Final Report for
Amphibian Management and Monitoring at
Palo Corona Regional Park,
Monterey County, California
2005

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Executive Summary

Work Conducted

Given the need to understand the entire community of pond-breeding amphibians, we conducted surveys in spring and summer 2005 to measure: A) occurrence and breeding activity of amphibians in all known stock ponds, seeps, creeks, and cattle troughs 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) hybridization status of California tiger salamanders (CTS) on the property, and E) compositions of both invertebrate prey and invertebrate and vertebrate predator communities.

A. The Palo Corona property contains ten ponds and five streams that could provide appropriate habit for water-breeding amphibians. The contract for this project was finalized too late in the 2004-2005 winter season to allow surveys of early-season steam-breeding amphibian activity, but we conducted adult activity surveys in all ponds and sampled for larvae and metamorphs in all 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 two of ten ponds, PTF at nine of ten ponds, and CN at four of nine ponds. CRLF adults were not observed in any cattle troughs, but larvae were observed in the cattle trough below Animas Pond. PTF larvae were observed in five of nine cattle troughs. No introduced American bullfrogs were detected at PCRP. We were not able to adequately sample streams on the property due to the late rains that blocked our access to the remote stream sites.
C. We found the greatest relative abundance of larval California red-legged frogs, California tiger salamanders, California newts, and Pacific treefrogs in Salamander Pond. Dead Pig Pond had the second greatest relative abundance of larval California red-legged frog and California newt larvae. 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 is some evidence for infection with the chytrid *Batrachochytrium dendrobatidis* in CRLF larvae in Animas Pond, Animas Cattle Trough, and Dead Pig Pond.
E. Testing of tissue samples taken from CTS showed no hybridization with introduced tiger salamanders
F. 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

The data collected in 2004-2005 provide evidence of relative numbers and habitat use for breeding. However, this 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 a second year of monitoring of amphibian use of aquatic habitats at PCRP. While some data collection can be less extensive than in the 2004-2005 season, we recommend that sampling for both larvae and adult activity be conducted in all ponds and streams in the 2005-2006 season. In particular, we suggest:

A. Monitoring be used to provide feedback for management activities and early detection of invasive species such as bullfrogs, 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. Following the 2005-2006 season, we suggest that a scaled-back set of observations be taken in ponds closest to potential sources of bullfrog invasion, while continuing to monitor amphibian use of ponds were habitat improvements have been made.
B. We recommend continued monitoring of larval amphibians and expanded methods to include a stratified sampling method in 2005-2006. This will allow feedback for management activities and a standardized method for a proxy of breeding success.
C. Further tests to determine if amphibians at PCRP are infected with chytrid should be undertaken. Decontamination methods for all equipment that comes into contact with water bodies should be followed.
D. Testing for hybridization of CTS with introduced tiger salamanders should be continued in the future as hybrids occur on neighboring properties.
E. Improvement should be made to upland habitats for CTS, particularly removal of French broom.
F. 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.
G. 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.
H. Cooperation with neighboring land managers on control and eradication of invasive species will boost the efficacy of these measures. As noted above, invasion of bullfrogs, non-native tiger salamanders, and possibly disease from adjacent areas will be an ongoing concern at the 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, and 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 4300 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, perhaps 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 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.

<u>Amphibian Species Descriptions</u>

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 (Hanselmann 2004).

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 the listing of the California tiger salamander as a Federally Threatened Species.

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 utilize a variety of habitats, including woodlands, grasslands, chaparral, and farmland, but are primarily found in low foliage near water (Stebbins 2003). Pacific treefrogs are susceptible to infection by the trematode parasite *Ribeiroia ondatrae* (Johnson 2002; Hemingway 2004). Investigation is ongoing as to their susceptibility to infection with *Batrachochytrium dendrobatidis* (Vance Vrendenburg, pers. comm. 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, 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. It moves from *Planorbella sp.* freshwater snails and infects larval amphibians, causing 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 completes its life cycle in the bird's digestive tract when eggs are passed back into the water in feces (Yamaguti 1975). Presence of all three hosts is required for persistence of the parasite, a condition that, in part, leads to its patchy distribution across the landscape. It is suspected that mortality occurs in tadpoles through massive infection by parasites and to malformed metamorphosing amphibians through increased risk of predation by the next parasite host or through starvation (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 larval and in the skin adult amphibians. Infection with *B. dendrobatidis* can lead to mortality via a reduction in the amphibian's ability to osmoregulate water and electrolytes and/or through production of toxic compounds that are absorbed by the amphibian (Berger 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). Infection from *B. dendrobatidis* can be detected with through changes in the pigmentation of the mouthparts of larval amphibians (a quite imperfect detection method) or by genetic laboratory tests on tissue samples (which is expense and time-consuming).

Ranaviruses

Ranaviruses consist of several 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 only a small number of ponds that could 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. Location of the ponds is given in latitude and longitude coordinates in Table 1.

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, this 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 banks. 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 is located further up the front slope, with about two-thirds of its perimeter surrounded by grassland and a third chaparral. Although similar in size to Entrance Pond, it is shallower throughout. 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 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 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 no open water.

Animas Pond is located behind the front slopes along the main trail, and 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 rush, aquatic iris, duckweed, willow, and other aquatic plants. While completely covered, the plant cover is not as dense as is the vegetation at River Pond.

Roadrunner Pond is located along the access road and is surrounded by grassland, chaparral, and French broom. One of the smaller ponds, it is about 200 square meters. Roadrunner pond is unfenced and about 10% 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 is located further along the main road, receiving runoff from the road and grasslands above. It is about 1000 square meters. Surrounded completely by tall French broom, oaks, poison oak, and grassland, it is unfenced, though not likely accessible to cattle as the surrounding vegetation is quite thick. 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 stream from the road runs into the pond.

Salamander Pond is adjacent to the Santa Lucia Conservancy 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 along its other side. It is surrounded by French broom with grasses. 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 growing in the pond and about 25% of the pond has submerged aquatic vegetation.

Wire Corrals Pond is located up from 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 deep. The pond has about 10% coverage with submerged aquatic vegetation and a very small amount of floating vegetation.

Van Winkleys Pond is located along Van Winkleys Creek in the middle of mixed hardwood forest. It has no vegetation and is quite silty. It is about 80 square meters.

Echo Ridge Pond is more of a seep than a pond. It was not more than 10 centimeters deep in any place and was about 50 square meters. It maintains riparian vegetation, notably rush, and appears to be fed by a spring.

Pond	**Latitude**	**Longitude**
Entrance	36.5303050	121.9170270
Boundary	36.5275550	121.9130550
River	36.5346380	121.8996380
Animas	36.5224440	121.8946940
Roadrunner	36.5133330	121.8896110
Dead Pig	36.5099160	121.8878330
Salamander	36.5073880	121.8786660
Wire Corrals	36.4760000	121.8829720
Van Winkleys	36.4616940	121.8673330
Echo Ridge	36.4614160	121.8634440

Table 1: Latitude and longitude coordinates for the ten ponds at Palo Corona Regional Park.

Creek Descriptions

A total of five significant creeks occur on the Palo Corona property. While during this project we focused more effort on the ponds, we also did some surveys in these creeks, and 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 much in the way of 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 as well as large cobble. Much of Seneca Creek runs through redwood forest.

San Jose Creek is the largest of the creeks at PCRP. Again, it includes a variety of habitats, including riffles, pools, and runs with sand, gravel, and coarse cobble. 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.

Cattle Troughs

We also performed some surveys in cattle troughs on the property. Aquatic-breeding amphibians will frequently use cattle troughs, sometimes at extremely high densities, so we sampled in at total of nine troughs identified on the Palo Corona property.

Methods

Much of PCRP is remote and can be challenging to access, especially in a year with high levels of precipitation, as in spring 2005. Due to the muddy condition of the roads on the property, we were unable to coordinate the first exploration of the ponds on the Ranch with Lynn Overtree, consulting land manager with MPRPD, until March 25. The property has been fairly accessible since that date. With Overtree, we identified 10 ponds, 5 creeks, and a total of nine cattle troughs that required amphibian surveys.

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

<u>Determination of Amphibian Species Composition</u>

An inventory of amphibian species using stock ponds, seeps, creeks, and cattle troughs 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, Pacific treefrogs, and bullfrogs. Habitats were quietly approached at least one hour after sunset and with no lights. 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 resting and movement. During nighttime surveys, flashlights were be used to locate amphibians by eye shine.

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 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 and creeks under permits from US Fish and Wildlife Service held by Antonia D'Amore, Valentine Hemingway, and Ben Fitzpatrick. Dipnets were pulled along the bank and deeper areas and larval amphibians identified.

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

Basic Methodology
Pitfall traps are often 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, it provides a good measure of which ponds are most important breeding sites for particular species.

We sampled larval amphibians in ponds on sunny to lightly overcast days during an 18-day period. We used two d-shaped dipnets with a 1.8 meter handle length. The large dipnet had a net opening of 1056 square centimeters and the small one had an opening of 437.5 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. This method allows the net to gather species inhabiting both the deep and shallow portions of the water column. Each larval species in the net was identified, counted, and placed in a bucket with water from the pond. To best characterize the animals in a pond, we took standard net samples (e.g., using the larger of our two nets) from the entire perimeter of each pond, pacing the distance between sweeps. Ten paces were taken between samples at Animas, Roadrunner, Dead Pig, and Salamander Ponds.

Test for Sampling Effort

We undertook a trial quantitative census of larval amphibians at Dead Pig Pond. Dead Pig Pond is primarily open water, with about a third 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 only the large dipnet, sweeping every 10 paces and identifying and counting each larval amphibian encountered. This data was then used to determine a minimum number of dipnet samples necessary to obtain reliable amphibian density estimates.

Test for Equivalency of Different Net Sizes

Two other ponds, Animas and River, pose a challenge for employing standardized methods to dipnet larval amphibians due to the thick vegetation limiting access to water and potential harm to larval amphibians. Therefore, we conducted an experiment to standardize our sampling efforts for use of a smaller dipnet and a decreased number of samples. At Entrance and Boundary Ponds, five paces were taken between samples, alternating between large and small dipnets to provide data to determine a conversion factor for small netfulls to large netfulls; the smaller net is more useful in ponds with dense vegetation, so conversion was needed to compare with larger net samples from more open ponds. To compare and combine data from these large and small net sweeps, we tested two assumptions: either that the small net sampled an area equal to that of a large net and that the opening size of the nets equals the water area that they actually sample. Differences in the angle of the opening of the net as it was swept toward the bank make it likely that conversion between the small and large dipnet lies somewhere between equal and square centimeter to square centimeter.

<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 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. According to Lara Rachowicz, PhD candidate at U.C. Berkeley, the loss of pigment is seen in association with infection of the Mountain yellow-legged frog, *Rana muscosa*, with the chytrid fungus *Batrachochytrium dendrobatidis* (Fellers 2001; Rachowicz 2004). They have found that loss of mouthpart pigmentation in tadpoles is 95% effective in diagnosing chytrid infection in populations of *Rana muscosa* (pers. comm. L. Rachowicz.) We followed up any potential positive findings by returning to sites suspected to have positive larval amphibians and searching for evidence of metamorph die-off, an event common in amphibian populations affected by chytridiomycosis.

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.

<u>Assessment of California Tiger Salamander Hybridization Levels With Non-Native Salamanders</u>

We took tissue samples from 9 California tiger salamander larvae at Salamander Pond and 38 at Roadrunner Pond. Each was assayed for native *Ambystoma californiense* alleles and introduced *A. tigrinum* alleles at two unlinked nuclear DNA markers (DLX3 and SLC4a) and mitochondrial DNA (mtDNA) according to methods described in Riley et al. (2003) and Fitzpatrick and Shaffer (2004). A total of 47 salamander larvae were assayed.

<u>Aquatic Invertebrate Species Survey</u>

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 crustacean. 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 using PCRP's aquatic systems.

Results

<u>Species Composition</u>

All ponds and several cattle troughs at PCRP have been surveyed to determine amphibian community composition. Tables 2 and 3 outline the findings from those surveys, which collapse adult, larval, and egg mass data into an indicator of presence or absence of each species at each pond or cattle trough.

Entrance, River, Animas, Dead Pig, and Salamander Ponds have been confirmed as breeding sites for California red-legged frogs. In addition, adult California red-legged frogs have been observed using Boundary and Roadrunner Ponds with no sign of breeding activity this season. Salamander and Roadrunner Ponds have been confirmed as California tiger salamander breeding habitats. We observed California newts using Boundary, Roadrunner, Dead Pig, and Salamander Ponds for breeding. Pacific treefrogs used all ponds to breed with the exception of Van Winkleys. No American bullfrogs have been detected in any of the ponds on the property using dipnet, evening listening, and nighttime eyeshine surveys, though they are known to use surrounding aquatic habitats including the Carmel River area and Rancho San Carlos property.

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	No	Yes**	No	Yes**	No
Roadrunner	No	Yes**	Yes	Yes**	Yes**	No
Dead Pig	No	Yes**	Yes**	No	Yes**	No
Salamader	No	Yes**	Yes**	Yes**	Yes**	No
Wire Corrals	No	No	No	No	Yes**	No
Van Winkleys	No	No	No	No	No	No
Echo Ridge	No	No	No	No	Yes**	No

Table 2: Detection of Amphibians in Ponds at Palo Corona Regional Park.
*Unknown indicates that further surveys will be performed to determine presence or absence of these amphibians.
**Indicates breeding activity in the form of calling, larvae, or egg masses.

We also surveyed nine of the cattle troughs at Palo Corona Regional Park. We observed California red-legged frog tadpoles and a young-of-the-year in the Animas trough, the largest and deepest of the troughs. Most of these tadpoles were quite large and it is suspected that they over-wintered from the previous year. Pacific treefrogs used five of the nine troughs for breeding. No other amphibians were observed using cattle troughs.

Cattle Trough	American Bullfrogs	California Newts	California Red Legged Frogs	CaliforniaTiger Salamanders	Pacific Treefrogs	Western Toads
Entrance	No	No	No	No	Yes**	No
Western Boundary	No	No	No	No	Yes**	No
Eastern Boundary	No	No	No	No	No	No
Frontlands Barn	No	No	No	No	No	No
Frontlands Corral	No	No	No	No	Yes**	No
Animas	No	No	Yes**	No	Yes**	No
Chavity Homestead	No	No	No	No	No	No
Chavity Canyon	No	No	No	No	No	No
North Malapaso	No	No	No	No	Yes**	No

Table 3: Detection of Amphibians in Cattle Troughs at Palo Corona Regional Park
*Unknown indicates that further surveys will be performed to determine presence or absence of these amphibians.
**Indicates breeding activity in the form of calling, larvae, or egg masses.

Surveys of the creeks, including Van Winkleys, Seneca, San Jose, Animas, and Panoche, were performed after the optimal period as late rains caused several surveys to be rescheduled due to inaccessibility. Therefore we do not feel confident that these surveys are representative of the amphibian species composition in these streams and further surveys should be undertaken to ascertain the species using streams and creeks at PCRP.

Management recommendations are conservative accordingly, assuming the presence of these amphibian species. These surveys yielded amphibians only in Animas Creek, at the crossing. There we encountered California red-legged frogs and Pacific treefrogs. Above the previously dammed portion of San Jose Creek, we twice observed an animal jumping into the creek from the bank that appeared to be a frog, although positive identification could not be made. While surveys of these streams were not particularly fruitful this year, it is likely that amphibians are using the creeks at PCRP. Species likely to use PCRP's stream habitats include California giant salamanders (*Dicamptodon ensatus*), Coast Range California newts (*Taricha torosa torosa*), Pacific treefrogs (*Pseudacris regilla*), California red-legged frogs (*Rana draytonii*), and bullfrogs (*Rana catesbeiana*). There is also a remote possibility of use by Foothill yellow-legged frogs (*Rana boylii*). Future surveys may reveal a wider use of these streams and creeks by amphibians.

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

Test for Sampling Effort

Analysis of dip net data taken at Dead Pig Pond indicate that while collection of up to 20 net samples still results in decreasing variability, approximately 15 net samples are adequate to achieve good reliable amphibian density estimates (Figure 1). In Appendix 1 we show similar results for other ponds and species.

Equivalency of Different Net Sizes

Our data supports use of the large dipnet whenever possible as it is more likely to detect rarer species than the small Dipnet with the equivalent number of sweeps (Tables 4 and 5). For Entrance, Boundary, and Animas we present both a net size-corrected estimate of absolute abundance and an uncorrected estimate; these two estimates bracket the likely range of possible net size effects (Tables 4 and 5). The assumption that the two different sized nets have the same catch rate of amphibians basically is equivalent to the assumption that amphibians are only present at the top or bottom of each swept area, or in some other way are little affected by net area. Conversely, the assumption that catch rate scales with net mouth area assumes that amphibians are distributed throughout the water column. To our surprise, the best assumption seems to be that a sweep is a sweep – small and large net samples catch the same number of amphibians in general (Figures 4 and 5). Even with area-corrected samples, the rankings of relative abundance are consistent for Pacific treefrogs and California red-legged frogs, but the corrections change the estimated relative number of each species considerably. This leads us to conclude that the uncorrected results from the small versus large dipnet are comparable. Generally we prefer to use the large dipnet whenever possible as its likelihood of capturing rarer species is greater, but in conditions that prohibit the use of a large dipnet, a small dipnet can be used successfully.

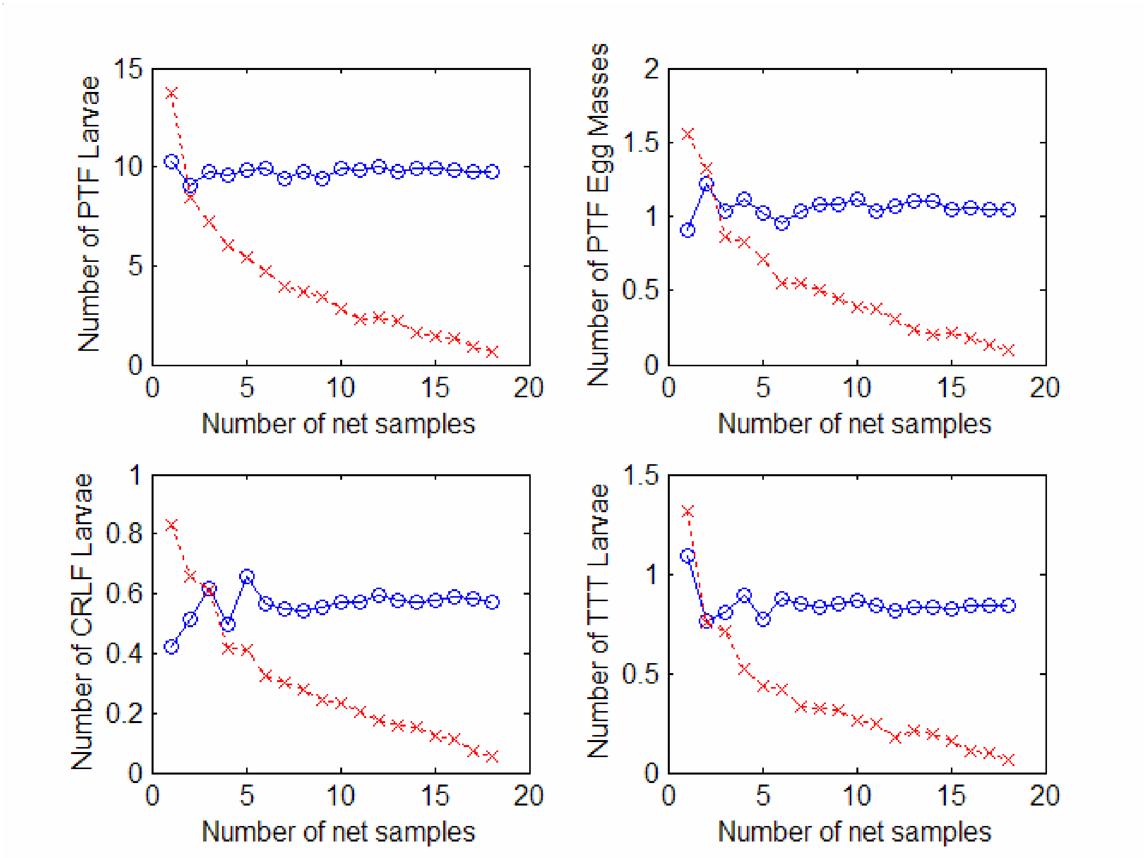

Figure 1. Effects of dip net sample number on means (blue lines) and standard deviations (red lines) across 100 randomized subsets of samples generated with data taken from Dead Pig Pond. PTF refers to the Pacific treefrog (*Pseudacris regilla*), CRLF refers to the California red-legged frog (*Rana aurora draytonii*), and TTT refers to the California Coast range newt (*Taricha torosa torosa*).

Abundance Results

Animas pond was surveyed with the small dipnet only, while Entrance and Boundary were surveyed with both the small and large dipnets. Roadrunner, Dead Pig, Salamander, and Wire Corrals were surveyed with the large dipnet only. River Pond proved to be too thickly vegetated to dipnet, so has been excluded from the analysis of relative abundance. No larval amphibians occurred in Van Winkleys Pond and Echo Ridge Pond was too small to sample by this method. The remaining ponds were surveyed by a standard method for assessing amphibian larvae in ponds, as discussed above in the methods section.

Below we have presented the relative abundance rankings from our dipnetting surveys of Entrance, Boundary, Animas, Roadrunner, Dead Pig, Salamander, and Wire Corrals Ponds (Tables 4 and 5). Of these ponds, Salamander Pond topped all other ponds for relative abundance for all four aquatic breeding amphibian larvae. Dead Pig Pond had the second greatest ranking for California red-legged frog and California Newt larvae. 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 had a ranking of third for both Pacific treefrog and California red-legged frog larvae. Again, this pond has a mosaic of open and vegetated water, both shallow and deep. It is too close to the coast for California tiger salamanders, as they tend to prefer warmer, drier conditions a bit further inland. Boundary Pond had the lowest relative abundances for California newt larvae, one of the lowest rankings for Pacific treefrog larvae, and no California red-legged frog larvae occurred there. While the pond has some shallow and deeper regions, its lack of vegetation along most of the bank and sparse emergent vegetation may leave amphibians with few egg attachment sites and larvae with few refuge sites from predators such as large wading birds and adult amphibians.

Table 4: Relative Abundance of Pacific Treefrogs and California Red-Legged Frogs in Palo Corona Regional Park Ponds

	Pacific Tree Frog Larvae				California Red-Legged Frog Larvae			
	Net Size Corrected		Uncorrected		Net Size Corrected		Uncorrected	
Pond	**R.A.***	**S.E.****	**R.A.***	**S.E.****	**R.A.***	**S.E.****	**R.A.***	**S.E.****
Entrance	2857.6	252	2044.8	252.8	23.2	6.4	12.8	0.001
Boundary	1540.875	489.825	794.85	112.875	0	n/a	0	n/a
Animas	5380.8	2120.4	2229.65	878.75	30.4	8.55	12.35	0.0009474
Roadrunner			267.6	68.4			0	n/a
Dead Pig			925.3	291.65			55.1	0.0024211
Salamander			5664	1068.8			76.8	0.0013125
Wire Corrals			235.2	42.42			0	n/a

*Relative Abundance
**Standard Error

Table 5: Relative Abundance of California Newts and California Tiger Salamanders in Palo Corona Regional Park Ponds

	California Newt Larvae				California Tiger Salamander			
	Net Size Corrected		Uncorrected		Net Size Corrected		Uncorrected	
Pond	**R.A.***	**S.E.****	**R.A.***	**S.E.****	**R.A.***	**S.E.****	**R.A.***	**S.E.****
Entrance	0	n/a	0	n/a	0	n/a	0	n/a
Boundary	26.25	0.0025	13.13	0.0020952	0	n/a	0	n/a
Animas	0	n/a	0	n/a	0	n/a	0	n/a
Roadrunner			0	n/a			5.2	0.00225
Dead Pig			79.8	0.0029474			0	n/a
Salamander			179.2	0.0023125			6.4	0.00025
Wire Corrals			0	n/a			0	n/a

*Relative Abundance
**Standard Error

Disease Assessment

Loss of pigmentation was observed in 1 of the 11 California red-legged frog larvae encountered at Dead Pig Pond, 2 of 16 in Animas Pond, and in 5 of 12 California red-legged frog larvae encountered in the cattle trough below Animas Pond. While this may indicate infection with chytridiomycosis, we did not observe any dead metamorphs as is often typical in chytridiomycosis outbreaks. Some depigmentation can occur as larval amphibians metamorphose and through wear. Further testing is merited to ensure accurate diagnosis in this case.

No limb malformations typical of infection with *Ribeiroia ondatrae* were observed in any larval amphibian. The snail hosts, *Planorbidae spp.* and *Physidae spp.*, and definitive host, large wading birds, 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).

Assessment of CTS Hybridization Levels With Non-native Salamanders

All alleles detected were native *A. californiense* alleles; there is no evidence of introduced DNA in these samples. However, introduced alleles have been detected at the neighboring Santa Lucia Preserve. Further, higher frequencies of introduced alleles have been observed near Hwy 68, within 10 miles to the North-East of Santa Lucia Preserve.

Other Vertebrate and Invertebrate Species Observed

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. A coyote also frequents the grassland 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, barn swallow, brewers blackbirds, mourning doves, and a great blue heron. 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); Mollusca: physidae; and leeches.

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); Mollusca: physidae; and leeches.

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 and harriers were observed around Dead Pig pond. In addition, an alligator lizard was seen on the road above the 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 and mallards were again observed using the Salamander Pond. 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.

We noticed several Santa Cruz aquatic garter snakes in the Wire Corrals pond, feeding on Pacific treefrog larvae and insects. Aquatic invertebrates in the pond include Diptera: chironomonidae (midge) and culicidae (Mosquito larvae); Crustacea: ostracoda; Hemiptera: notonectidae (backswimmer) and corixidae (water boatman); Ephemeroptera (mayfly); Odonata: anisoptera (dragonfly nymph) and zygoptera (damselfly nymph); Coleoptera: hydrophilidae (Water scavenger beetle); Nematoda (Nematode); and Hemiptera: gerridae (water striders) and ranatra (water scorpion).

Van Winkleys pond hosts Diptera: chironomonidae (midge); Hemiptera: dytiscidae (predaceous diving beetle) and gerridae (water striders); Odonata: anisoptera (dragonfly nymph); and an unidentified clam-like invertebrate.

At the Echo Ridge spring, the only invertebrate observed was Hemiptera: gerridae (water striders).

Recommendations

We have submitted with this report a TNC CAP workbook for PCRP (see below). While this worksheet summarizes the threats, status, and management for amphibians, we also provide a narrative explanation of our management recommendations below.

Home	Conservation Action Planning Workbook A tool for developing strategies, taking action, and measuring success Version 4.b © 2005 The Nature Conservancy	The Nature Conservancy SAVING THE LAST GREAT PLACES ON EARTH	Basic Version

Project Scope and Targets		
Project:		
Ecoregion(s):		California Central Coast
Conservation Targets	1:	CA red legged frogs
	2:	CA tiger salamaders
	3:	pond habitats
	4:	upland habitats
	5:	stream habitats
	6:	aquatic-breeding amphibian community
	7:	
	8:	
Contact name:		
Title, Office:		
Organization:		
Email Address:		
Date:		October-05

Links

Type of Target	Habitat Association	
Species: Amphibians	Freshwater - Lakes	
Species: Amphibians	Freshwater - Lakes	
Plant community	Riparian	
Plant community	Grassland	
Plant community	Riparian	
Animal community	Riparian	
Approved to share within The Nature Conservancy?		-
Approved to share outside The Nature Conservancy?		-
"Beta" version:	ConPrjMgmt_v4b.xls	25-Apr-05

Disease Assessment and Management

With approval from U.S. Fish and Wildlife Service, in 2006 we will take samples from mouthparts of amphibians with and without depigmentation with the use of cotton swabs to test for the presence of *Batrachochytrium dendrobatidis* DNA through a standardized real-time PCR technique (Boyle 2004). This will allow us to definitively determine if amphibians at Palo Corona Regional Park are infected with *Batrachochytrium dendrobatidis.* It will also allow us to begin a project to test mouthpart depigmentation in the larval amphibians of this region as a diagnostic indicator of infection with *Batrachochytrium dendrobatidis*.

When any human contact is made with the ponds at Palo Corona, decontamination of all equipment should be conducted as described in Appendix B of the U.S. Fish and Wildlife Services' "Revised Guidance on Site Assessments and Field Surveys for the California Red-legged Frog" from August 2005. These are included in Appendix B of this report. Importantly, this procedure should be followed not just by amphibian researchers, but by others visiting these ponds, as human transportation of amphibian diseases is suspected to be a major method of spread.

More specific actions in relation to disease are described in species sections below.

Recommendations to Maintain and Enhance Existing Populations

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

California Red-Legged Frogs

California red-legged frogs are widespread at PCRP, using seven of the ten ponds. PCRP appears to lack bullfrogs and introduced fish, common invasive predators that are a problem for California red-legged frogs. We recommend continued monitoring for these invasive species and immediate eradication activities should they be detected. Collaboration with owners of neighboring properties on beginning or continuing eradication projects, particularly bullfrogs, may prove helpful in avoiding future problems with these invasive species at PCRP. Further, we recommend an educational program for employees, volunteers, and visitors of PCRP regarding the threat of invasive species to California red-legged frogs and California tiger salamanders and steps they can take to avoid release of invasives, and what they can do if they see an invasive. We also recommend collaboration with neighboring landowners on education and monitoring projects.

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.

Currently chytrid infection is suspected in tadpoles in two of the ponds, Animas and Dead Pig, and one cattle trough, Animas. While there is no treatment method for wild animals at this time, we recommend continued monitoring and testing of amphibians in all ponds to remain current on the status of the disease at PCRP. Further, we recommend limited contact with aquatic habitats and the amphibians by the public, management, and researchers. Avoid moving materials from one aquatic habitat to another. All clothing and equipment that comes in contact with soil, vegetation, water, or animals in the proximity of an aquatic habitat should be decontaminated according to the procedure outlined in Appendix B of the U.S. Fish and Wildlife Services' "Revised Guidance on Site Assessments and Field Surveys for the California Red-legged Frog" from August 2005, before leaving the site to decrease the possibility of humans being a vector of amphibian pathogens. These are included in Appendix B of this report.

While cattle have a positive impact on upland grassland habitat for California red-legged frogs, they can prove harmful to aquatic habitats for amphibians, decreasing vegetative cover and egg attachment sites. They may also dislodge and trample eggs on vegetative attachment sites and trample tadpoles. We therefore recommend the fencing of ponds to decrease cattle traffic and promote vegetative growth in the ponds. This will have the added benefit of decreasing the possibility of cattle spreading pathogens such as chytrid between sites in the mud caked in their hooves.

River Pond has become overgrown with vegetation, with very little open water, and appears to be quite shallow in areas where it is accessible. River Pond is also located in close proximity to the Carmel River, a location known to harbor bullfrogs. While American bullfrogs have not been detected at River Pond, continued monitoring is essential to avoid a population becoming established. Further, some removal of vegetation, and possibly allowing cattle access to the pond between September through November post-metamorphosis and during the non-breeding season for California red-legged frogs, will allow for some thinning of the vegetation and more open water to allow frog to use and find refuge within the pond. Following the 2005-2006 season, we suggest that a scaled-back set of observations be taken in ponds closest to potential sources of bullfrog invasion, while continuing to monitor amphibian use of ponds were habitat improvements have been made.

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 approached. 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 from this pond and potentially spreading chytrid and other pathogens in the mud on their shoes.

Finally, 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.

California Tiger Salamanders

California tiger salamanders currently breed 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, although we did not find dragonfly larvae in either of these ponds. 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, we are unclear on the status of Salamander pond. We recommend avoiding any changes to either of these ponds that will cause them to remain wet year-round.

Invasive plants, particularly French broom (*Genista spp.*), are invading the grassland habitat around Salamander and Roadrunner Ponds. This change decreases the upland grassland habitat that California tiger salamanders depend 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. Further, California tiger salamanders appear to prefer to use ground squirrel burrows. It is a common practice to eradicate ground squirrels for agriculture and cattle, but we strongly recommend against this practice.

Ponds with introduced fish and American bullfrogs are unsuitable breeding habitat for California tiger salamanders (Shaffer 2005). While Salamander and Roadrunner ponds do not currently have any invasive fish or bullfrogs, we recommend continued monitoring for these species and immediate eradication should they be detected.

We recommend an education program for the visiting public about the negative effects of introducing species and an aggressive program to deter visitors from releasing any animals to the property and ponds. We also encourage collaboration with land managers from neighboring properties in this education program and in monitoring for invasions. Finally, we recommend continued genetic monitoring of tiger salamander populations at PCRP.

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.

Recommendations for Species Not Found, but Potentially Occurring at PCRP

Foothill Yellow-Legged Frogs

Foothill yellow-legged frogs have been extirpated from much of their previous habitat through conversion of habitat, grazing in the riparian zone, and introduction of invasive fish, bullfrogs, and crayfish (Fellers 2005). It is also thought that dams are problematic for Foothill yellow-legged frogs, by providing refuge for invasive species (Fellers 2005). Minimizing or eliminating cattle grazing along the streams and creeks at PCRP will help maintain healthy habitat for Foothill yellow-legged frogs and avoid trampling of eggs, larvae, or adults around and under river stones and gravel. Continuing with a monitoring program for invasive species and eradication will also help to increase appropriate habitat for these frogs. Streams and creeks in particular often cross property boundaries and collaborating with neighboring landowners on monitoring and eradication will greatly increase effectiveness of efforts. Foothill yellow-legged frogs are also prone to chytrid infection, though the effects on them have not been closely studied (Fellers 2005). They are closely related to the Mountain yellow-legged frog in the Sierra Nevada Range that have had sharp population declines in response to infection with this pathogen (Fellers 2005). We recommend following the decontamination procedures described in Appendix B to avoid human spread of pathogens. Again, working with neighboring landowners to encourage them to take similar measures may decrease the incidence of infection by human and cattle vectors. Finally, should this species be detected at PCRP, we recommend continued inspection and testing of individual amphibians in stream habitats to monitor for invasion of this pathogen.

Western Toads

Western toads are particularly sensitive to trampling of wetland breeding habitat by livestock (Muths 2005). Fencing ponds will help to provide breeding habitat for Western toads. Western toads are also particularly prone to predation by introduced fish (Muths 2005). We recommend strongly against the addition of fish to any aquatic habitats. Further, continued monitoring of aquatic habitats for invasive fish and immediate eradication efforts will help to keep any inadvertent introductions under control and benefit most aquatic-breeding amphibian species at PCRP.

Annual Monitoring Program

The data collected in 2004-2005 provide evidence of relative numbers and habitat use for breeding. However, this 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 amphibian use of aquatic habitats at PCRP. While some data collection can be less extensive than in the 2004-2005 season, we recommend that sampling for both larvae and adult activity be conducted in all ponds and streams in the 2005-2006 season. 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. One change we would recommend is to add a stratified sampling design at Entrance, Boundary, Roadrunner, Dead Pig, Salamander, and Wire Corrals Ponds, which are open enough to allow this type of sampling (see chapter 6 item 10 in "Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians" for a detailed description of this design). For the balance of the ponds, 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 circumference.

In addition, 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. Nightime 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. Nightime eyeshine surveys are particularly rich for viewing adult pond-breeding amphibians that tend to be nocturnal in this region. If possible, take a photograph of any California red-legged frog adults. Their markings are unique and one may be able to identify individuals from them.

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.

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. Further, trails and roads should be constructed to minimize contact with aquatic habitats by people and vehicles. 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 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.

Grazing by cattle or some other method should be included to create patchiness in invasive grasses, mimicking patchiness of native bunch grasses that are easier for migrating amphibians to move through. Goats are an option to keep French broom under control. Avoid the control of ground squirrels that is a common management practice in areas where cattle are grazing.

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.

Berger, L., R. Speare, P. Daszak, D. E. Green, A. A. Cunningham, C. L. Goggin, R. Slocombe, M. A. Ragan, A. D. Hyatt, K. R. McDonald , H. B. Hines, K. R. Lips, G. Marantelli, H. Parkes (1998). "Chytridiomycosis Causes Amphibian Mortality Associated with Population Declines in the Rain Forests of Australia and Central America." PNAS **95**(14): 9031-9036.

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.

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 , and J. E. Longcore (2001). "Oral Chytridiomycosis n the Mountain Yellow-Legged Frog (*Rana muscosa*)." Copeia **4**: 945-953.

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

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.

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.
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., 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." Emerg Infect Dis [serial on the Internet].
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: Effects of Number of Dip Net Samples On Mean and Standard Deviation

<u>Entrance Pond Large Dipnet</u>

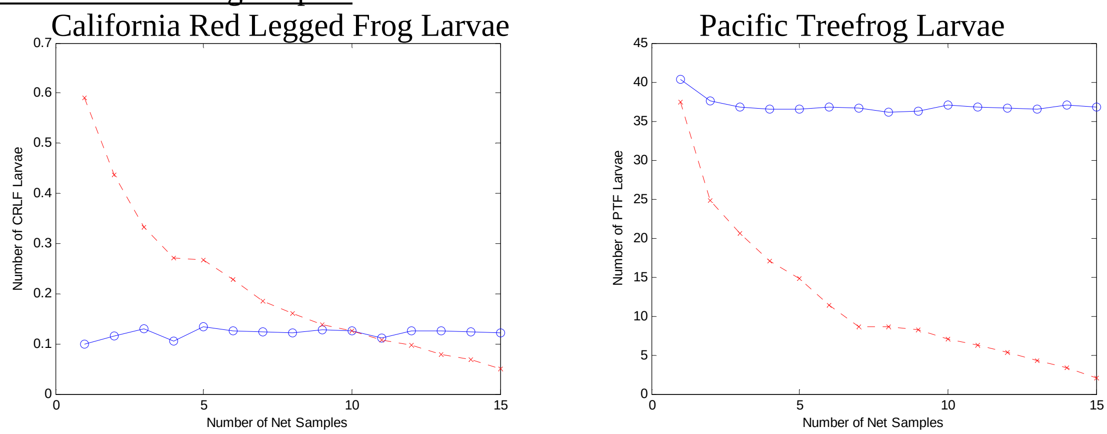

Figure 1. Effects of large dip net sample number on means (blue lines) and standard deviations (red lines) across 100 randomized subsets of samples generated with data taken from Entrance Pond.

<u>Entrance Pond Small Dipnet</u>

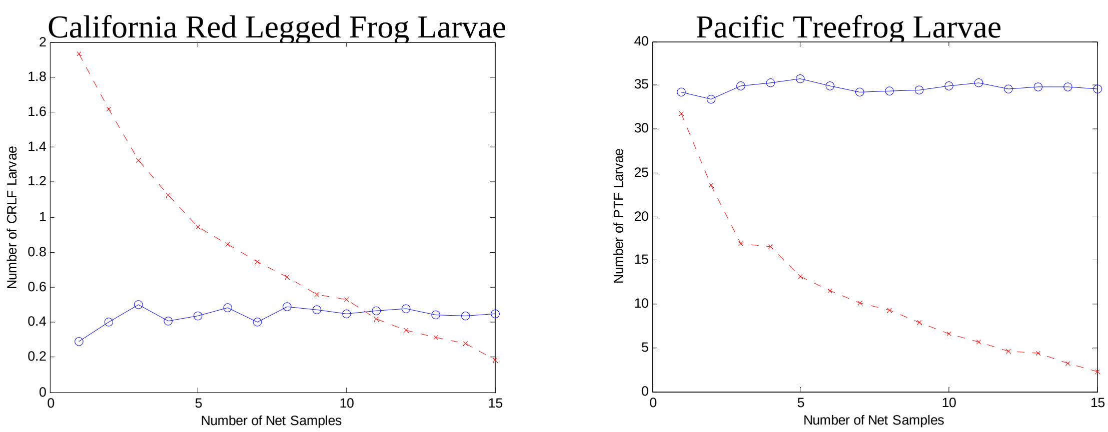

Figure 2. Effects of small dip net sample number on means (blue lines) and standard deviations (red lines) across 100 randomized subsets of samples generated with data taken from Entrance Pond.

Boundary Pond Large Dipnet

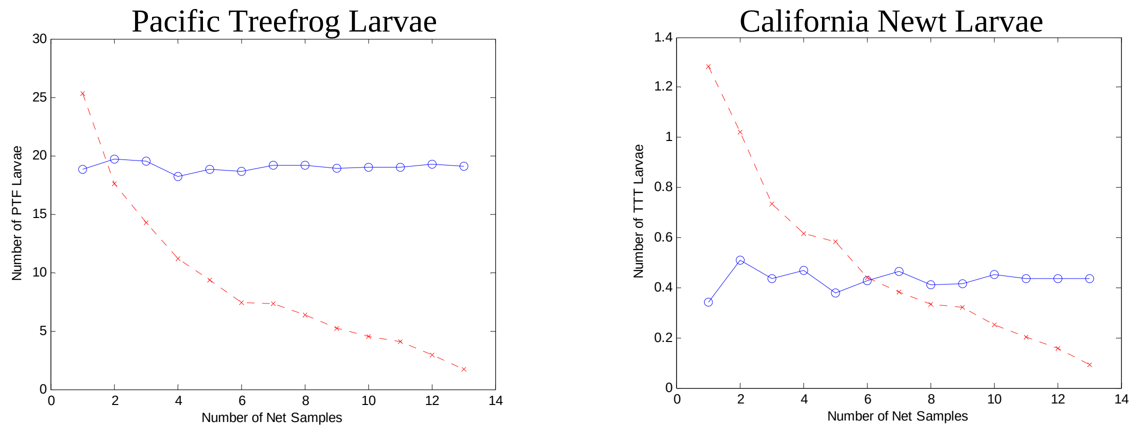

Figure 3. Effects of large dip net sample number on means (blue lines) and standard deviations (red lines) across 100 randomized subsets of samples generated with data taken from Boundary Pond.

Boundary Pond Small Dipnet

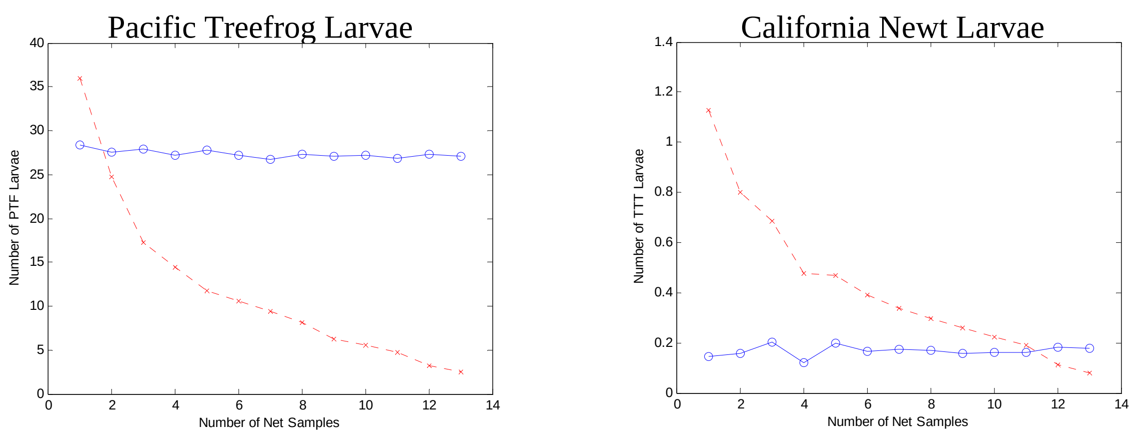

Figure 4. Effects of small dip net sample number on means (blue lines) and standard deviations (red lines) across 100 randomized subsets of samples generated with data taken from Boundary Pond.

Animas Pond Small Dipnet

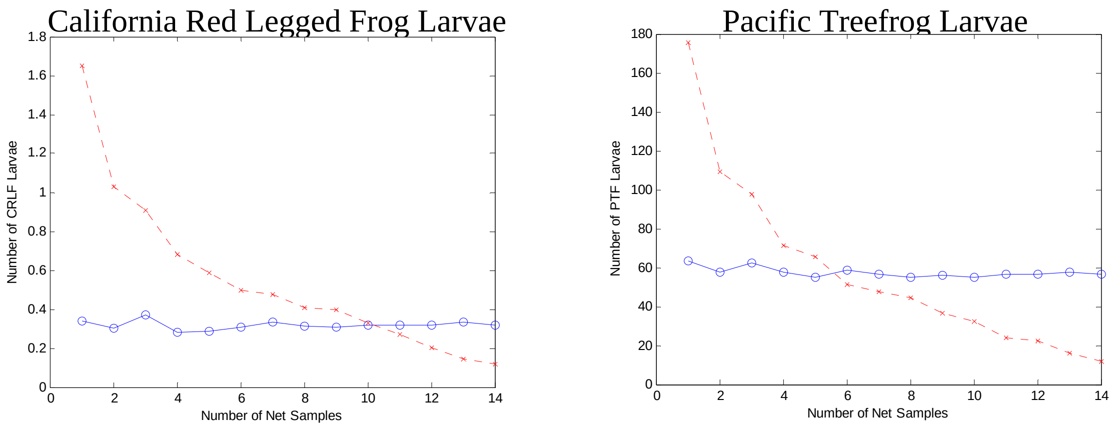

Figure 5. Effects of small dip net sample number on means (blue lines) and standard deviations (red lines) across 100 randomized subsets of samples generated with data taken from Animas Pond.

Roadrunner Pond Large Dipnet

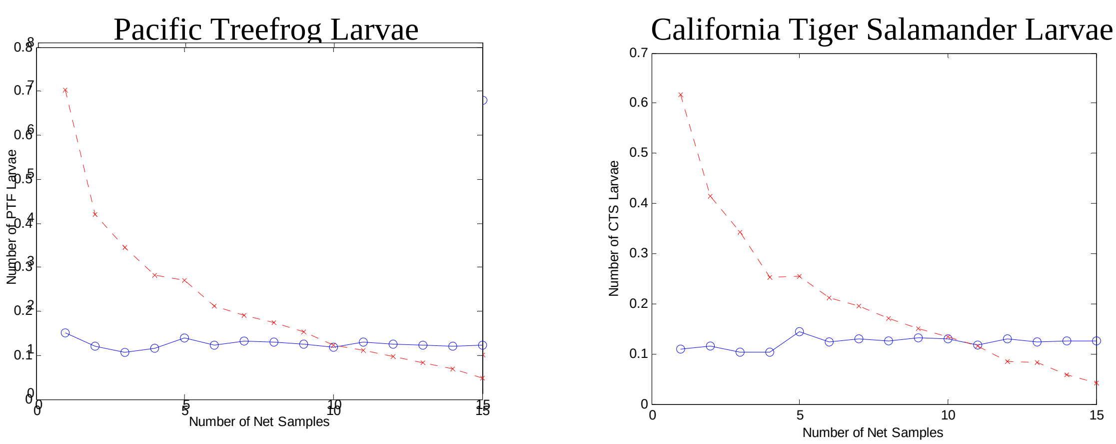

Figure 6. Effects of large dip net sample number on means (blue lines) and standard deviations (red lines) across 100 randomized subsets of samples generated with data taken from Roadrunner Pond.

Dead Pig Large Dipnet

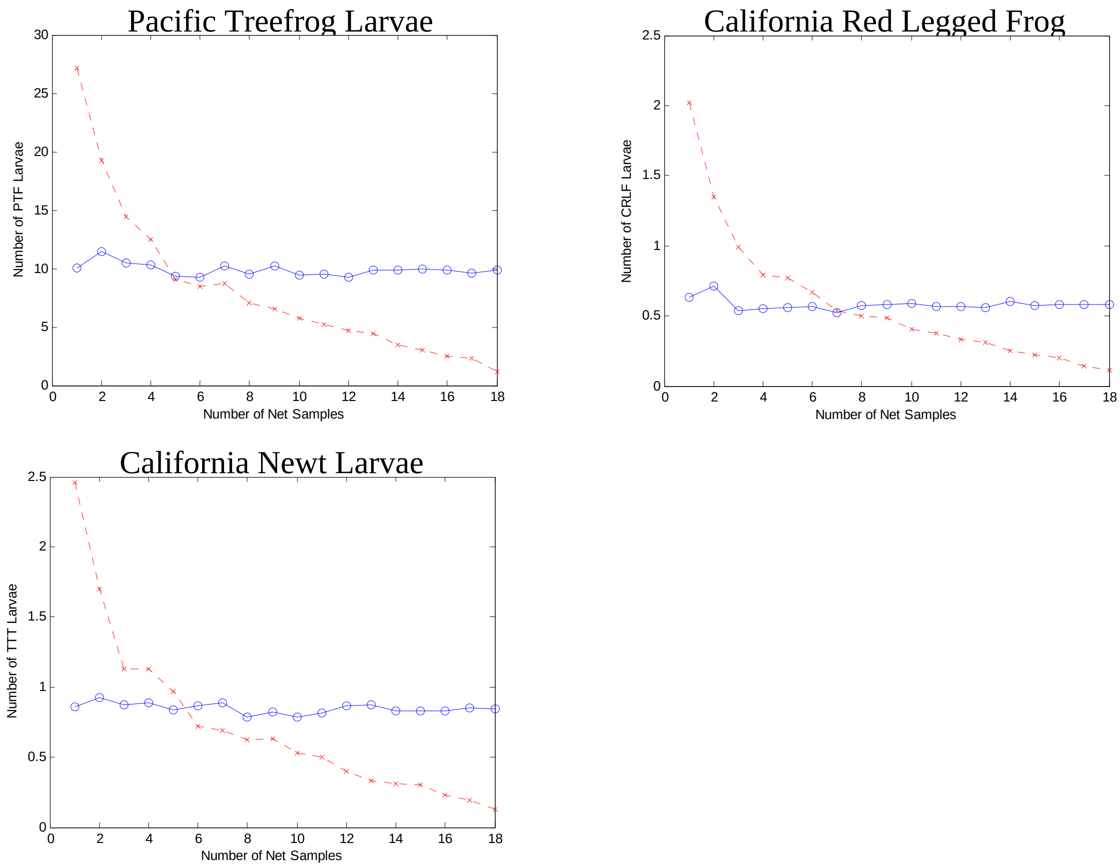

Figure 7. Effects of large dip net sample number on means (blue lines) and standard deviations (red lines) across 100 randomized subsets of samples generated with data taken from Dead Pig Pond.

Salamander Pond Large Dipnet

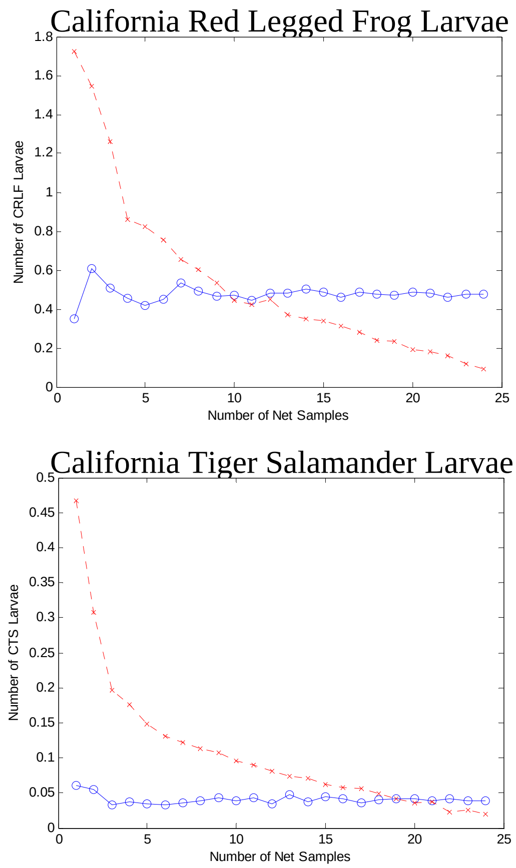

Figure 8. Effects of large dip net sample number on means (blue lines) and standard deviations (red lines) across 100 randomized subsets of samples generated with data taken from Salamander Pond.

Appendix B: Decontamination Procedures
From Appendix B of the U.S. Fish and Wildlife Service's "Revised Guidance on Site Assessments and Field Surveys for the California Red-legged Frog," August 2005

Recommended Equipment Decontamination Procedures

In an effort to minimize the spread of pathogens that may be transferred as result of activities, surveyors should follow the guidance outlined below for disinfecting equipment and clothing after entering a pond and before entering a new pond, unless the wetlands are hydrologically connected to one another:

i. All organic matter should be removed from nets, traps, boots, vehicle tires and all other surfaces that have come into contact with water or potentially contaminated sediments. Cleaned items should be rinsed with clean water before leaving each study site.

ii. Boots, nets, traps, hands, *etc*. should be scrubbed with either a 75% ethanol solution, a bleach solution (0.5 to 1.0 cup per 1.0 gallon of water), Quat-128TM (1:60), or a 6% sodium hypochlorite 3 solution. Equipment should be rinsed clean with water between study sites. Cleaning equipment in the immediate vicinity of a pond or wetland should be avoided (*e.g.*, clean in an area at least 100 feet from aquatic features). Care should be taken so that all traces of the disinfectant are removed before entering the next aquatic habitat.

iii. Used cleaning materials (liquids, *etc.*) should be disposed of safely, and if necessary, taken back to the lab for proper disposal. Used disposable gloves should be retained for safe disposal in sealed bags.

iv. Additionally, the surveyors shall implement the following when working at sites with known or suspected disease problems: disposable gloves should be worn and changed between handling each animal. Gloves should be wetted with water from the site or distilled water prior to handling any amphibians. Gloves should be removed by turning inside out to minimize cross-contamination.