# Pesticides and Population Declines of California Alpine Frogs


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## Abstract


Atmospherically deposited pesticides from the intensively cultivated Central Valley of California, USA, have been implicated as a cause for population declines of several amphibian species, with the strongest evidence for the frogs *Rana muscosa* and *Rana sierrae* at high elevation in the Sierra Nevada mountains. Previous studies on these species have relied on correlations between frog population status and either a metric for amount of upwind pesticide use or limited measurements of pesticide concentrations in the field. The present study tested the hypothesis that pesticide concentrations are negatively correlated with frog population status (i.e., fraction of suitable water bodies occupied within 2 km of a site) by measuring pesticide concentrations in multiple media twice at 28 sites at high elevation in the southern Sierra Nevada. Media represented were air, sediment, and *Pseudacris sierra* tadpoles. Total cholinesterase (ChE), which has been used as an indicator for organophosphorus and carbamate pesticide exposure, was also measured in *P. sierra* tadpoles. Results do not support the pesticide-site occupancy hypothesis. Of 46 pesticide compounds analyzed, nine were detected with ≥ 30% frequency, representing both historically- and currently-used pesticides. In stepwise regressions with a chemical metric and linear distance from the Central Valley as predictor variables, no negative association was found between frog population status and the concentration of any pesticide or tadpole ChE activity level. By contrast, frog population status showed a strong positive relationship with linear distance from the Valley, a pattern that is consistent with a general west-to-east spread across central California of the amphibian disease, chytridiomycosis, observed by other researchers.


## Introduction

Agricultural pesticides are being transported by air many tens of kilometers to remote locations in mountain areas, and they have been implicated as a cause for recent, dramatic population declines of several amphibian species in such areas. The strongest case is for the frogs (*Rana muscosa and R. sierrae*), which taken together were formerly nearly ubiquitous among high-elevation waters bodies throughout the Sierra Nevada of California (Fig. 1). Evidence for this pesticide effect, however, relies primarily on correlations between frog population status and either a metric for amount of upwind pesticide use [1] or limited measurements of pesticide concentrations in the field [2]. Largely unmeasured have been the magnitude and temporal variation of pesticide concentrations in the habitat of these species, and associations between the distributions of pesticides and the distribution of population declines.

Figure. 1. *Rana muscosa*, a species that has disappeared from over 90% of its historic localities in the Sierra Nevada of California [11].

## Objectives

The present study tested the hypothesis that pesticide concentrations are negatively correlated with *Rana* population status in the high elevation environment of these species in the southern Sierra Nevada. The idea was that the processes determining the patterns of distribution of pesticides in the high-elevation environment today are likely to be similar to those that operated over the past several decades when many *Rana* populations disappeared. Thus, correspondence between current pesticide distributions and population status of *Rana* spp. during the decline period would provide support for the hypothesis that pesticides have contributed to population declines. We also tested for associations between frog population status and a bioindicator of exposure to some pesticides (i.e., depression in total acetyl cholinesterase activity [ChE]) in tadpoles of a different but abundant frog, *Pseudacris sierra*.

## Methods

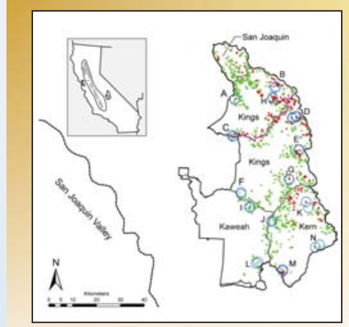


Fig. 2. Water bodies deemed suitable habitat for *Rana* spp. (green triangles and red circles). Green triangles depict water bodies with frogs absent during surveys in 1997-2002; red circles depict water bodies with frogs present. Open blue circles designate the area within 2 km of sites sampled for chemical measurements (at the center of each circle). Letters refer to 14 areas containing the sampled sites. The heavy black line represents the joint outer border of Sequoia and Kings Canyon National Parks; light black lines delineate watersheds of the San Joaquin, Kings, Kaweah, and Kern Rivers. The purple line (i.e., line extending east from area C) indicates the boundary between the geographic range of *R sierrae* (north of line) and *R. muscosa* (south of line).

- The study area was Sequoia and Kings Canyon National Parks at 2785 – 3375 m elevation (Fig. 2).
- Twenty-eight water bodies (e.g., Fig. 3) were sampled in 14 dispersed areas (A to N in Fig. 2).
- Media sampled were air, sediment, and tadpoles of Pseudacris sierra. Air was sampled by passive air samplers for 30 d.
- Samples were collected twice during summer 2005.
- Target analytes were 46 pesticides or their metabolites for sediment, 48 for tadpoles, and 22 for air; estimated detection limits were extremely low [3,4].
- Population status of Rana spp. was represented by the percent of suitable water bodies (defined by elevation, depth, and absence of fish) within 2 km of a site that were inhabited by one of the species during surveys in 1997-2002 [1].
- We tested for associations between percent of water bodies occupied by Rana spp. and pesticide metrics using logistic regression. Distance from the San Joaquin Valley was included in the models because population declines were first observed relatively close to the Valley, and some evidence suggested a decrease in pesticide concentrations with distance from the Valley [4,5].
- Eleven pesticide metrics were used: concentrations for nine pesticide compounds, principal component 1 from a principal components analysis of concentrations of all compounds, and cholinesterase activity in P. sierra tadpoles.
- Forty-two logistic regressions were conducted, one for each combination of pesticide metric, medium (air, sediment, tadpoles), and sampling period (n=2).

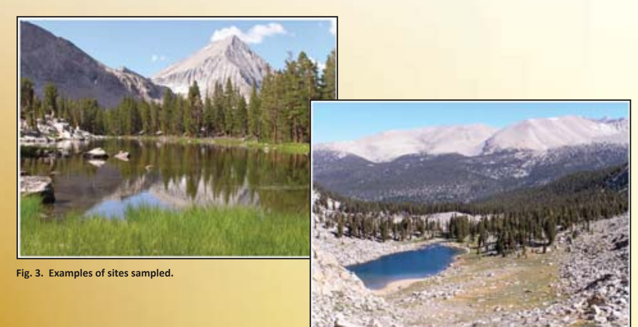

Fig. 3. Examples of sites sampled.

## Results

Table 1. Pesticide compounds detected in >30% of samples in designated media.

| Pesticide Compound | Historical or Current Use | Class | Medium: Air | Sediment | Tadpoles |
|---|---|---|---|---|---|
| Chlorpyrifos | Current | Organophosphate insecticide | | X | X |
| Dacthyl | Current | Herbicide | | X | X |
| α-endosulfan | Current | Organochlorine sulfide insecticide | | X | X |
| β-endosulfan | Current | Organochlorine sulfide insecticide | X | X | X |
| Endosulfan sulfate | Current | Organochlorine sulfide insecticide | | X | X |
| p,p'-DDE | Historical | Organochlorine insecticide | | X | X |
| *Trans*-chlordane | Historical | Organochlorine insecticide | | X | X |
| *Cis*-nonachlor | Historical | Organochlorine insecticide | | X | X |
| *Trans*-nonachlor | Historical | Organochlorine insecticide | | X | X |

- Nine pesticide compounds were detected frequently in sediment and *P. sierra* tadpoles, representing both historic- and current-use pesticides in four classes (Table 1). Only β-endosulfan was detected frequently in air.
- An average of 10 water bodies comprising suitable habitat for *Rana* spp. occurred within 2 km of a site.
- The percent of these water bodies occupied by *Rana* spp. varied widely among sites, from 0 to 100% (average 31%; Figs. 2 and 4).
- The percent of water bodies occupied was strongly related to linear distance from the San Joaquin Valley (logistic regression, p<0.0001; Fig. 4).
- In contrast, the percent of water bodies occupied was not related to a pesticide metric in the predicted direction (i.e., negative) in any of the 42 logistic regressions that included distance.
- In the 42 logistic regressions that excluded distance, percent of sites occupied was significantly related to only one chemical metric (i.e., *trans*-nonachlor in sediment, period 2; negative relationship, p=0.0117; Figs. 5 and 6).

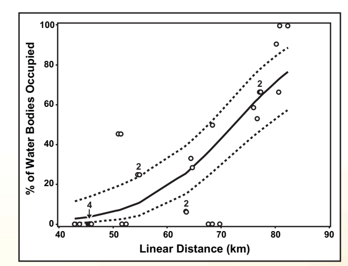


Fig. 4. Population status of *Rana* spp. (i.e., percent of suitable water bodies occupied within 2 km of site) of as a function of distance from the San Joaquin Valley. Solid line depicts logistic regression fit; dashed lines indicate 95% confidence limits for regression; p=0.0001.

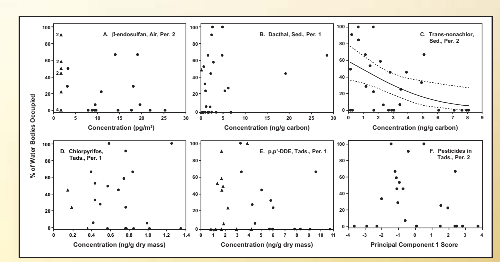


Fig. 5. Population status of *Rana* spp. as a function of selected pesticide metrics. Circles represent concentration values above estimated detection limits (EDL); triangles represent values below detection limits (nondetects), represented as 1/2 EDL. Media represented are air, sediment (Sed.), and tadpoles (Tads.) of *Pseudacris sierra* during sampling period (Per.) 1 or 2. For *trans*-nonachlor in sediment, period 1 (C), solid line depicts logistic regression fit, dashed lines indicate 95% confidence limits for regression; p<0.0117.

## Discussion / Conclusions

- Evidence does not support the hypothesis that pesticides have contributed to the dramatic population declines of *Rana* spp. in the study area.
  - The present study showed no association between the distribution of *Rana* spp. and distributions of pesticides:
    - includes nine pesticide compounds in four classes representing both historic- and current-use pesticides
    - includes a metric for pesticides as a group (principal component)
    - includes a bioindicator of exposure to certain classes of pesticides (acetyl cholinesterase)
    - includes samples from multiple media and multiple times during a period of intensive use of some pesticides
  - Concentrations of endosulfan compounds and chlorpyrifos in *P. sierra* tadpoles in the present study were many orders of magnitude lower than the tissue concentrations know to be toxic for this species [6]
  - Concentrations of several pesticides in water from multiple lakes in the study area during most of the ice-free period were extremely low (mostly 1 ng/L [parts per trillion] or less) [2,7,8]. These values are orders of magnitude below benchmarks for effects on aquatic life and known toxic levels for amphibians [7].
- An alternative hypothesis for the population declines of *Rana* spp. in the study area is chytridiomycosis, a disease discovered in 1997 that is affecting amphibian populations throughout much of the world.
  - Chytridiomycosis has been documented as the proximal cause of decline and extinction in numerous Rana populations in the study area since the early 2000s [9,10].
  - Available data suggests that chytridiomycosis spread west-to-east across the study area (i.e., away from San Joaquin Valley), a pattern that is consistent with the increase in percent of water bodies occupied with increasing distance from the Valley during 1997-2002.
- We suggest that locations at lower elevation and nearer the San Joaquin Valley are much more likely to have had amphibian populations affected by pesticides than the high-elevation environment represented in the present study.

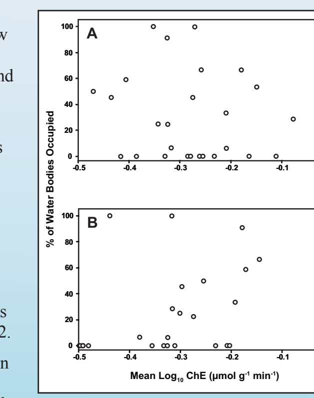


Fig. 6. Population status of *Rana* spp. as a function of total cholinesterase activity (ChE) for *Pseudacris sierra* tadpoles at each site during the two sampling periods (A and B).

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