Progress Report 2 for
Amphibian Management and Monitoring Plan for Palo Corona Ranch, Monterey County, California

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Palo Corona Ranch is situated in the foothills of the Santa Lucia Range. The undeveloped property provides and variety of upland and aquatic habitats for many plants and animals, including amphibians. Conversely it can be challenging to access, especially in a year with great amounts of rain. 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, Wildlands Manager, until March 25. The property has been accessible since that date. With Overtree, we have identified 10 ponds and 5 cattle troughs that require amphibian surveys. We are coordinating with Overtree to locate and access the streams.

The following is a summary of our primary field survey activities to date:

- March 4 review of maps of the property and driving tour along the boundary of the property with Lynn Overtree
- March 25 daytime visual survey of Entrance Pond, Boundary Pond, Cattle troughs below Boundary Pond, River Pond, and Cattle Trough along the road beyond the barn
- March 30 daytime dipnetting of Entrance Pond, Boundary Pond, Cattle troughs below Boundary Pond, River Pond, Cattle Trough along the road beyond the barn, Las Animas Pond, Cattle Trough below Las Animas Pond, and Roadrunner Pond
- March 31 Night time listening and eyeshine surveys at Entrance Pond, Boundary Pond, Cattle troughs below Boundary Pond, River Pond, Cattle Trough along the road beyond the barn, Las Animas Pond, and Cattle Trough below Las Animas Pond
- April 7 Nightime listening and eyeshine surveys at Roadrunner Pond and Dead Pig Pond
- April 8 Seining of Entrance Pond, Boundary Pond, Wire Corrals Pond, Salamander Pond, Dead Pig Pond, and Roadrunner Pond
- April 14 Daytime dipnetting of Dead Pig Pond and Las Animas Pond
- April 19 Daytime dipnetting of Entrance Pond
- April 20 Daytime dipnetting of Entrance Pond
- April 21 Daytime dipnetting of Boundary Pond
- April 22 Daytime dipnetting of Boundary Pond and Cattle Troughs
- April 25 Daytime dipnetting of Roadrunner and Salamander Pond
- April 29 Evening listening and nighttime eyeshine at Roadrunner, Dead Pig, and Salamander Ponds
- April 30 Daytime dipnetting at River and Animas Ponds
- May 2 Daytime dipnetting at Animas Pond, Animas River Crossing, and Cattle Trough
- May 12 Nightime eyeshine at Wire Corrals, Van Winkleys, and Echo Ridge Ponds and evening listening at River Pond, Animas Pond, Wire Corrals, Van Winkleys, and Echo Ridge Ponds with Lynn Overtree
- May 13 Daytime dipnetting of Wire Corrals, Van Winkleys, and Echo Ridge Ponds, cattle troughs, and overview of stream sites with Lynn Overtree

These field activities have resulted in substantial information. Below we report on the progress we have made toward the ten deliverables outlined in Exhibit A of the contract for amphibian surveys at Palo Corona Ranch.

1. A census of native and exotic amphibians (larval stage only) in all stock ponds, seeps, creeks and other potential amphibian habitats found on the Ranch.

All ponds and several stock ponds have been surveyed to determine amphibian community composition. Figure 1 outlines 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 and cattle trough.

Figure 1: Detection of Amphibians in Ponds at Palo Corona Ranch

Pond	Pacific Treefrogs	California Red Legged Frogs	California Newts	CaliforniaTiger Salamanders	American Bullfrogs
Entrance	Yes**	Yes**	No	No	No
Boundary	Yes**	Yes	Yes**	No	No
River	Yes**	Yes**	Unknown*	No	No
Animas	Yes**	Yes**	No	No	No
Roadrunner	Yes**	Yes	Yes**	Yes**	No
Dead Pig	Yes**	Yes**	Yes**	No	No
Salamader	Yes**	Yes**	Yes**	Yes**	No
Wire Corrals	Yes**	No	No	No	No
Van Winkleys	No	No	No	No	No
Echo Ridge	Yes**	No	No	No	No

*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.

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 signs 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 bullfrogs have been detected in the ponds on the property through 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.

We have surveyed nine of the Cattle Troughs on the Palo Corona Ranch. We observed California red legged-frog tadpoles and a young-of-the-year only in the Animas trough. Most of these tadpoles were quite large and it is suspected that they over wintered from the previous year. Pacific treefrog are using five of the nine troughs for breeding. No other amphibians were observed using the cattle troughs.

Figure 2: Detection of Amphibians in Cattle Troughs at Palo Corona Ranch

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

*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 undertook a trial quantitative census of larval amphibians at Dead Pig Pond. Dead Pig Pond is primarily open water, with about a quarter of the pond covered with tall emergent vegetation. The entire margin of the pond can be accessed for dipnet surveys of larval amphibians. 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 to dipnet without harming the larval amphibians. We conducted an experiment to standardize our sampling efforts for use of a smaller dipnet and a decreased number of samples. River Pond proved to be too thickly vegetated to dipnet, so has been excluded from the analysis of relative abundance. The balance of the ponds were surveyed by a standard method for assessing amphibian larvae in ponds (see item 6 below).

Below we have presented the relative abundance rankings from our dipnetting surveys of Entrance, Boundary, Animas, Roadrunner, Dead Pig, Salamander, and Wire Corrals Ponds. Animas pond was surveyed only with the small dipnet, 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. 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. 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 3 and 4). 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 capture rarer species is greater, but in conditions that prohibit the use of a large dipnet, a small dipnet can be used successfully.

Figure 3: Relative Abundance of Pacific Treefrogs and California Red-Legged Frogs in Palo Corona Ranch 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

Figure 4: Relative Abundance of California Newts and California Tiger Salamanders in Palo Corona Ranch 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

2. A assessment of the disease status (including trematodes and chytrid fungus) of California red-legged frogs (CRLF) and California tiger salamanders (CTS), including presence/absence data and an estimate of the proportion of the population showing disease symptoms in each pond

Mouthparts on Pacific treefrog and California red-legged frog tadpoles have been inspected for chytrid infection. We performed these inspections with all larval amphibians encountered that were sufficiently large, or ones that were developing legs, providing a standardized quantification of disease incidence in ponds. Loss of pigmentation was observed in 1 of the 11 California red-legged frog larvae encountered at Dead Pig Pond, 2 of 16 in Las Animas Pond, and in 5 of 12 California red-legged frog larvae encountered in the cattle trough below Las Animas Pond. According to Lara Rachowicz, PhD Candidate at UCBerkeley, the loss of pigment is consistent with that seen in association with infection of *Rana muscosa* with the chytrid *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 plan to take swab samples of the oral parts of amphibians with depigmentation to test for the presence of *Batrachochytrium dendrobatidis* DNA through a standardized real-time PCR technique. We are awaiting approval from US Fish and Wildlife Service to take swab samples. We could not locate any researchers who have or have sought approval to swab California red-legged frogs, but our contact at US Fish and Wildlife Service was encouraging of our application. We anticipate that we will have approval to take swab samples when California red-legged frog tadpoles are present again in Spring 2006.

All larval amphibians with limbs are inspected for healthy growth. No limb malformation has been observed in any larval amphibian thus far.

3. An assessment of vertebrate and invertebrate faunas found in ponds

The Entrance Pond is used be a variety of birds and insects. We observed red tailed hawks, barn swallows, dark-eyed junkos, turkey vultures, mallard, 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. An aquatic insect dipnet 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 visually identify all of the birds using the pond during our visits, but mallards and red-winged black birds were identified. Flame skimmer dragonflies and boreal bluet damselflies also use the pond. We observed the following aquatic invertebrates 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 *Thamnophis atratus atratus* (Santa Cruz Aquatic Garter Snake) 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).

4. An assessment of CTS hybridization levels with non-native salamanders
We took tissue samples from 8 California tiger salamander larvae at Salamander Pond and 38 at Roadrunner Pond. We will analyze these tissue samples for hybridization with non-native Tiger salamanders. The samples are being processed and results will be reported in the final report in August.

5. A detailed reporting and analysis of all resulting data, including but not limited to: spatial autocorrelation and incidence function analyses of amphibian and disease occurrence patterns and relative risk analysis for ponds and pond complexes
Reporting and analysis of data will occur after censuses of larval amphibians in all aquatic habitats have been performed.

6. A protocol for a future annual monitoring program (including data storage and analysis) of all known or potential amphibian-inhabited ponds, including mark-recapture techniques

We sampled larval amphibians in ponds using dipnets with a 1.8 meter handle length. The large dipnet had a net opening of 1056 square centimeters and the small an opening of 437.5 square centimeters. The dipnet was swept perpendicular to the bank, from deep to shallow, leveraging the dipnet to get a deeper sample 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. Ten paces were taken between 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 data from the small net was treated both as equal to the large net and size corrected to bracket the range of possible values. 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. Our data supports use of the large dipnet whenever possible as it is more likely to detect rarer species than the small dipnet. The small net was used at Animas Pond, where the large dipnet could not be pulled through due to the dense vegetation. Use of even the small dipnet at River Pond was unsuccessful as the pond is completely choked with vegetation.

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 of amphibian density estimates.

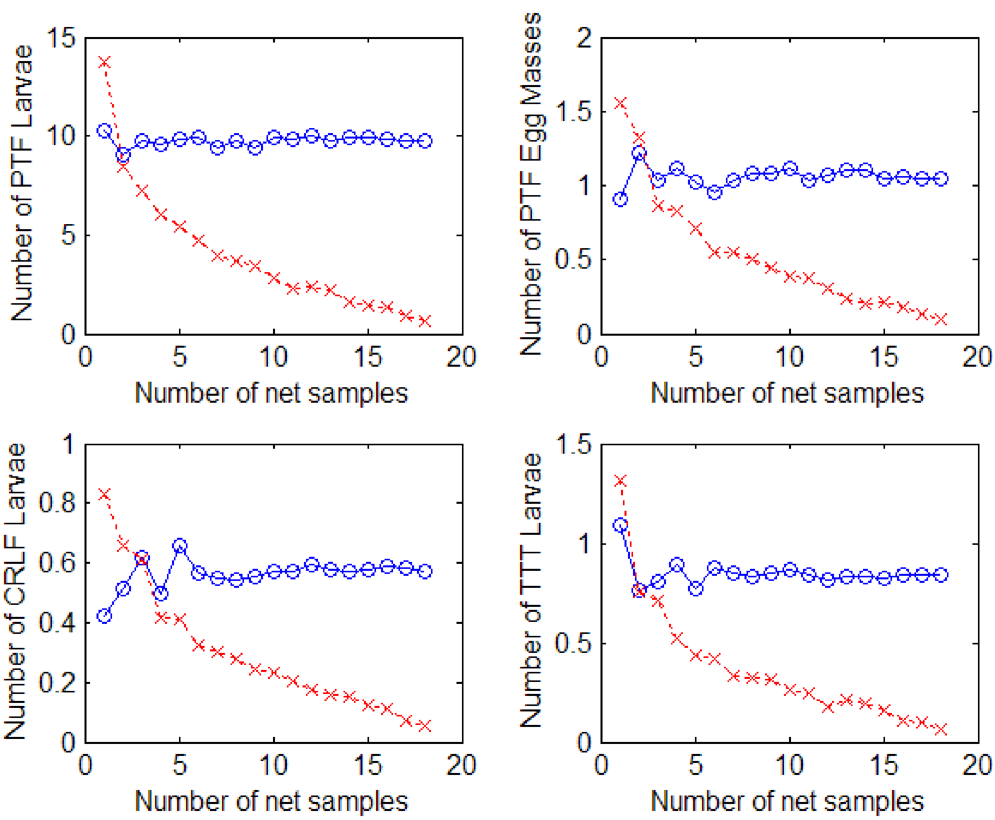

Figure 5. Effects of dip net sample number on means (blue lines) and standard deviations (red lines) across 100 randomized subsets of samples generated with data taken from Dead Pig Pond. PTF refers to the Pacific treefrog (*Pseudacris regilla*), CRLF refers to the California red-legged frog (*Rana aurora draytonii*), and TTT refers to the California Coast range newt (*Taricha torosa torosa*).

For future assessment of relative abundance of larval amphibians, we recommend following the dipnet method described above. 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, a version of which will accompany the final version of this report, and relative abundance estimated using the mean number of larvae per dipnet multiplied by the estimated circumference.

In addition, knowledge of amphibian 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 usage 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". 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 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. Data on the location, lifestage, and estimated size of each amphibian should be recorded and entered into a database. If possible, take a photograph of any California red-legged frogs. Their markings are unique and adults may be identified by these markings.

7. Recommendations for enhancing existing populations

We will make recommendations after performing censuses of larval amphibians in all aquatic habitats and analyzing the data.

8. A detailed viability assessment of CTS and CRLF populations (using The Nature's Conservancy's e5S planning tool)

We will perform a viability assessment of CTS and CRLF populations after we census larval amphibians in all aquatic habitats and analyze the data.

9. A discussion of local and regional metapopulation dynamics for CTS and CRLF

We will discuss local and regional metapopulation dynamics for CTS and CRLF following our censuses of larval amphibians in all aquatic habitats and data analysis.

10. Recommendations for management of upland habitat (e.g. grazing strategies, brush encroachment, control of exotic plants, roads, trails, etc.).

We will make recommendations for the management of upland habitat after our census of larval amphibians in all aquatic habitats and data analysis.

References Cited

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.

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.

Appendix 1

Entrance Pond Large Dipnet

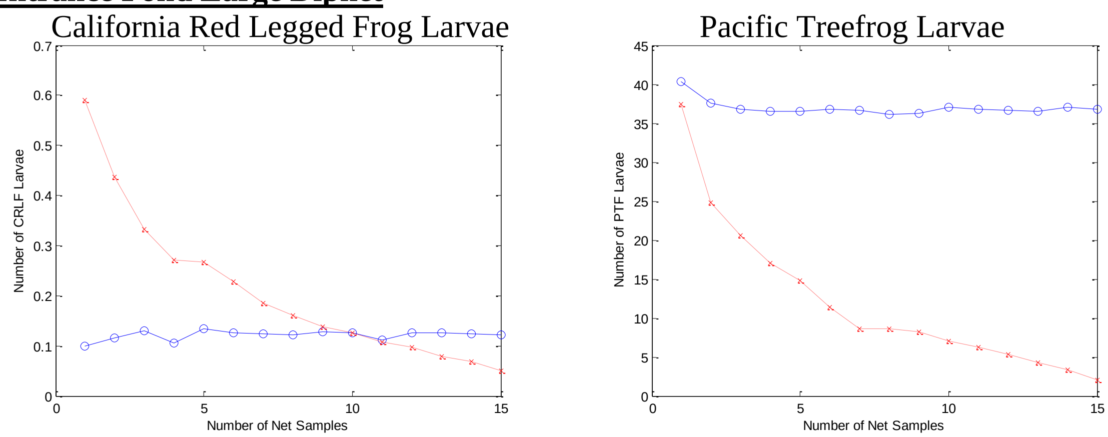

Figure 1. Effects of large dip net sample number on means (blue lines) and standard deviations (red lines) across 100 randomized subsets of samples generated with data taken from Entrance Pond.

Entrance Pond Small Dipnet

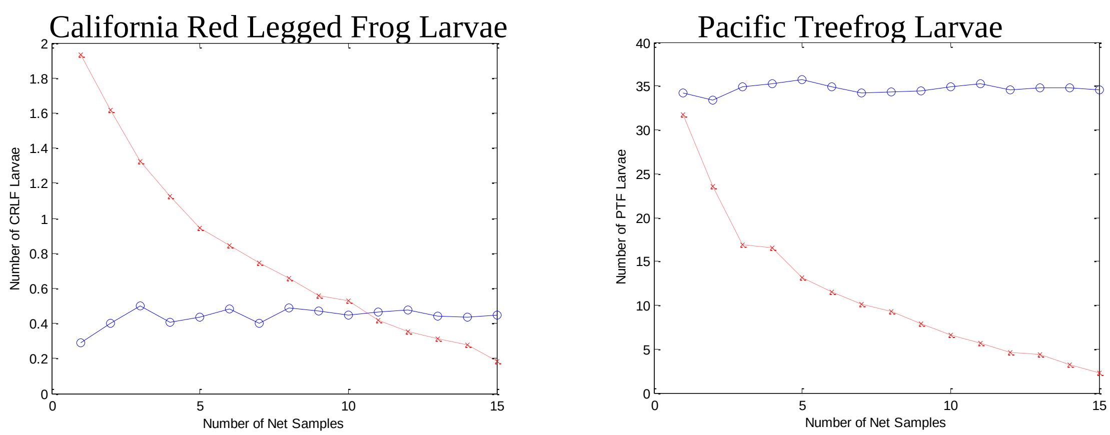

Figure 2. Effects of small dip net sample number on means (blue lines) and standard deviations (red lines) across 100 randomized subsets of samples generated with data taken from Entrance Pond.

Boundary Pond Large Dipnet

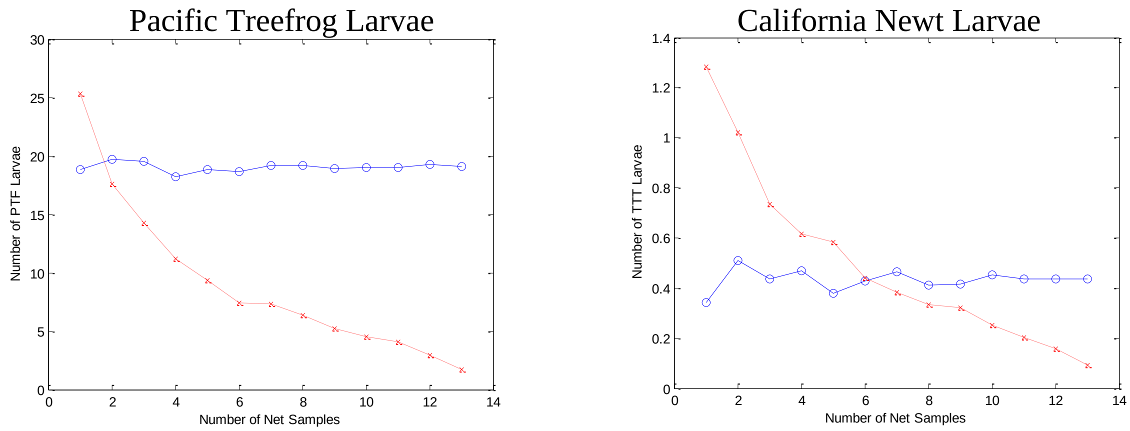

Figure 4. Effects of large dip net sample number on means (blue lines) and standard deviations (red lines) across 100 randomized subsets of samples generated with data taken from Boundary Pond.

Boundary Pond Small Dipnet

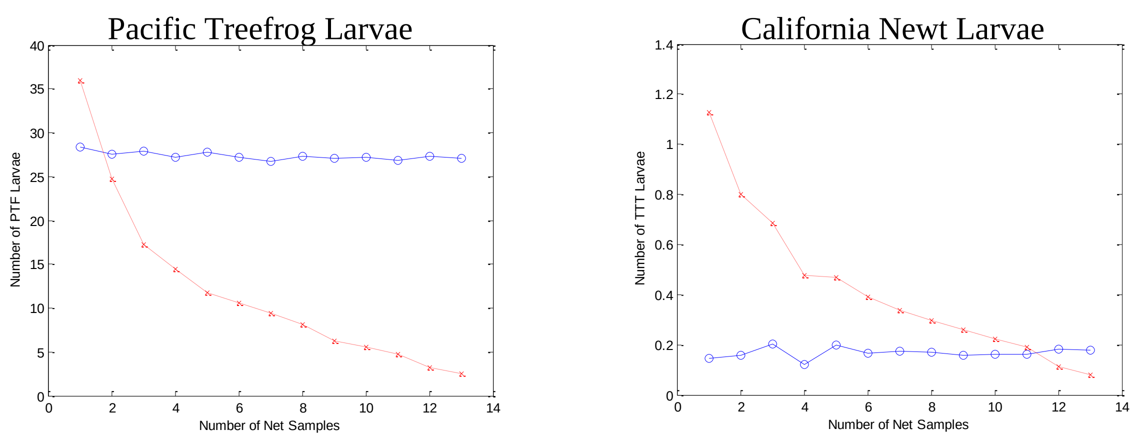

Figure 5. Effects of small dip net sample number on means (blue lines) and standard deviations (red lines) across 100 randomized subsets of samples generated with data taken from Boundary Pond.

Animas Pond Small Dipnet

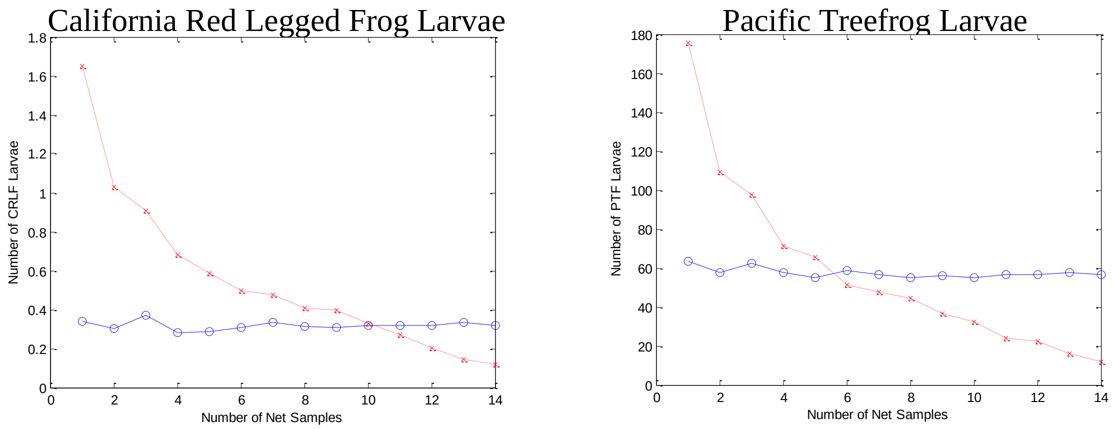

Figure 6. Effects of small dip net sample number on means (blue lines) and standard deviations (red lines) across 100 randomized subsets of samples generated with data taken from Animas Pond.

Roadrunner Pond Large Dipnet

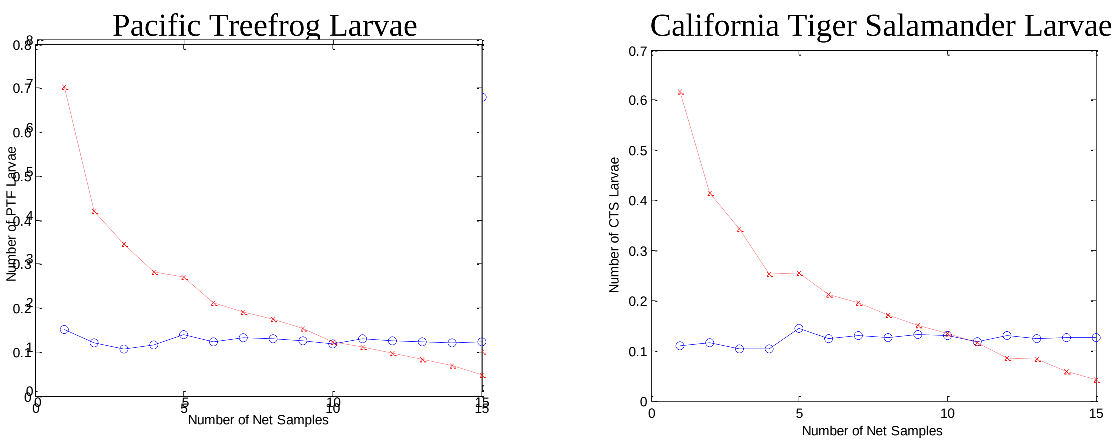

Figure 7. Effects of large dip net sample number on means (blue lines) and standard deviations (red lines) across 100 randomized subsets of samples generated with data taken from Roadrunner Pond.

Dead Pig Large Dipnet

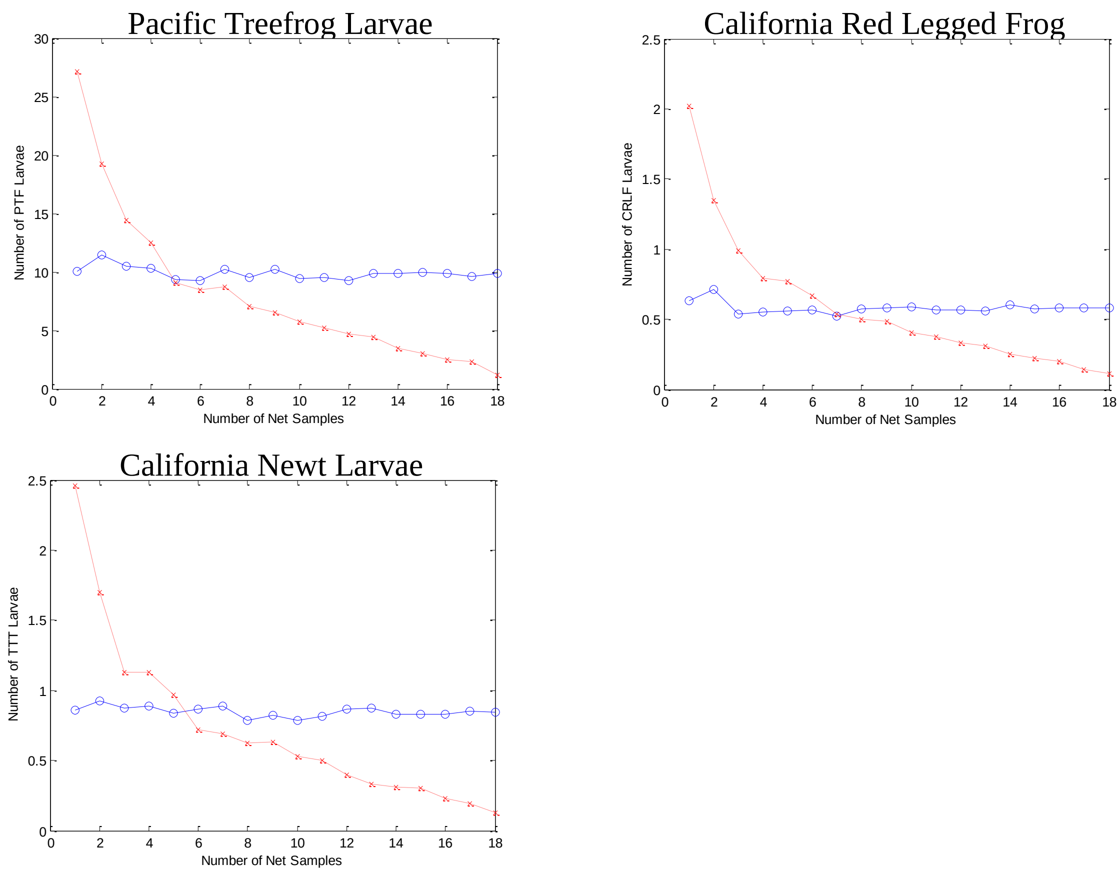

Figure 8. Effects of large dip net sample number on means (blue lines) and standard deviations (red lines) across 100 randomized subsets of samples generated with data taken from Dead Pig Pond.

Salamander Pond Large Dipnet

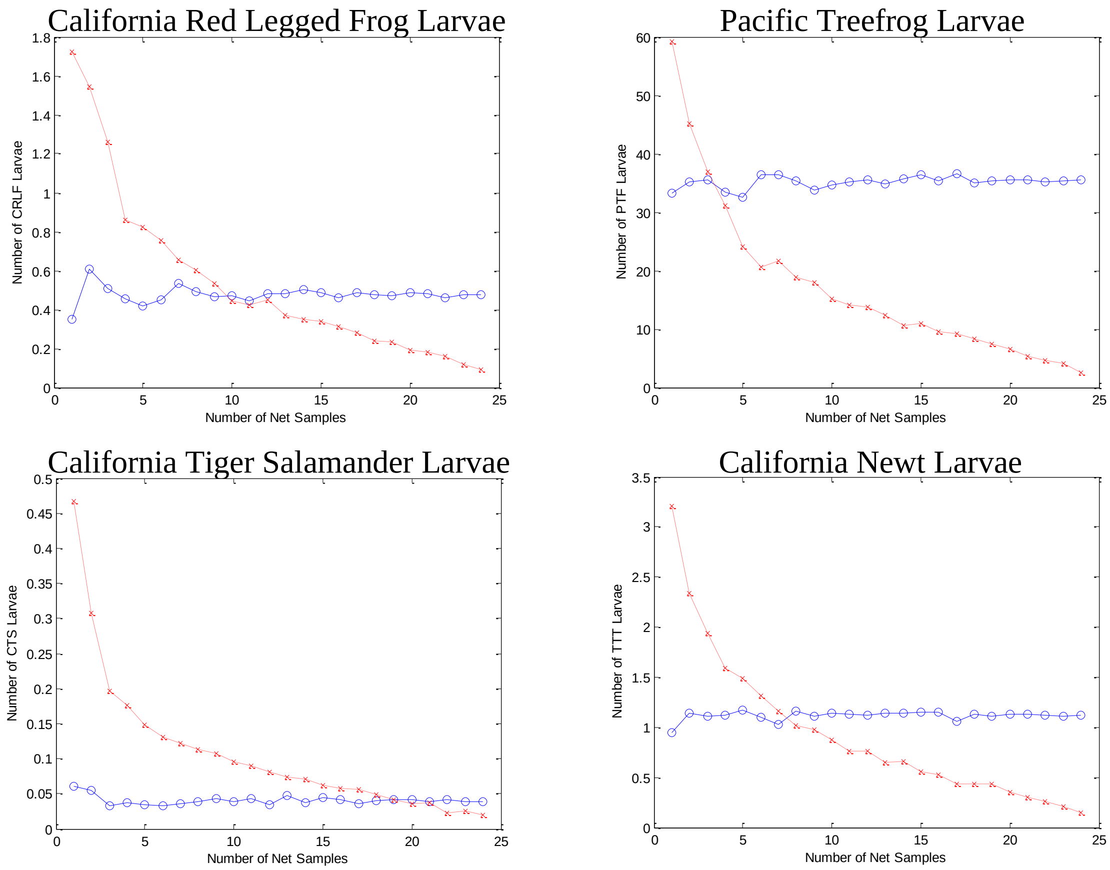

Figure 9. Effects of large dip net sample number on means (blue lines) and standard deviations (red lines) across 100 randomized subsets of samples generated with data taken from Salamander Pond.