Ephemeroptera (mayflies); Odonata: anisoptera (dragonfly nymph) and zygoptera (damselfly nymph); Coleoptera: hydrophilidae adult and larvae (water scavenger beetle) and dytiscidae larvae (predaceous diving beetle or water tiger); Mollusca: physidae; and leeches.

We saw several birds using the Boundary Pond, including the song sparrow, cliff swallow, Anna's hummingbird, black phoebe, Brewers blackbirds, mourning doves, mallard ducks, American goldfinchs, and great blue herons. Further, aquatic invertebrates include Hemiptera: notonectidae (backswimmer) and corixidae (water boatman); Ephemeroptera (mayflies); Diptera: chaoboridae and chironomonidae (midges); Conchostracan: *Cyzicus californicus* (Clam shrimp); Collembolla (globular springtail); Crustacea: copepoda, ostracoda, and cladocera (water flea); Coleoptera: dytiscidae (Predaceous diving beetle larvae or water tiger) and hydrophilidae (water scavenger beetle); and Mollusca: physidae.

Heavy vegetative cover at the Animas Pond made it difficult to identify most of the birds flitting around in the vegetation during our visits, but mallards and red-winged black birds were observed using the pond area. Flame skimmer dragonflies and boreal bluet damselflies also use the pond. We observed the following aquatic invertebrate families in the aquatic insect dipnet: Coleoptera: dytiscidae (Predaceous diving beetle larvae or water tiger) and hydrophilidae (water scavenger beetle); Crustacea: amphipoda (scud) and copepoda; Diptera: chironomonidae (midge), Ephemeroptera (mayfly); Odonata: anisoptera (dragonfly nymph); and Mollusca: physidae.

Spider observed at Animas Pond.

Cliff swallows, doves, and vultures all used Roadrunner Pond while we observed it. We also noted an abundance of blue dragonflies mating around the pond and laying their eggs in the pond. Aquatic invertebrates seen at the Roadrunner Pond include Diptera: chironomonidae and chaoboridae (midges); Crustacea: copepoda; Collembolla (globular springtail); Hemiptera: notonectidae (backswimmer); Ephemeroptera (mayflies); Odonata: zygoptera (damselfly nymph); Coleoptera: dytiscidae (Predaceous diving beetle larvae or water tiger); and Mollusca: physidae.

Red-winged blackbirds, mallard ducks, and harriers were observed around Dead Pig pond. Aquatic invertebrates using the pond include Crustacea: copepoda, amphipoda, ostracoda, cladocera (water fleas); Hemiptera: gerridae (water striders), corixidae (water boatmen), and notonectidae (backswimmers); Coleoptera: hydrophilidae (water scavenger beetles); Odonata: anisoptera (dragonfly nymph) and zygoptera (damselfly nymph); Ephemeroptera (mayfly); Diptera: chaoboridae (midge) and culicidae (mosquito larvae); and Mollusca: planorbidae and physidae.

Red-winged blackbirds, coots, and mallards were observed using the Salamander Pond. We also briefly observed a species of garter snake, but it disappeared in the grass before we could identify it. We also saw Ephemeroptera (mayfly); Hemiptera: notonectidae (backswimmer) and corixidae (water boatman); Diptera: culicidae (mosquito larvae); Crustacea: cladocera, amphipoda (scud), ostracoda, and copepoda; and Mollusca: physidae.

Recommendations

General Recommendations

Palo Corona Regional Park (PCRP) is home to several species of amphibians, including the California red-legged frog (*Rana draytonii*), California tiger salamander (*Ambystoma californiense*), Pacific treefrogs (*Pseudacris regilla*), and California newts (*Taricha torosa*). Of these, California red-legged frogs are currently listed as threatened at the California State and Federal levels. California tiger salamanders are listed as species of special concern at the California State level and threatened at the Federal level; they are currently being considered for listing as endangered at the California State level. The special consideration afforded these two species requires coordination by representatives of Palo Corona Regional Park with the California Department of Fish and Game and the US Fish and Wildlife Service on activities that may impact these species. As Palo Corona Regional Park maintains active grazing, it has some flexibility under Section 4(d) of the Endangered Species Act, as the US Fish and Wildlife Service deems appropriate for ranching activities. Under the Endangered Species Act, any animals listed as endangered are automatically protected and Section 4(d) does not allow flexibility. If activities are specifically related to the maintenance of grazing and involve habitat of animals that are listed as threatened or of lesser protection status, US Fish and Wildlife does not require special take permits (pers. comm. Jacob Martin, USFWS, 19 Dec. 2006). In this situation, it is advisable to discuss the activity with a USFWS contact prior to the beginning of the project to ensure they consider it to fall under the Section 4(d) rules. Any activities that impact California red-legged frog or California tiger salamander habitat that are not related specifically to maintenance of grazing for cattle require discussion with USFWS to determine the proper permitting and personnel are on hand. California Department of Fish and Game has their own set of requirements and permits, and they should be contacted prior to any activities that may impact these amphibians, regardless of whether activities involve maintenance of cattle grazing land. Coordination with both USFWS and CDFG on activities that affect either amphibian species is required.

Currently, some of the most serious threats to native amphibians in central California are absent or of minimal importance at Palo Corona: habitat loss, bullfrogs, and fish. This makes the property of considerable importance for California red-legged frogs and

California tiger salamanders, in particular. However, the relative health of these populations is almost certainly due to the limited human traffic onto this property in the past, and thus calls for careful consideration of increasing human use and its attendant threats as land management evolves on the site.

Invasive Species

Ponds with introduced fish and American bullfrogs are unsuitable breeding habitat for California tiger salamanders (Shaffer 2005) and California red-legged frogs (Lawler 1999). PCRP appears to lack bullfrogs and introduced fish. We recommend continued monitoring for these invasive species and immediate eradication by trained personnel should they be detected. Collaboration with owners of neighboring properties on beginning or continuing eradication projects, particularly bullfrogs, would certainly help avoid future problems with these invasive species at PCRP. In 2005, Denise Duffy and Associates encountered bullfrogs in the Robinson and Las Garzas Watersheds (Harwayne, Keegan et al. 2005). San Jose Creek runs within a kilometer of Las Garzas Creek. It is possible that bullfrogs from Santa Lucia Preserve could move onto the Palo Corona property via San Jose Creek; we recommend continued monitoring for bullfrogs at Palo Corona, particularly using evening audio surveys during summer months.

Invasive plants, particularly French broom (*Genista spp.*), are invading the grassland habitat around Salamander, Dead Pig, and Roadrunner Ponds. This change decreases the upland grassland habitat that California tiger salamanders depend heavily upon for eleven months of the year. Active removal of invasive plants and preservation of grassland will greatly enhance the habitat for California tiger salamanders. CRLF depend strongly on their cryptic coloration and often hunker down instead of fleeing when they sense danger. Remove vegetation within 3 meters of ponds by hand to avoid harming amphibians using this habitat. Permitted personnel should precede any mowing to remove any amphibians in vegetation. These activities should be conducted between the end of August and the first heavy rains to avoid periods of heavy usage of upland habitat by amphibians. Vegetation removal should be balanced with changes in water quality due to sedimentation. We recommend consultation with experts in this area prior to activities.

Further, California tiger salamanders appear to prefer to use gopher and ground squirrel burrows. It is a common practice to eradicate burrowing mammals for agriculture and cattle, but we strongly recommend against this practice. We have noticed a large number of burrows in the road along Salamander Pond and have noted Pacific treefrogs and California newts emerging from them. We suspect they could also be used by California tiger salamanders. We recommend drivers avoid using this section of the road, opting to walk when possible. If driving is unavoidable, have a person walk ahead and watch for emerging amphibians and avoid driving during dusk and early evening when large numbers of amphibians will be emerging from the burrows to minimize harm to species using these burrows.

We recommend an educational program for employees, volunteers, and visitors of PCRP regarding the threat of exotic species to native flora and fauna, particularly California red-legged frogs and California tiger salamanders steps they can take to avoid release of exotics, and what they can do if they see an exotic. These activities can include workshops, printed materials, and signage on the property.

Vegetation Management In Ponds
Ponds, such as River Pond, that have become overgrown with vegetation and have very little open water can be in jeopardy of becoming meadows. As loss of freshwater habitat is a serious problem for amphibians, we recommend maintenance of current freshwater habitats. Thinning or removal of sections of vegetation may help with this situation, but should be considered outside prime breeding periods of January through August. Additionally, removal of sediment may be required to re-establish or maintain a pond. Any work like this should be discussed with local biologists and government agencies to ensure minimization of incidental take and proper permitting.

We strong advise against the use of herbicides, pesticides, and fertilizers in PCRP, particularly in close proximity to aquatic habitats and watersheds and on windy days. Working with land owners in watersheds above the ponds on this issue will help to keep the aquatic habitats healthy for California red-legged frogs and other plants and animals using these habitats.

<u>Recommendations to Maintain and Enhance Existing Populations</u>

California Red-Legged Frogs
Most of the comments in the recommendations section above apply to all amphibians at PCRP. We have an additional specific recommendation for CRLF at PCRP. Currently California red-legged frog juveniles and adults use the Animas pond, often resting on the bank along the trail. They are startled off these refuge sites when humans approach. Fencing this pond will help keep visitors from meandering to the pond edge and disturbing the California red-legged frogs resting there. It will also keep visitors from having contact with soil and water from this pond and potentially spreading chytrid and other pathogens in the mud on their shoes at PCRP and to other properties they visit subsequently.

California Tiger Salamanders
California tiger salamanders have bred in two ponds at PCRP, Roadrunner Pond and Salamander Pond. Both of these ponds host a variety of native food sources and predators for California tiger salamanders. Shaffer et al. (2005) demonstrated that resident California newt and dragonfly larvae populations are capable of eating all California tiger salamander embryos in a pond where they co-occur. Of some concern are the California newt adults we observed in these ponds and the breeding dragonflies at Roadrunner Pond in 2006. We did not find dragonfly larvae in either of these ponds, but we would advise watching for them in subsequent years. Ponds that dry down in the fall, even every few years, may discourage adult California newts from taking up year-round residence in the ponds, allowing California tiger salamander greater breeding success. While Roadrunner pond is reported to dry-down regularly, Salamander pond rarely dries-down. We recommend avoiding any changes to either of these ponds that will cause them to remain wet year-round. We also recommend PCRP consider using a pump to

artificially dry Salamander Pond during the fall every few years to discourage overwintering newt larvae and large populations of native competitors whose populations may be bolstered in perennial ponds. This would require discussion with a hydrologist and coordination with governmental agencies, including CDFG and UCFWS. Neighboring Santa Lucia Preserve only has 5 known breeding ponds and 2 located near the border with PCRP. Studies there show the breeding adult population to be low. The animals there, like the ones at PCRP appear not to be hybridized. Thus these are very important populations and key to preserve (Allaback, pers comm. 2006.)

California Newts
California newts will be benefited by many of the actions aimed at improving habitat and protection for California red-legged frogs and California tiger salamanders. Common threats to California newt populations include degradation of breeding sites, habitat fragmentation, upland habitat conversion, and introduced predators that feed on their eggs and larvae including crayfish, mosquitofish, green sunfish, and rainbow trout (Kuchta 2005). They also use stream habitats, where alteration of sedimentation can be problematic (Kuchta 2005). This can be caused by upland agriculture, deforestation, or fire. Monitoring for and mitigation of high sediment flows in streams caused by upland activities will improve habitat for California newts using streams and creeks.

Metapopulation Dynamics for CTS and CRLF

Preservation of amphibian populations through conservation of habitat at places such as PCRP is essential to the persistence of threatened amphibians like California red-legged frogs and California tiger salamanders. Just as essential is collaboration with neighboring landowners on conservation strategies that focus on maintenance of connectivity of appropriate habitat for these amphibians. While PCRP has seven ponds with California red-legged frogs and two with California tiger salamanders, this does not constitute a local metapopulation that is safe from the threat of population decline from any number of threats, including stochastic factors. Maintenance of connectivity between habitat sites is essential for healthy amphibian populations.

Bulger et al. (2003) found California red-legged frogs moving between ponds and streams and via a variety of upland habitat along straight lines. Maintaining connectivity between ponds through promotion of native vegetation is important for healthy California red-legged frog populations. Heavy usage of roads will decrease connectivity between ponds and should be avoided.

Disease Assessment and Management

Batrachochytrium dendrobatidis does occur in amphibians on the Palo Corona Regional Park property, so we recommend following the strictest procedures to avoid increasing the rate of spread of this pathogen between sites and between individual animals. Until more is known about the effects of this pathogen on the species of amphibians at PCRP, we urge all staff, researchers, volunteers, and visitors of the park to avoid contact with amphibians and pond and creek waters, as human transport of amphibian diseases is suspected to be a method of spread. Should contact be unavoidable, follow the decontamination procedures outlined in Appendix A. These procedures were authored by Valentine Hemingway and Cammy Chabre. They are based on the U.S. Fish and Wildlife Services' "Revised Guidance on Site Assessments and Field Surveys for the California Red-legged Frog" from August 2005, with amendments based on information from Dr. Richard Speare and Dr. David Green, both veterinarians specializing in amphibian disease and wildlife health.

Management of Upland Habitat

General recommendations for management of upland habitat include strategies to discourage invasive plants and maintain native grasses, such as grazing, eradication of French broom around and between ponds, and care to avoid activities that will cause sedimentation of aquatic habitats (see "Invasive Species" section above). Further, trails and roads should be constructed to minimize contact with aquatic habitats by people and vehicles. To avoid direct mortality of amphibians and spread of pathogens, we recommend avoiding crossing through creeks by vehicles and installation of bridges for vehicle crossing if at all possible. This will help avoid molestation of amphibians and potential spread of pathogens.

California tiger salamanders are particularly dependent upon appropriate upland habitat. This includes grassland and oak woodland with California ground squirrel and gopher burrows. Removal of invasive plants, particularly French broom (*Genista spp.*) from these habitats is essential for maintenance of appropriate habitat for this species. Dead Pig Pond lies between the two breeding sites for California tiger salamanders. We recommend the removal of French broom around Dead Pig Pond and between it, Salamander, and Roadrunner Ponds to increase connectivity and restore the grassland favored by California tiger salamanders between the ponds. This action will encourage the use of Dead Pig Pond as a breeding site for California tiger salamanders and upland habitat for juvenile and adults.

We are not by any means experts in vegetation control, but offer a few suggestions here that would best be checked with an expert in the field of vegetation control. Grazing by cattle or some other vegetation control method may be used to create patchiness in invasive grasses, mimicking patchiness of native bunch grasses that are easier for migrating amphibians to move through. Goats may be an option to keep French broom under control. Vegetation within about 3 meters of the pond edge should be removed by hand to avoid harm to amphibians. Any mowing should be accompanied by someone to help remove amphibians ahead of mowers. All vegetation removal should be conducted between late August and the first heavy rains of the season. Avoid the control of gopher and ground squirrels that is a common management practice in areas where cattle are grazing.

Management Recommendations Specific To Cattle Access to Ponds

Unfortunately, there are few studies on the impact of cattle grazing on amphibian populations. Most recommendations are based on antecdotal or observational evidence. An important factor to consider when thinking about cattle grazing and pond use is the positive results for *Batrachochytrium dendrobatidis* at PCRP.

There are several important questions to be studied that directly impact cattle management activities:
1. Does cattle grazing of ponds impact amphibians using the ponds?
2. Does chytrid affect amphibian species of PCRP?
3. Do cattle play a role in chytrid transmission between sites?

There are some researchers who are currently studying these questions. Their results will be helpful to PCRP, and maintaining a conservative and flexible management plan will allow managers at PCRP to adapt their strategy to new information as it is made available.

For these reasons, we recommend fencing the entire perimeter of ponds with the addition of gates. The ponds could be bisected with fencing as well, with gates on either side of the bisected pond to allow for the controlled cattle access. This would allow for an adaptave management tool that has flexibility to incorporate new management strategy as the studies on these topics come in. Here is our reasoning:

1. It may be that cattle are very disruptive to California red-legged frogs and California tiger salamanders, crushing or dislodging egg masses or harming tadpoles. Cattle may also have important positive impacts on them, such as opening up banks and water for tadpole and adult use. This has not been formally studied at this point. Further, we do not know the optimal vegetation scheme for California red-legged frogs. It is also not known how increases in turbidity affect development in eggs and tadpoles; it is harmful to some species and not in others, but it is not known for California red-legged frogs and California tiger salamanders. Fencing the entire pond now with the bisecting fence and gates on both sides will allow control of access, restricting cattle entrance during key breeding season (January through August) and allowing cattle admittance when tadpoles are larger and more able to escape danger. This fencing scheme allows for a variety of management options to adapt as research results come in.

2. Until there is a better sense for how *Batrachochytrium dendrobatidis* impacts California red-legged frogs and California tiger salamanders and for how *Batrachochytrium dendrobatidis* moves between sites, fencing the pond and allowing limited access by the cattle will limit the possibility for increased rate of spread of this pathogen by humans and human activity. While there is not a way to control this pathogen in wild populations, decreasing the possibility we, our tools, and our animals are spreading is strongly advised. By limiting access to the ponds by cattle, and allowing them a day or so to allow caked-in mud and sand to dry and flake out of their hooves before they have access to another pond, we will hopefully avoid a possible human source of spread of this disease.

Annual Monitoring Program

The data collected in 2004-2006 provide evidence of relative numbers and habitat use for breeding. However, 2004-2005 was an exceptionally wet winter season and therefore changes in amphibian numbers that might be observed in subsequent years must be viewed in light of the normal variability in amphibian activity that is driven by rainfall patterns across years. Because of this variation and the need to account for it in establishing baseline abundance and presence/absence patterns, we recommend continued monitoring of amphibians in aquatic habitats at PCRP. While some data collection can be less extensive than in the 2004-2006 seasons, we recommend that sampling for both larvae and adult activity be conducted in all ponds and streams. Monitoring should be used to provide feedback for management activities and early detection of invasive species such as bullfrogs, which occur on lands surrounding PCRP.

For future assessment of relative abundance of larval amphibians, we recommend following a similar dipnet method as described in the methods section of this document. A minimum of 15 evenly spaced dipnet samples should be taken per water body, identifying and counting each larval amphibian in each dipnet sweep, noting the composition of each dipnet. The circumference of the water body should be estimated by paces and converted to meters based on the surveyor's average pace distance. This data should be input into an Excel spreadsheet and relative abundance estimated using the mean number of larvae per dipnet multiplied by the estimated number of dipnet widths in the circumference.

Following 2006, we suggest that a scaled-back set of audio and visual surveys be taken in ponds closest to potential sources of bullfrog invasion, while continuing to monitor amphibian use of all ponds with particular attention to ponds were habitat improvements have been made.

Knowledge of amphibians' use of the ponds can be greatly expanded by use of daytime and nighttime visual inspection surveys and evening listening surveys. This will reveal information on adult frog use of the ponds and breeding attempts. For a thorough treatment of these survey methods, please refer to "Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians" (Heyer 1994). We recommend following the method outlined in the U.S. Fish and Wildlife Service's "Revised Guidance on Site Assessments and Field Surveys for the California Red-legged Frog" from August 2005. Evening listening surveys are made between January and August to determine the composition of adult frogs breeding in the pond area. Daytime visual inspection surveys involve using binoculars and a sit-and-wait technique to locate and identify amphibians using the ponds. Nighttime eyeshine surveys use binoculars while holding a flashlight just below or alongside them to detect the eyeshine of amphibians and other animals using the pond and its perimeter and to positively identify those individuals. Nighttime eyeshine surveys are particularly rich for viewing adult pond-breeding amphibians that tend to be nocturnal in this region. These are the methods we have used and described in our methods section above. Trained personnel will be most effective at performing the audio and visual surveys and any surveys involving handling of amphibians, such as dipnetting, require permitted individuals to perform them.

References Cited

Allen, M. F., T. Tennant (2000). Evaluation of Critical Habitat for the California red-legged frog (*Rana aurora draytonii*). University of California, Riverside, Center for Conservation Biology.

Berger, L., R. Speare, and A. Hyatt (1999). Chytrid Fungi and Amphibian Declines: Overview, Implications, and Future Directions. Declines and Disappearances of Australian Frogs. A. Campbell. Canberra, ACT, AU, Environment Australia**:** 23-33.

Blaustein, A. R., J. M. Romansic, et al. (2005). "Interspecific Variation in Susceptibility of Frog Tadpoles to the Pathogenic Fungus Batrachochytrium dendrobatidis." Conservation Biology **19**(5): 1460-1468.

Boyle, D. G., D. B. Boyle, V. Olsen, J. A. T. Morgan, and A. D. Hyatt (2004). "Rapid Quantitative Detection of Chytridiomycosis (*Batrachochytrium dendrobatidis*) in Amphibian Samples Using Real-Time Taqman PCR Assay." Diseases of Aquatic Organisms **60**: 141-148.

Bulger, J. B., N. J. Scott, Jr., and R. B. Seymour (2003). "Terrestrial Activity and Conservation of Adult California Red-Legged Frogs *Rana aurora draytonii* in Coastal Forests and Grasslands." Biological Conservation **110**: 85-95.

Bury, R. B. (2005). *Dicamptodon ensatus*. Amphibian Declines: The Conservation Status of Unites States Species. M. Lannoo. Berkeley, Ca, University of California Press**:** 653-654.

Casper, G. S. and R. Hendricks (2005). *Rana catebbeiana* Shaw 1802. Amphibian Declines: The Conservation Status of United States Species. M. Lannoo. Berkeley, University of California Press**:** 540-546.

Daszak, P., L. Berger, A. A. Cunningham, A. D. Hyatt, D. E. Green, and R. Speare (1999). "Emerging Infectious Diseases and Amphibian Population Declines." Emerging Infectious Diseases **5**(6): 735-748.

Daszak P, S. A., Cunningham AA, Longcore JE, Brown CC, Porter D. (2004). "Experimental evidence that the bullfrog (Rana catesbeiana) is a potential carrier of chytridiomycosis, an emerging fungal disease of amphibians." Herpetological Journal **14**(4): 201-207.

Doubledee, R. A., E. B. Muller, and R. M. Nisbet (2003). "Bullfrogs, Disturbance Regimes, and the Persistance of California Red-Legged Frogs." Journal of Wildlife Management **67**(2): 424-438.

Fellers, G. M. (2005). *Rana boylii*. Amphibian Declines: The Conservation Status of United States Species. M. Lannoo. Berkeley, CA, Univesity of California Press**:** 534-536.

Fellers, G. M. (2005). *Rana draytonii*. Amphibian Declines: The Conservation Status of United States Species. M. Lannoo. Berkeley, University of California Press**:** 552-554.

Fellers, G. M., D. E. Green, et al. (2001). "Oral Chytridiomycosis in the Mountain Yellow-Legged Frog (Rana muscosa)." Copeia **1**(4): 945-953.

Garner, T., M. Perkins, et al. (2006). "The emerging amphibian pathogen *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American bullfrog, *Rana catesbeiana*." Biol. Lett.: FirstCite-FirstCite.

Hanselmann, R., A. Rodriguez, M. Lampo, L. Fajardo-Ramos, A. A. Aguirre, A. M. Kilpatrick, J. P. Rodriguez, and P. Daszak (2004). "Presence of an Emerging Pathogen of Amphibians in Introduced Bullfrogs *Rana catesbeiana* in Venezuela." Biological Conservation **120**: 115-119.

Harwayne, J., D. Keegan, et al. (2005). 2005 Stock-Pond Survey Report for the Santa Lucia Preserve, Monterey County, California. Monterey, Denise Duffy and Associates, Inc.

Biosearch Associates.

Hemingway, V. A., A. D'Amore, D. Reis, and K. Wasson (2004). Threatened Amphibians: Trends in Distribution and Abundance at Elkhorn Slough National Estuarine Research Reserve 1997-2004. Report to ESNERR, Resource Conservation District, Monterey County Mosquito Abatement District, Monterey County Planning Commission, and USFWS.

Heyer, W. R., M.A. Donnelly, R.W. McDiarmid, L.-A. C. Hayek, & M.S. Foster, Ed. (1994). Measuring and Monitoring Biological Diversity Standard Methods for Amphibians. Washington & London, Smithsonian Institution Press.

Jennings, M. R., and M. P. Hayes (1985). "Pre-1900 Overharvest of California Red-Legged Frogs (*Rana aurora draytonii*): The Inducement for Bullfrog (*Rana catesbeiana*) Introduction." Herpetologica **41**(1): 94-103.

Johnson, P. T. J., and D. R. Sutherland (2003). "Amphibian Deformities and *Ribeiroia* infection: an Emerging Helminthiasis." Trends in Parasitology **19**(8): 332-335.

Johnson, P. T. J., K. B. Lunde, D. A. Zelmer, and J. K. Werner (2003). "Limb Deformities as an Emerging Parasitic Disease in Amphibians: Evidence from Museum Specimens and Resurvey Data." Conservation Biology **17**(6): 1724-1737.

Johnson, P. T. J., K. B. Lunde, E. G. Ritchie, and A. E. Launer (1999). "The Effect of Trematode Infection on Amphibian Limb Development and Survivorship." Science **284**: 802-804.

Johnson, P. T. J., K. B. Lunde, E. M. Thurman, E. G. Ritchie, S. N. Wray, D. R. Sutherland, J. M. Kapfer, T. J. Frest, J. Bowerman, and A. R. Blaustein (2002). "Parasite (*Ribeiroia ondatrae*) Infection Linked to Amphibian Malformations in the Western United States." Ecological Monographs **72**(2): 151-168.

Kuchta, S. R. (2005). *Taricha torosa*. Amphibian Declines: The Conservation Status of United States Species. M. Lannoo. Berkeley, CA, University of California Press**:** 653-654.

L. Berger, R. Speare, et al. (1998). "Chytridiomycosis Causes Amphibian Mortality Associated with Population Declines in the Rain Forests of Australia and Central America." PNAS **95**(14): 9031-9036.

Lawler, S. P., D. Dritz, T. Strange, and M. Holyoak (1999). "Effects of Introduced Mosquitofish and Bullfrogs on the Threatened Calfornia Red-Legged Frog." Conservation Biology **13**(3): 613-622.

Loredo, I., and D. Van Vuren (1996). "Reproductive Ecology of a Population of the California Tiger Salamander." Copeia **1996**: 895-901.

Loredo, I., D. Van Vuren, and M. L. Morrison (1996). "Habitat Use and Migration Behavior of the California Tiger Salamander." Journal of Herpetology **30**(2): 282-285.

Muths, E., and P. Nanjuppa (2005). *Bufo boreas*. Amphibian Declines: The Conservation Status of United States Species. M. Lannoo. Berkeley, CA, University of California Press**:** 392-396.

Parris, M. J., and J. G. Beaudoin (2004). "Chytridiomycosis Impacts Predator-Prey Interactions in Larval Amphibian Communities." Oecologia **140**: 626-632.

Rachowicz, L. J., and V. T. Vrendenburg (2004). "Transmission of Batrachochytrium dendrobatidis within and between amphibian life stages." Diseases of Aquatic Organisms **61**: 75-83.

Riley, S. P. D., H. B. Shaffer, S. R. Voss, and B. M. Fitzpatrick (2003). "Hybridization Between A Rare, Native Tiger Salamander (*Ambystoma californiense*) and its Introduced Congener." Ecological Applications **13**(5): 1263–1275.

Scott, N. J. and G. B. Rathburn (2002). Stockpond Management for the Benefit of California Red-Legged Frogs (*Rana aurora draytonii*).

Sessions, S. K., and S. B. Ruth (1990). "Explanation for Naturally Occurring Supernumary Limbs in Amphibians." The Journal of Experimental Zoology **254**: 38-47.

Shaffer, H. B., R. A. Alford, et al. (1994). Quantitative Sampling of Amphibian Larvae. Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L.-A. C. Hayek and M. S. Foster. Washington, Smithsonian Institution Press**:** 130-141.

Shaffer, H. B., P. C. Trenham (2005). Ambystoma califoriense. Amphibian Declines: The Conservation Status of United States Species. M. Lannoo. Berkeley, University of California Press**:** 605-608.

Shaffer, H. B., R. N. Fisher, and S. E. Stanley (1993). Status Report: The California Tiger Salamander (*Ambystoma californiense*). Final Report for the California Department of Fish and Game. Unpublished Report.

Stebbins, R. C. (1985). A Field Guide to Western Reptiles and Amphibians. Boston, Massachusetts, Houghton Mifflin Company.

Stopper, G. F., L. Hecker, R. A. Franssen, and S. K. Sessions (2002). "How Trematodes Cause Limb Deformities in Amphibians." Journal of Experimental Zoology **294**: 252-263.

Storer, T. I. (1925). A Synopsis of the Amphibia of California. Berkeley.

Trenham P.C., W.D. Koenig, H.B. Shaffer (2001). "Spatially autocorrelated demography and interpond dispersal in the salamander Ambystoma californiense." Ecology **82**(12): 3519-3530.

Trenham, P. C. (2001). "Terrestrial Habitat Use By Adult *Ambystoma californiense*." Journal of Herpetology **35**: 343-346.

U.S. Fish and Wildlife Service (2002). Recovery Plan for the California Red-legged Frog (Rana aurora draytonii). Portland, Oregon, U.S. Fish and Wildlife Service.

Webb, C., and J. Joss (1997). "Does Predation by the Fish *gambusia holbrooki* (Atheriniformes: Poeciliidae) Contribute to Declining Frog Populations?" Australian Zoologist **30**(3): 316-324.

Weldon, C., L. H. du Preez, A. D. Hyatt, R. Muller, and R. Speare (2004). "Origin of the Amphibian Chytrid Fungus." Emerging Infectious Diseases **10**(12): 2100-2105.

Wright, A. H. and A. A. Wright (1949). Handbook of Frogs and Toads of the United States and Canada. Ithaca, New York, Comstock Publishing Associates.

Wright, K. M. and B. R. Whitaker (2001). Amphibian Medicine and Captive Husbandry. Malabar, Florida, Kreiger Publishing Company.

Yamaguti (1975). A Synoptical Review of Life Histories of Digenetic Trematodes of Vertebrates with Special Reference to the Morphology of Their Larval Forms. Tokyo, Keigaku Publishing Co.

Appendix A: Decontamination Procedures

Don't Be A Vector: Field Methods to Avoid Spreading Amphibian Diseases

Valentine Hemingway and Cammy Chabre
August 2006

Just as emerging infectious disease has become a prime concern for human health, new pathogens have become a concern for wildlife populations, including amphibians. Amphibians in our region have a variety of threats to contend with, from loss of habitat to poor water quality to introduced predators to a variety of diseases. Many researchers are beginning to find that synergisms between these threats pose unexpected consequences for amphibians. Until we understand what these threats mean to the amphibians in our region, we advise erring on the side of caution.

We have put together a list of recommendations that are easy to incorporate to your fieldwork routine. These recommendations are based on recent research and recommendations made by the USFWS. The main amphibian diseases that are of concern are chytrid and ranaviruses. By following these guidelines you will decrease the possibility of becoming a vector of these diseases. The goal is not to cease transmission of infection here, as this would be an impossibility for us at this time. Instead, the idea is not to increase the rate of transmission both between sites and at sites by our activities.

Recommendations

Between Sites

Option 1: Dedicated Equipment for Each Site

One strategy is to use dedicated equipment for each site. This includes waders, nets, boots, and any other equipment that may contact the site. Few will have the luxury of this option.

Option 2: Decontaminate Between Sites

When using the same equipment for different sites, decontaminate between sites. Also, if you do not wear waders and your skin and clothing come into contact with the water, mud, or other materials from the site, you should decontaminate clothing and skin between sites.

A. Use a **stiff bristle brush** to scrub off any organic debris, mud or dirt from your equipment while at the site. Use fresh, clean water for this scrubbing.

B. **Spray down**, soak, or wipe your equipment (nets, boots, waders, float tubes, boats, measuring devices, traps, syringes, etc.) with decontamination solution of choice. Dr. David Green of the USGS National Wildlife Health Center strongly recommends the use of household bleach as it is broad-spectrum, breaks down quickly, and it definitely kills both chytrid and ranavirus when used properly. If your skin or clothing has come in contact with water or mud from the site, spray this also to kill pathogens you may be carrying. After the appropriate amount of time for the particular decontamination solution you are using (see below), **rinse the equipment with clean water** to avoid adding toxins to your next site. Applying the solution and rinsing should be done into a container to dispose of the effluent later or in a location where the effluent will not enter the water body, at least 100 feet/35 meters away from the water. We recommend garden sprayers as a convenient way to store, move, and apply fresh water and solution. Standing in a large Rubbermaid container while spraying will allow for the collection of the effluent and its safe disposal later.

C. Should you not be able to decontaminate equipment or clothing between sites, place those items in **waterproof bags or containers** to avoid potential contamination of the next site.

Decontamination of Vehicles:

If you are driving **vehicles** into the water bodies, drive 100 feet/35 meters away from the water body and spray the vehicle, especially the tires and undercarriage, with decontamination solution. If it is impossible to do this between each water body, at a minimum do it between catchments.

Dry Down Equipment Between Sites Not Recommended:

Drying down equipment between sites is another recommendation effective against chytrid, but this does not appear to be effective for all strains of ranavirus. Ranavirus can survive up to 90 days out of water. Thus, we recommend using one of the above procedures for decontamination between sites.

At Sites

Option 1: Bag Hands

When hand-catching animals, use a bag around your hand to catch the individual and hold it for processing. Some water in the bag can help dilute any peptides they may release that could be harmful to them. Individuals can be measured and weighed (if the bag does not contain water and other materials) and pittagged while in the bag. The bag can be used to hold amphibians to swab for chytrid. An added benefit to using these bags is they can be decontaminated later and reused. After trying a variety of bags, we recommend using “Kyjen UnScented Cornstarch Pooch Pickup Bags,” available at Petsmart in Santa Cruz and online. They are thin enough to get a good hold while catching and have handles that can be tied to hold the amphibian until you are ready to process it. You can also write the site where the amphibian was caught on the bag for later release.

Option 2: Use Gloves

Use nitex, latex, or vinyl gloves to catch and process each animal singly. Gloves should be changed between animals by pulling them off, making them inside out, and disposed of properly after use (i.e. soaked in decontamination solution or autoclaved and disposed.)

Using Dipnet or Seine

When using a dipnet or seine, process the animals as quickly as possible to avoid prolonged closer-than-normal contact

Surgical and Non-Surgical Equipment

Decontaminate equipment that comes into contact with the animals. For example, if you are toe clipping, soak scissors in decontamination solution for the recommended amount of time and rinse with fresh water before using on another animal. Consider using several pairs of scissors to speed the processing of the animals, so one can be soaking while using another. Measuring devices, syringes, and other pieces of equipment that come in contact with individual amphibians or sites should also be treated this way. Using bags to catch and hold amphibians have the added benefit of allowing the amphibian to be measured without coming in contact with the measuring device.

Decontamination Solutions

There are several solutions for decontamination for both chytrid and ranavirus. They vary in price, toxicity, and how long they need to remain on the equipment for them to be effective. Below we have made a table of them and have provided some additional information on them in Appendix A.

Disinfectant	Concentration	Time Needed for Decontamination	Source
Heat [c]	60^{o}C	15 minutes	N/A
Hot Wash[c]	60^{o}C +	15 minutes	N/A
Ethanol[b]	70%	1 minute	
Virkon[c]	1mg/ml	1 minute	http://www.pbsanimalhealth.com http://www.aquaticeco.com
Sodium hypochlorite/ bleach[a, c, d]	4%	15 minutes	Any drug or grocery store.
Tec-Quat 128/DDAC[a]*	1:60	1 minute	http://www.ptthings.com
TriGene Virucidal Surface Disinfectant[a,c]*	0.5ml/L	1 minute	Not currently available from a US supplier, order directly from the manufacturer at: rebecca.wilson@medichem.co.uk http://www.medi-chem.com
F10 Super Concentrate Disinfectant[a,c]*	1ml/L	1 minute	Not currently available from a US supplier, order from any of these suppliers: formten@icon.co.za www.healthandhygiene.net chemicalessentials@bigpond.com iant@healthandhygiene.co.za

a. Nets, waders, and other field equipment
b. Metal instruments

c. Enclosures and tanks
d. Dr. David Green of the USGS National Wildlife Health Center strongly recommends the use of household bleach as it is broad-spectrum, breaks down quickly, and it definitely kills both chytrid and ranavirus when used properly.
*Not tested against ranavirus

Literature Cited

Australian Government Department of Environment and Heritage (2006). “Background Document for the Threat Abatement Plan: Infection of Amphibians with the Chytrid Fungus Resulting in Chytridiomycosis.”
http://www.deh.gov.au/biodiversity/threatened/publications/tap/chytrid/

Johnson, M. L., L. Berger, et al. (2003). "Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid Batrachochytrium dendrobatidis." Diseases of Aquatic Organisms 57: 255-260.

Lynch, S., and A. Fesnock (2006). “Decontaminate Your Equipment Between Ponds.” Part of a packet created for a Long-toed Salamander meeting

Speare, R., L. Berger, et al. (2004). “Hygiene Protocol for Handling Amphibians In Field Studies.” From Protocol for Handling *Frogs*
www.jcu.edu.au/school/phtm/PHTM/frogs/field-hygiene.pdf

Speare R. (ed) Recommendations from Workshop in Getting the Jump on Amphibian Disease. Attachment 5: In: Speare R and Steering Committee of Getting the Jump on Amphibian Disease. Developing management strategies to control amphibian diseases: Decreasing the risks due to communicable diseases. School of Public Health and Tropical Medicine, James Cook University: Townsville. 2001:131-147. At
http://www.jcu.edu.au/school/phtm/PHTM/frogs/adrecommendations.htm#betweenpop

U.S. Fish and Wildlife Service (2005). “Revised Guidance on Site Assessments and Field Surveys for the California Red-legged Frog” http://
www.dfg.ca.gov/hcpb/species/stds_gdl/amp_sg/CRF%20Survey%20Guidance%20Aug2005_Final.pdf

Webb, R., D. Mendez, L. Berger, and R. Speare (2006). “Additional Disinfectants Effective Against the Amphibian Chytrid Fungus, *Batrachochytrium dendrobatidis*.” In submission.