SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 30, No. 3, pp. 682–691, 2011

Printed in the USA
DOI: 10.1002/etc.425

PESTICIDE DISTRIBUTIONS AND POPULATION DECLINES OF CALIFORNIA, USA, ALPINE FROGS, *RANA MUSCOSA* AND *RANA SIERRAE*

DAVID F. BRADFORD,*† ROLAND A. KNAPP,‡ DONALD W. SPARLING,§ MALIHA S. NASH,† KERRI A. STANLEY,‖ NITA G. TALLENT-HALSELL,† LAURA L. MCCONNELL,# and STACI M. SIMONICH‖ ††

†U.S. Environmental Protection Agency, National Exposure Research Laboratory, Las Vegas, Nevada
‡University of California, Mammoth Lakes, California, USA
§Southern Illinois University, Carbondale, Illinois, USA
‖Oregon State University, Corvallis, Oregon, USA
#U.S. Department of Agriculture, Beltsville, Maryland 20705
††Oregon State University, Corvallis, Oregon, USA

(*Submitted* 18 *June* 2010; *Returned for Revision* 16 *August* 2010; *Accepted* 28 *September* 2010)

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. Among 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. Environ. Toxicol. Chem. 2011;30:682–691.
© 2011 SETAC

Keywords—Cholinesterase *Pseudacris sierra* Sediment Sierra Nevada Tadpole

INTRODUCTION

The Sierra Nevada and adjacent Cascade mountains of California and Nevada, USA, have been a global hotspot for amphibian population declines in recent decades, with at least seven species strongly affected (*Anaxyrus* [= *Bufo*] *canorus*, *Lithobates* [= *Rana*] *pipiens, Rana boylii*, *Rana draytonii*, *Rana muscosa*, *Rana sierrae*, *Rana cascadae*) [1]. *Rana muscosa* and *R. sierrae* (southern mountain yellow-legged frog and Sierra Nevada yellow-legged frog, respectively; formerly both *R. muscosa* [2]) in the Sierra Nevada provide particularly dramatic examples of population declines. These two closely related species were once nearly ubiquitous in water bodies at high elevation (mostly > 2200 m) but remain extant at only 5 to 7% of their historical locations [2]. A number of causes for the declines of these species have been evaluated, with substantial support provided for introduced game fishes [3] and the emerging amphibian disease chytridiomycosis [4,5]. Atmospherically deposited pesticides originating from the adjacent intensively cultivated Central Valley of California have also been implicated, acting either separately or in conjunction with chytridiomycosis. Many pesticides, whose likely primary origin is the Central Valley, have been found in multiple media throughout all elevations in the Sierra Nevada [6–11].

Evidence that airborne contaminants have contributed to population declines of *R. muscosa* and *R. sierrae* derives from two types of studies. First, occurrence of these species has been shown to be negatively related to the amount of upwind pesticide use [12,13]. In particular, frog site occupancy among 6,831 water bodies in the Sierra Nevada was strongly negatively related to a distance-weighted metric for amount of pesticide applied upwind [13]. Second, ranid frog population persistence was negatively related to contaminant levels in water and *R. muscosa* adults [14] and a bioindicator of pesticide exposure (i.e., depression in total cholinesterase activity [ChE]) in tadpoles of a widespread frog, the Sierra chorus frog, *Pseudacris sierra* [7]. Depression in ChE can result from exposure to organophosphorous and carbamate pesticides commonly used in the Central Valley [7].

The inference from these studies that atmospherically deposited pesticides have contributed to population declines of *R. muscosa* and *R. sierrae*, however, has several limitations. In particular, the premise that exposure of frogs to pesticides is related to the metric used to represent amount of upwind pesticide use [12,13] has not been validated with measurements in any medium. Moreover, the chemical measurements taken to evaluate associations with population declines came from only one area with disappearing populations and one where populations were stable [14], and frogs have subsequently disap-

All Supplemental Data may be found in the online version of this article.

* To whom correspondence may be addressed (bradford.david@epa.gov).

Published online 1 December 2010 in Wiley Online Library (wileyonlinelibrary.com).

peared from most sites within the second area where pesticide concentrations were generally lower [5]. Also, analysis of *Rana* frog population status relative to the ChE indicator in *P. sierra* combined several *Rana* species and covered a geographic region much larger than the combined range of *R. muscosa* and *R. sierrae* [7].

Despite these limitations, it remains plausible that atmospherically deposited pesticides might have adversely affected populations of *R. muscosa* and *R. sierrae* in the high-elevation, alpine environment of the Sierra Nevada. Many mountain ranges, including the Sierra Nevada, can act as convergence zones for some organic chemicals as a result of diurnal mountain winds, increased precipitation, and lower temperatures in comparison with surrounding terrain [15]. Moreover, evidence is growing that amphibians in general are at greater risk than previously thought from pesticide exposure through multiple pathways. For example, adverse effects on amphibians have been documented by studies of pesticides on individuals, parasites, and community interactions [16]; multiple stressors can exacerbate pesticide effects [17]; pesticide mixtures can be more toxic than individual pesticides [18]; standard 96-h toxicity testing does not capture lag effects [19]; breakdown products of pesticides may be much more toxic than the tested parent compound [20]; and species vary substantially in their responses to pesticide exposure [21]. Consequently, concern is rising that amphibians may be experiencing adverse effects of pesticide exposure in locations where current regulatory guidelines indicate no concern.

The purpose of the present study was to evaluate the distribution of atmospherically deposited pesticides in the southern Sierra Nevada relative to the distributions of *R. muscosa* and *R. sierrae* collectively. 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 have operated over the past several decades, when many frog 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. The present study tests the specific hypotheses that currently and historically used pesticide concentrations are negatively correlated with frequency of occurrence of remaining populations of these two amphibian species and that ChE in *P. sierra* tadpoles is positively correlated with frequency of population occurrence.

The study was conducted in the southern Sierra Nevada (Sequoia and Kings Canyon National Parks, CA, USA), because population declines and the distribution of remaining populations of the two frog species have been well documented here [3,13,22], several potential causes for population declines in this area have been extensively studied [3,4,13,23–25], and contaminant concentrations are generally greater at the southern end of the Sierra Nevada than farther north [7,26]. The current study was restricted to high-elevation, alpine sites (>2,750 m elevation, which is near or above treeline) because the vast majority of water bodies comprising habitat for *R. muscosa* and *R. sierrae* in this region occur above this elevation, and this elevation threshold includes most of the sites where pesticides have been implicated in population declines of these species [13,14]. Neither species is known at low elevations (<1,400 m [1]). Metrics for distance from the Central Valley were included in the analyses because population declines in the southern Sierra were first observed relatively close to the Valley [22], and some evidence suggests a decrease in pesticide concentrations with distance from the Valley [6,8]. Although the latter study [8] did not find a general relationship for pesticide concentrations among high-elevation sites as a function of distance from the valley, concentrations for some pesticide–medium–time combinations showed significant negative relationships with distance from the Valley.

MATERIALS AND METHODS

Study sites and Rana *population status*

Two sites were sampled for pesticides and ChE in each of 14 areas dispersed throughout the high-elevation (>2,750 m) portion of Sequoia and Kings Canyon National Parks, all west of the Sierra Nevada crest (Fig. 1). The 14 areas were selected to ensure that they were dispersed throughout the three major watersheds in the Parks, to represent distances both relatively near and far from the San Joaquin Valley, and to contain abundant populations of the target species, *P. sierra*. Within each area, two water bodies (i.e., sites) were selected in which *P. sierra* was common, one with a depth of at least 1 m and one of any depth located at least 200 m away. Sampling was conducted twice during the summer of 2005. Water bodies sampled were generally small (median surface area = 0.24 ha) and shallow (median depth = 1.5 m) and ranged in elevation from 2,786 to 3,375 m. Within three of the 14 areas, the sites sampled differed between the two sampling periods because tadpoles of *P. sierra* could not be obtained at one of the two sites both times. Additional site details are provided elsewhere [8].

The population status for *R. muscosa* and *R. sierrae*, collectively, in the vicinity of each site was derived from a survey for amphibians, fish, and aquatic habitat for all water bodies (lakes and marshes; $n = 3170$) in Sequoia and Kings Canyon National Parks conducted between 1997 and 2002 [13]. Although this time interval predates the chemical sampling in 2005, Many populations of *Rana* spp. had declined and many had not declined at this time, a condition useful for evaluating factors associated with the decline. *Rana* spp. (any life stage) presence or absence was determined by visual-encounter surveys of the entire shoreline and first 100 m of inlet and outlet streams, a technique shown to be an accurate method for determining presence or absence of these species [13]. Population status was defined as the percentage of suitable water bodies occupied by either of these species within 2 km of the site, excluding water bodies outside the watershed containing the site (Kings, Kaweah, or Kern Rivers; Fig. 1). Suitable water bodies were defined as those lacking fish, ≥ 1.5 m deep, and $\leq 3{,}660$ m elevation [27]. All lakes at high elevation in the Parks were historically devoid of fish but introduced trout were widespread by the 1950s [3]. *Rana muscosa* or *R. sierrae* occurred in each of the 14 sampled areas until at least sometime between the 1960s and 2000s [2,13,22] (Sequoia and Kings Canyon National Parks, unpublished data). *Rana* spp. populations in all areas have since disappeared or been drastically reduced in numbers [2,13,22] (R. Knapp, unpublished data).

Pesticide sample collection and analysis

Pesticide concentrations were determined in three media: air, sediment, and whole *P. sierra* tadpoles at developmental stages up to Gosner [28] stage 41. Details for sampling methods, chemical analysis, detection limits, and concentrations are provided elsewhere [8] and are summarized here. Water was not sampled because previous sampling in the study area showed that the likelihood of detecting pesticides in water was much lower than in sediment [10]. *Rana muscosa* and

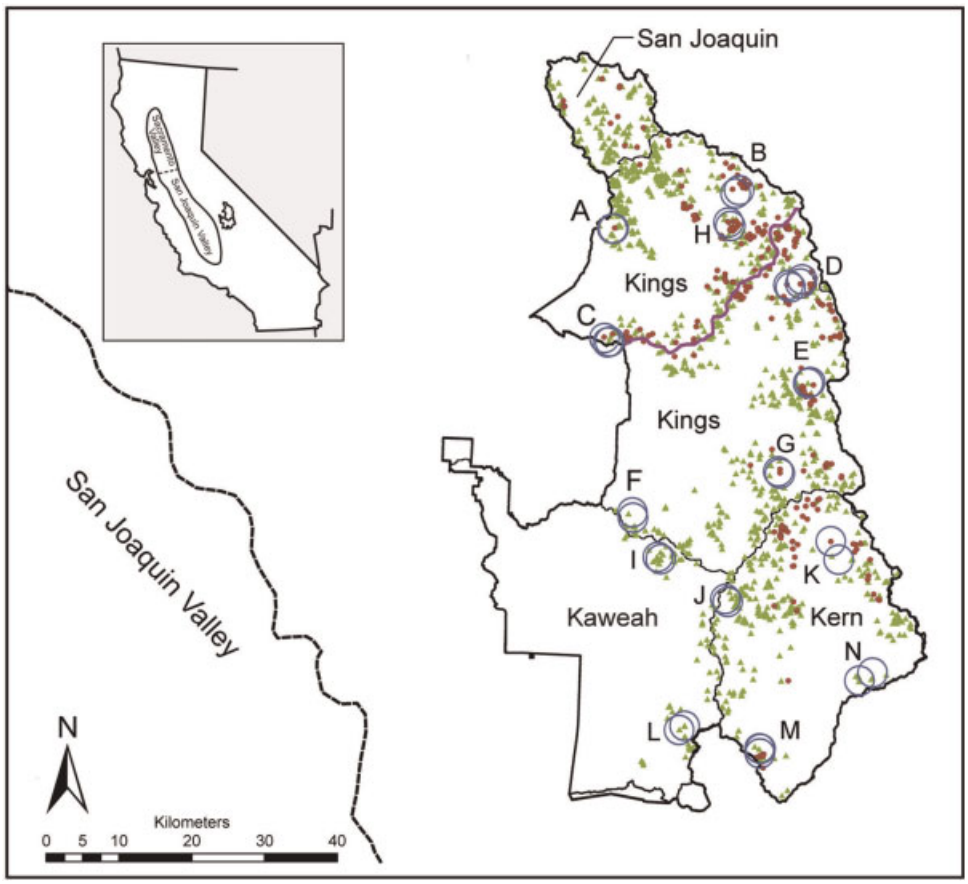

Fig. 1. Water bodies deemed suitable habitat for *Rana muscosa* or *R. sierrae* (green triangles and red circles). Green triangles depict water bodies with frogs absent during surveys in 1997 to 2002; red circles depict water bodies with frogs present. Open blue circles designate the area within 2 km of sites sampled for chemical measurements (at the center of each circle). This area was used to compute the metric for population status of *R. muscosa* and *R. sierrae* collectively. Letters refer to 14 areas containing the sampled sites. The thick black line represents the joint outer border of Sequoia and Kings Canyon National Parks (CA, USA); thin black lines delineate watersheds of the San Joaquin, Kings, Kaweah, and Kern Rivers (CA, USA). 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 2).

R. sierrae were not suitable for sampling because they no longer occur over most of the study area, and most remaining populations are small [27]. By contrast, *P. sierra* is widespread and abundant throughout the study area, and it is an aquatic breeding frog like *R. muscosa* and *R. sierrae*. Stage 41 was selected as a cutoff because it is the last stage before metamorphosis begins, and this threshold can be easily recognized at a distance in the field [28]. Sediment and tadpoles were collected at both sites in each of the 14 areas, whereas air was sampled at two points within 100 m of each other at one site in each of the 14 areas. Sediment was collected with a hand corer at 1.0 m water depth, and the top 2.5 cm was taken as the sample [8]. Air samples were collected with a passive sampling device consisting of a polyurethane foam disk within a metal housing [8]. Disks were deployed for approximately 30 d ending at the time of sample collection for other media. Samples of all media were collected during two periods in 2005: July 30 to August 12 (period 1) and August 29 to September 12 (period 2).

Target analytes were 46 pesticides or their metabolites for sediment, 48 for tadpoles, and 22 for air [8]. A pesticide was included in subsequent analyses if the detection frequency was >30%. The 30% threshold was selected because, for statistical analyses, concentration values below the estimated detection limit were replaced with half the detection limit, and this technique yields adequate summary statistics at detection frequency >30% [29]. Nine pesticide compounds were detected in sediment and tadpoles at ≥30% of sites during at least one of the sampling periods. Five of these represent currently used pesticides: chlorpyrifos, dacthal, α-endosulfan, β-endosulfan, and endosulfan sulfate. Four represent historically used pesticides: dichlorodiphenyldichloroethylene (*p,p′*-DDE), *trans*-chlordane, *cis*-nonachlor, and *trans*-nonachlor. In air samples, only β-endosulfan was detected at ≥30% of sites. Concentrations were low in all media (i.e., parts-per-billion range in sediment and tadpoles and pg/m^3 in air [8]).

The pesticide data set comprised 36 combinations of chemical (nine pesticide compounds), medium (air, sediment, tadpole), and time (period 1 or 2). Chlorpyrifos was not detected in tadpoles in period 2, and α-endosulfan did not meet the detection frequency criterion in sediment during period 2 [8]. Sample sizes for analysis varied among chemical–medium–time combinations largely because of the elimination of samples when laboratory blanks were high relative to concentrations in sediment and tadpoles [8]. Median sample size among the 36 combinations of chemical, medium, and time was 27 (range 8–28; Table 1). The median detection frequency was 82%; only three combinations were between 30 and 50% (α-endosulfan in sediment in period 1, α-endosulfan in tadpoles in period 2, and β-endosufan in air in period 1).

To derive a metric that represented all pesticide compounds together, principal components analysis was conducted for each of the four combinations of medium (sediment or tadpoles) and sampling period ($n = 17$ to 27; Table 1 [8]). Principal component 1 (PC_1) was used as the metric because all pesticide

Table 1. Results of logistic regressions for frequency of *Rana* spp. site occupancy as a function of individual chemical metrics with and without linear distance included as a predictor variable in the model[a]

		Sampling period 1				Sampling period 2			
				Linear distance included				Linear distance included	
Medium		*n*	Linear distance omitted	Linear distance	Chemical Metric	*n*	Linear distance omitted	Linear distance	Chemical Metric
Air	β-Endosulfan	27	NS	0.0002/+	NS	28	0.0901/−	<0.0001/+	NS
Sediment	Chlorpyrifos	8	NS	0.0719/+	NS	25	NS	<0.0001/+	NS
	Dacthyl	28	NS	<0.0001/+	NS	23	NS	<0.0001/+	NS
	α-Endosulfan	28	NS	<0.0001/+	NS	—	—	—	—
	β-Endosulfan	28	NS	<0.0001/+	NS	28	NS	<0.0001/+	NS
	Endosulfan sulfate	27	NS	<0.0001/+	NS	28	NS	<0.0001/+	NS
	p,p′-DDE	28	NS	<0.0001/+	NS	28	NS	<0.0001/+	NS
	trans-Chlordane	28	NS	<0.0001/+	0.0343/+	28	NS	<0.0001/+	0.0343/+
	cis-Nonachlor	27	NS	<0.0001/+	0.0587/+	28	NS	<0.0001/+	0.0587/+
	trans-Nonachlor	28	NS	<0.0001/+	NS	28	0.0117/−	<0.0001/+	NS
	PC_1	27	NS	0.0001/+	NS	20	NS	0.0001/+	NS
Tadpole	Chlorpyrifos	24	NS	<0.0001/+	NS	—	—	—	—
	Dacthyl	21	NS	<0.0001/+	NS	23	NS	<0.0001/+	NS
	α-Endosulfan	19	NS	<0.0001/+	NS	26	NS	<0.0001/+	NS
	β-Endosulfan	27	0.0656/+	<0.0001/+	NS	26	NS	<0.0001/+	NS
	Endosulfan sulfate	27	NS	0.0001/+	NS	26	NS	<0.0001/+	NS
	p,p′-DDE	27	NS	<0.0001/+	NS	26	NS	<0.0001/+	NS
	trans-Chlordane	13	NS	0.0003/+	NS	26	NS	<0.0001/+	NS
	cis-Nonachlor	27	NS	<0.0001/+	NS	26	NS	0.0004/+	NS
	trans-Nonachlor	27	NS	<0.0001/+	NS	26	NS	<0.0001/+	NS
	PC_1	17	NS	<0.0001/+	NS	23	NS	<0.0001/+	NS
	Cholinesterase	26	NS	<0.0001/+	NS	24	NS	0.0002/+	NS

[a] Tadpoles were *Pseudacris sierra*. PC_1 represents principal component 1 from the principal component analysis of pesticides in each medium–period combination. Metrics were concentrations for individual chemicals, scores for PC_1, and activity level for total cholinesterase in tadpoles. Values shown are *p* value and direction of relationship between fraction of sites occupied and the variable. NS = not significant at $p = 0.10$. DDE refers to dichlorodiphenyldichoroethylene. In models with linear distance included, two outliers were removed to achieve an adequate fit for dacthal in sediment in period 1, and one was removed for *trans*-chlordane in tadpoles in period 1.

compounds loaded positively and mostly similarly on PC_1 in each of the four principal components analyses, indicating that chemical compositions were generally similar among sites [8]. The PC_1 accounted for 44.3 to 73.8% of the variation (i.e., eigenvalues ranged from 0.443 to 0.738 [8]).

Cholinesterase sampling and analysis

Approximately 16 *P. sierra* tadpoles per site (range 14–18) were collected for analysis of ChE simultaneously with collection of samples for pesticide analysis. Tadpoles were collected by hand or dip net and placed in plastic bags filled with water from the collection site. Tadpoles and bags were handled with clean, powder-free latex gloves at all times. Tadpoles at Gosner [28] stage >41 were excluded. Tadpoles were individually wrapped in aluminum foil, placed together in a 250-ml plastic jar, and frozen on dry ice. Samples were kept frozen on dry ice or at −80 °C until analysis.

In the laboratory, tadpoles were thawed and staged [28], the gut coil was removed, and the remaining body was weighed to the nearest 0.0001 g. Cholinesterase was determined through spectrophotometric methods [30] with reagent quantities adjusted for a microplate reader (Synergy model; Biotek Instruments).

Distance metrics

Two metrics were calculated to quantify the distance from each study site to the San Joaquin Valley, the southern arm of the Central Valley (i.e., linear distance and upslope distance [8]). Linear distance (measured using Arc map 9.2; ESRI) is the distance to the closest point on the mountain–valley boundary, defined as the boundary between mountain slopes and the relatively flat valley, roughly following certain contour levels but smoothed to eliminate prominent lateral deviations such as river valleys (Fig. 1). Elevations for this boundary were approximately 150 m near the Kings River, 170 m near the Kaweah River, and 300 m near the Kern River. Upslope distance was calculated using Arc Info (ESRI) as the path that runoff water would follow from the site to the mountain–valley boundary. Upslope distance was used as a surrogate for the flow path taken by daily upslope/downslope winds common in the southern Sierra Nevada during summer [31]. Linear distance for the sampled sites ranged from 42.9 to 82.5 km and upslope distance ranged from 59.6 to 187.3 km [8].

Statistical analysis

Comparing cholinesterase activity among sites. Because tadpole developmental stage was expected to affect ChE [7,20], analysis of covariance was conducted for $\log_{10}$-transformed ChE with tadpole developmental stage as a covariate (see Supplemental Data). This allowed adjustment of the observed ChE value to a constant stage if necessary for comparison among sites. To meet the assumption of normality, ChE values were $\log_{10}$-transformed and, for period 1, six outliers among 353 samples (identified by examination of residual plots) were omitted. Stage was significantly related to ChE during period 2, but not during period 1. The magnitude of the effect during period 2, however, was small. For example, the absolute difference between the geometric mean ChE at a site and the geometric mean adjusted to the median stage averaged only 3.9% (range 0.1–13.2% among sites). Given this small difference, all statistical tests among sites were conducted both ways (i.e., stage adjusted and not stage adjusted); the results were

nearly identical. Consequently, we report the simpler of these statistical approaches for ChE among sites, i.e., using the mean of $\log_{10}$ ChE without adjustment for stage effects.

Environmental temperature also can affect ChE in *P. sierra* [32]. Using elevation as a surrogate for environmental temperature at a site, the relationship between mean $\log_{10}$ ChE at a site and elevation was evaluated by Pearson correlation. The difference in $\log_{10}$ ChE between sampling periods was tested by paired *t* test.

Analysis of frog population status. Relationships between population status for *R. muscosa* and *R. sierrae* collectively and predictor variables were evaluated using logistic regression. Population status was represented as the frequency ratio: number of suitable water bodies occupied (*o*)/total number of suitable water bodies (*t*). The basic model was

$$g(u) = \text{Logit}(p(o/t)) = \beta_0 + \sum \beta_i x_i \quad (1)$$

where β_0 is the intercept, β_i represents the coefficient(s), and x_i represents the predictor variable(s). Three types of logistic regressions models were used, in which x_i represented a distance metric only, a chemical metric (plus tadpole developmental stage when the chemical metric was a pesticide concentration in tadpoles) and a distance and a chemical metric together (plus tadpole developmental stage when the chemical metric was a pesticide concentration in tadpoles). Developmental stage was included as indicated because stage was related to pesticide concentration in some cases [8]. Distance metrics were linear distance or upslope distance from the San Joaquin Valley. Elevation was not included because there was little evidence of a pesticide–elevation relationship separate from distance [8]. Chemical metrics were of three types: individual chemical concentrations in air, sediment, or *P. sierra* tadpoles; PC_1 from the principal components analyses for pesticide concentrations in sediment or tadpoles; and mean $\log_{10}$ ChE in *P. sierra* tadpoles. This resulted in 42 metric–medium–time combinations. For models with more than one predictor variable, interaction terms were included and selection of variables was conducted stepwise with entry probability = 0.3. To detect marginal associations among variables, model retention probability was set at 0.10 instead of 0.05. Collinearity among predictor variables was evaluated with a threshold of ||Pearson r|| > 0.7 for small sample sizes [33]; no pairwise predictor–variable correlations exceeded this threshold. To accommodate overdispersion of the data, the Williams scaling option was used [34]. The Williams option failed in two cases, so the Pearson option was used instead. The model Wald χ^2 was used to select the final model with $p \leq 0.10$. Goodness of fit was evaluated using the residual χ^2 test; residual $p(\chi^2) > 0.05$ indicated a good fit. In three models with a poor fit, outliers were identified by examining the change in deviance and Pearson χ^2. Outliers were removed before finalizing the model. Statistical analyses were performed in SAS 9.2 (SAS Institute).

RESULTS

Distribution of Rana *populations*

Among 3,170 water bodies surveyed in the two national parks in 1997 to 2002, 1,254 met the criteria defined as suitable habitat (i.e., depth, fish, elevation; Fig. 1). Among the 1,254 suitable water bodies, 266 (21.1%) were occupied by *R. muscosa* or *R. sierrae*. The number of suitable sites used for the population-status metric (that is, the number of suitable water bodies within 2 km of a site) averaged 10.1 (median = 9, range = 2–24). The frequency of suitable water bodies occupied by frogs within 2 km of a site averaged 30.5% (median = 25.0%, range = 0–100%).

A general geographic pattern of frog occurrence was evident, with most water bodies that contained frogs occurring relatively far from the San Joaquin Valley (Fig. 1). Indeed, among the sites sampled for pesticides and ChE, the frequency of suitable water bodies occupied by frogs within 2 km of a site was strongly positively related to linear distance from the San Joaquin Valley (logistic regression, $p < 0.0001$; Fig. 2A). By contrast, no relationship was evident between the population-status metric and the upslope distance (Fig. 2B).

Cholinesterase activity in P. sierra *tadpoles*

Cholinesterase activity varied considerably within and among sites. The coefficient of variation within a site averaged 18.1% for period 1 (Fig. 3A) and 26.5% for period 2 (Fig. 3B). Among sites, the median value ranged over twofold from 0.34 to 0.85 μmol g^{-1} min^{-1} for period 1 and from 0.32 to 0.75 μmol g^{-1} min^{-1} for period 2. Cholinesterase activity at a site did not differ significantly between periods 1 and 2 (paired *t* test of mean $\log_{10}$ ChE, $n = 21$ sites, $p = 0.30$), and ChE at a site was not significantly correlated between the two sampling periods (Pearson correlation of mean $\log_{10}$ ChE, $n = 21$ sites,

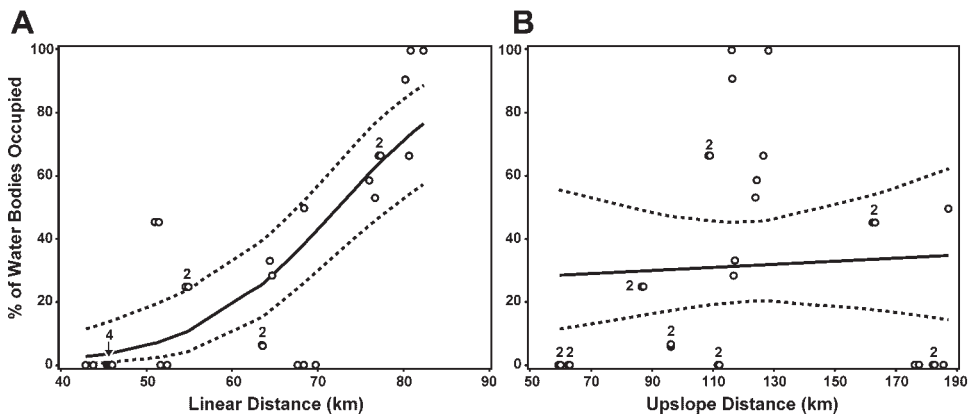

Fig. 2. Population status (i.e., percentage of suitable water bodies occupied within 2 km of site) of *Rana* frogs (*R. muscosa* and *R. sierrae*, collectively) as a function of distance from the San Joaquin Valley (CA, USA). Solid line depicts logistic regression fit; dashed lines indicate 95% confidence limits for regression. Numerals (2 and 4) indicate number of closely overlapping points. (**A**) Linear distance; model Wald $\chi^2 < 0.0001$, concordance = 84.4%. (**B**) Upslope distance; Wald $\chi^2 = 0.7703$.

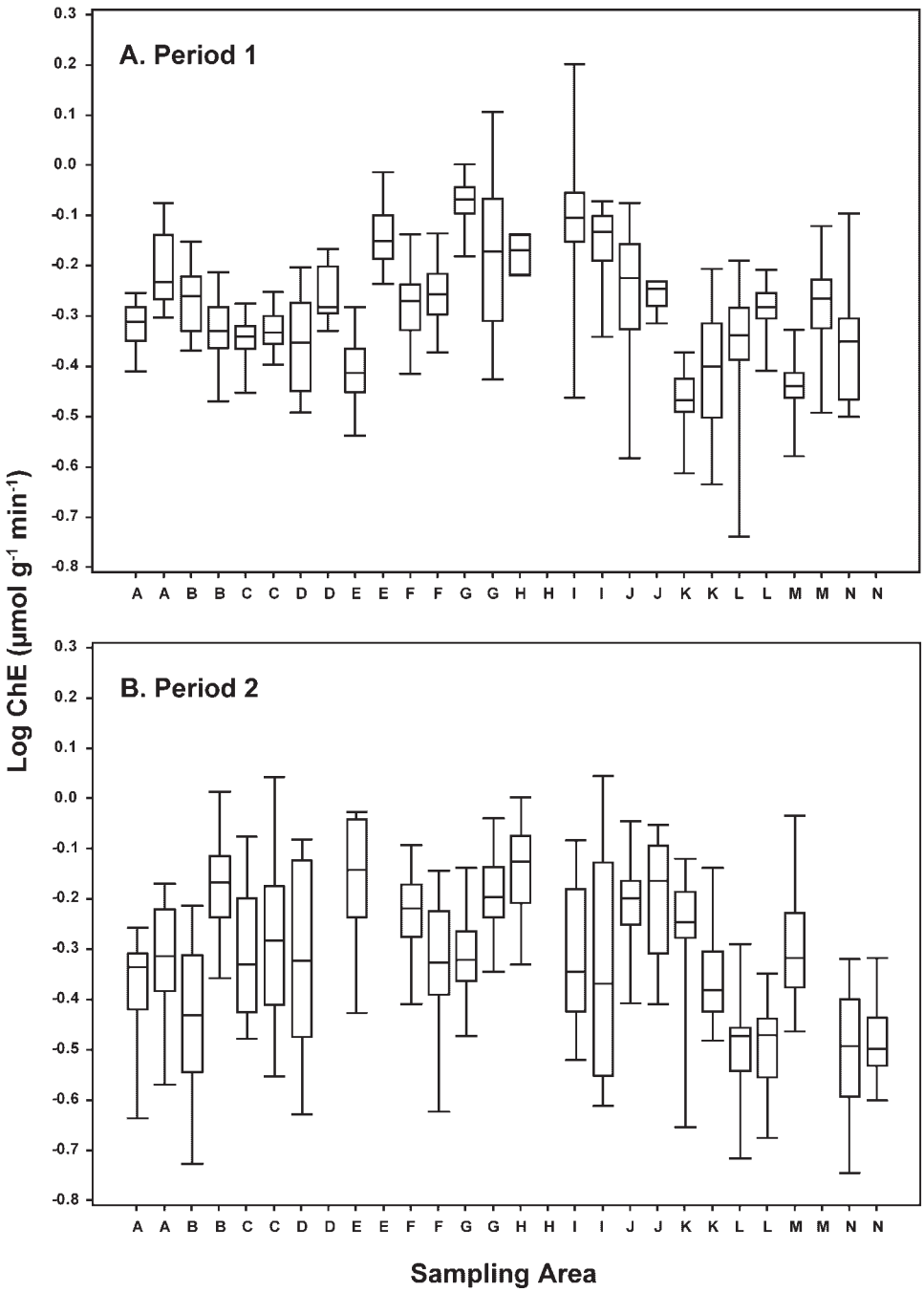

Fig. 3. Total cholinesterase activity levels (ChE; µmol g^{-1} min^{-1}) in *Pseudacris sierra* tadpoles among sites. Letters on the *x* axis refer to the 14 sampling areas shown in Figure 1. Outer box indicates interquartile range; horizontal line within box indicates median; vertical bars indicate range. Sample size per site averaged 14.3 (range 5–18). (**A**) Period 1, $n = 353$ tadpole samples. (**B**) Period 2, $n = 363$ tadpole samples.

$p = 0.55$). Median tadpole stage per sample averaged 31.5 during period 1 (range among individual tadpoles = 25–41) and 36.6 during period 2 (range among individuals = 25–41; tadpoles at stage >41 were purposely not collected).

Chlorpyrifos was the only cholinesterase-inhibiting pesticide found among the nine pesticide compounds evaluated in the present study. Cholinesterase activity at a site (mean $\log_{10}$ ChE) was not significantly related to concentration of chlorpyrifos in *P. sierra* tadpoles during period 1 ($n = 24$; Pearson correlation, $p = 0.66$; chlorpyrifos was not detected in tadpoles in period 2). Cholinesterase activity was also not significantly related to chlorpyrifos concentration in sediment during period 1 ($n = 7$, $p = 0.23$) or period 2 ($n = 21$, $p = 0.14$). Cholinesterase activity also showed no relationship with elevation during either period 1 (Pearson correlation, $p = 0.58$) or period 2 ($p = 0.99$).

Rana *population status versus chemical and distance metrics*

Rana population status showed few relationships with a chemical metric (i.e., chemical concentration, principal component, or ChE) during either sampling period (Table 1 and Figs. 4 and 5). In logistic regressions that did not contain a distance metric, a chemical metric was retained in only one model among the 42 combinations of chemical metric–medium–sampling period (i.e., *trans*-nonachlor in sediment, period 2; negative relationship; $p = 0.0117$; Table 1 and Fig. 4C). By contrast, in the 42 models that included both linear distance and a chemical metric, linear distance was

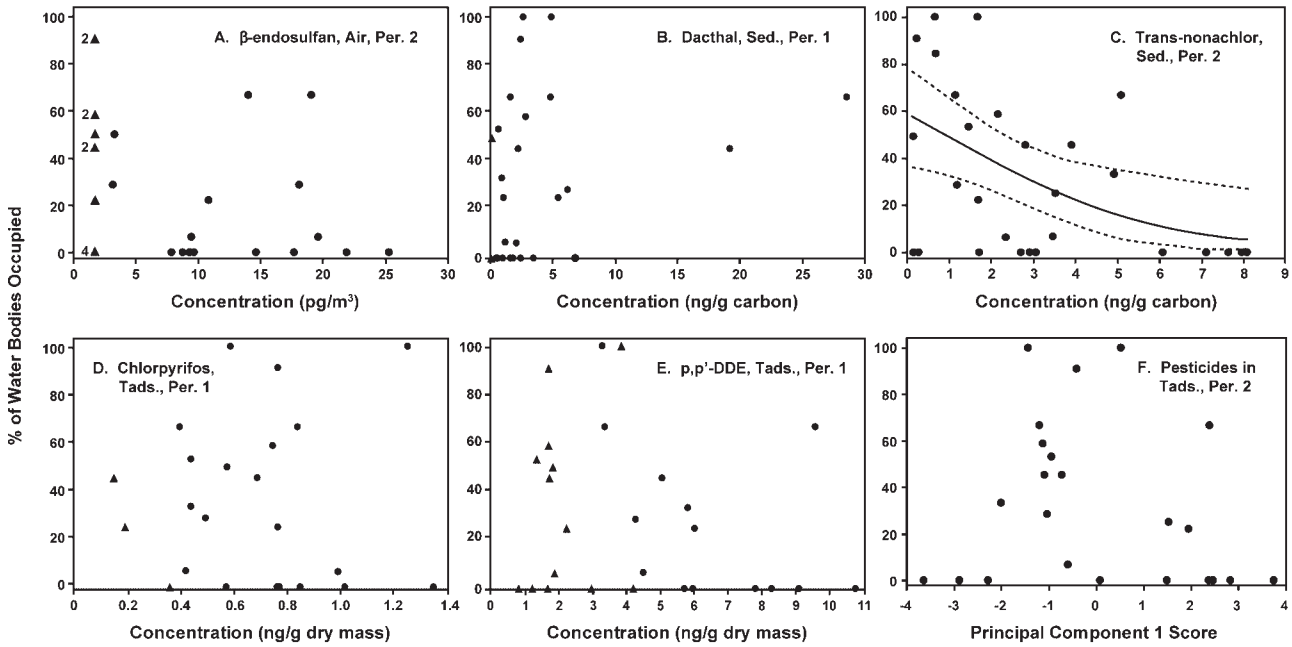

Fig. 4. Population status of *Rana muscosa* and *R. sierrae,* collectively, as a function of selected pesticide metrics. Circles represent concentration values above estimated detection limits (EDL); triangles represent values below detection limits (nondetects). Nondetects on a dry-mass basis varied and exceeded detected values in some cases because substitution of half of the EDL for values <EDL was done on a wet-mass basis, whereas samples varied in dry mass:wet mass ratio. (**A**) β-Endosulfan in air, period 2. (**B**) Dacthal in sediment, period 1. (**C**) *trans*-Nonachlor in sediment, period 1. Solid line depicts logistic regression fit; dashed lines indicate 95% confidence limits for regression; model Wald $\chi^2 = 0.0117$, concordance = 73.84%. (**D**) Chlorpyrifos in tadpoles, period 1. (**E**) *p,p′*-Dichlorodiphenyldichloroethylene (DDE) in tadpoles, period 1. (**F**) Principal component 1 (PC_1) for pesticides in tadpoles, period 2. A high PC_1 score indicates relatively high pesticide concentrations.

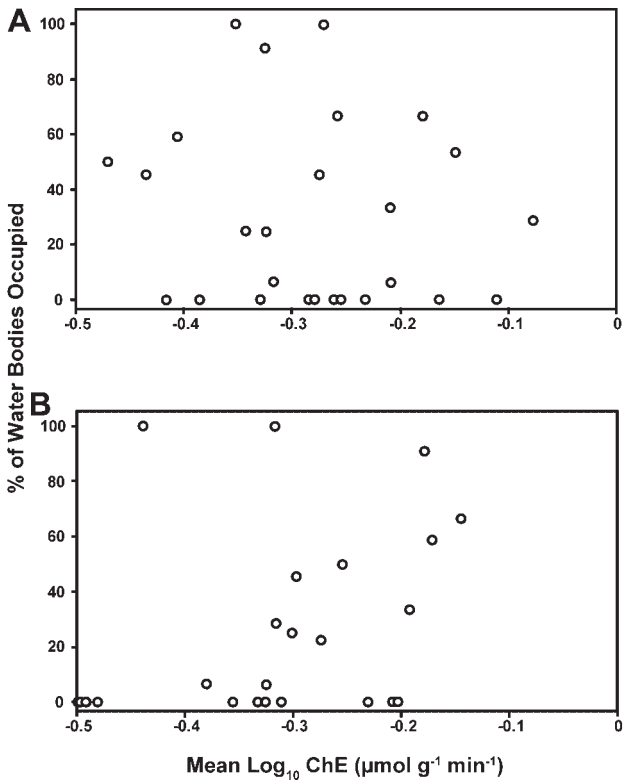

Fig. 5. Population status of *Rana* spp. as a function of total cholinesterase activity (ChE) for *Pseudacris sierra* tadpoles at each site. (**A**) Period 1. (**B**) Period 2.

retained in all models (Table 1). The *p* value for these relationships (all positive) was <0.0004 for all models except for chlorpyrifos in sediment, period 1 ($p = 0.0719$), for which sample size was small ($n = 8$). No chemical metric showed a negative association with *Rana* population status in these models (i.e., the direction predicted assuming chemicals have adversely affected *Rana* populations). Two chemical metrics were retained in the models with linear distance, but the direction of this relationship was positive (opposite the predicted direction). These were *trans*-chlordane in sediment, period 1 ($p = 0.0343$), and *cis*-nonachlor in sediment, period 1 ($p = 0.0587$). For the 42 models that included upslope distance and a chemical metric, upslope distance was not retained in any model. No interaction term was significant in any model with either distance metric.

DISCUSSION

Representativeness of observed pesticides

Although the distributions of the nine pesticide compounds in the present study obviously cannot represent the hundreds of pesticides possibly transported to high elevation in the Sierra Nevada in recent years, they do provide a diverse set for evaluating associations between pesticide distributions and frog population declines. The nine compounds represent six pesticides in four classes and include both current-use and historical-use pesticides. The three current-use pesticides were chlorpyrifos (an organophosphate insecticide), endosulfan (an organochlorine sulfide insecticide; parent compound for endosulfan sulfate), and dacthal (an herbicide). The three historical-use

pesticides are all organochlorine insecticides banned in the 1970s or early 1980s: DDT (parent compound for *p,p′*-DDE), chlordane, and nonachlor. Persistence in the environment is not long for chlorpyrifos, with a half-life on the order of days to weeks [35], whereas the other pesticides are generally more persistent, with half-lives in the environment on the order of weeks to years [36,37]. Indeed, dacthal, endosulfan, DDE, chlordane, and nonachlor were all detected in dated sediment cores in a previous study from two lakes in area I from the 1950s until the time of sampling in 2003 [10]. The distributions of the current-use pesticides in the present study are presumably determined primarily by recent use, transport, and deposition [11], whereas the distributions of the historical-use pesticides are presumably determined primarily by these processes operating decades ago and continual recycling in the local environment.

An unknown is the extent to which the distributions of observed pesticide compounds in the present study represent the distributions of these compounds during other times of the year or during previous years. For current-use pesticides, however, some evidence suggests that concentrations in the present study should be similar to those that occurred at times of intermediate or maximal pesticide use in previous years. Sources for these pesticides in the southern Sierra Nevada are primarily regional, such as the adjacent San Joaquin Valley [6,9–11]. Moreover, one of these studies [11] found that concentrations of two pesticides (endosulfan and propargite) in lake water in the study area closely tracked weekly pesticide application rates in the San Joaquin Valley with lag times of one to two weeks. For chlorpyrifos and dacthal, the two sampling periods in the present study overlapped the 7-d and monthly peaks in amount applied in the San Joaquin Valley during 2005, whereas for endosulfan sampling began 4 weeks after the 7-d application peak and overlapped weeks with intermediate amounts applied (California Department of Pesticide Regulation pesticide use reports; http://calpip.cdpr.ca.gov). It is also possible that pesticides are most likely to affect amphibians in early spring, when pesticides accumulated over the winter in the snowpack enter surface water and frog breeding begins. However, in a study during 2003 to 2005 concentrations of chlorpyrifos, dacthal, and endosulfan in the snowpack at two sites approximately 1 km apart in the study area were very low (<6 ng/L [9]), as they were in water of four lakes sampled during June through October of 2003 [11].

The amount of pesticide applied in the San Joaquin Valley in 2005 relative to previous years varied among the current-use pesticides. For dacthal, the amount applied in 2005 was nearly double the 10-year average (14,399 vs. 7,668 kg); for endosulfan, it was approximately half the 10-y average (28,370 vs. 56,259 kg); for chlorpyrifos, it was slightly above the 10-year average (682,022 vs 636,850 kg; California Department of Pesticide Regulation pesticide use reports; http://calpip.cdpr. ca.gov).

Lack of correspondence between Rana *and chemical metrics*

Virtually no association was evident between the distribution of *Rana* populations at high elevation and measured pesticide concentrations. Specifically, in the stepwise regressions that included linear distance, the predicted negative association was not found between frog population status and the concentration of any pesticide or PC_1 from the principal components analyses of all pesticides, and no association was found between frog population status and ChE in *P. sierra* tadpoles. Even when a distance metric was omitted from these regressions, only 1 of 42 chemical metric–medium–time combinations was significant at $p < 0.05$. By contrast, the conspicuous association between frog population status and linear distance from the San Joaquin Valley suggests that an unidentified factor related to distance was a cause for the *Rana* population declines. Historically, *Rana* were common in lake basins throughout the study area [2,13,22].

The lack of association between chemical metrics and frog population status is not consistent with the inference in three other studies [7,13,14] that windborne pesticides might have contributed to population declines of *R. muscosa* and *R. sierrae* in the Sierra Nevada. Davidson and Knapp [13] used a metric for amount of upwind pesticide use for all pesticides over a 10-year period, weighted inversely with distance from the point of application. They found that, after accounting for fish, habitat, and other factors, the metric was strongly negatively associated with site occupancy by frogs. However, their metric was also strongly negatively related to linear distance from the edge of the San Joaquin Valley ($r^2 = 0.5816$, $p < 0.0001$, $n = 6831$; see also Fig. 1 of Davidson and Knapp [13]), which may account for the strong association between their pesticide metric and frog site occupancy.

Fellers et al. [14] reported concentrations of several pesticides in 1997 in an area where *R. muscosa* populations had disappeared (Tableland; area I in Fig. 1) and in another area where frog populations were robust (Sixty Lake Basin; area E in Fig. 1). *Rana muscosa* populations disappeared in the Tableland over the period from 1977 to 1991 and were absent in 1993 [25,38,39]. In 1994, adult *R. muscosa* were translocated from Sixty Lake Basin to the Tableland. Many of these individuals persisted until 1997, when dead and dying frogs were found, and those that could be captured were collected for pesticide analysis [14,25]. These Tableland frogs showed significantly higher concentrations of several pesticides than frogs from Sixty Lake Basin, and concentrations of several pesticides were also higher in water from two lakes in the Tableland than in two lakes in Sixty Lake Basin [6,14]. The tendency for pesticide concentrations to be greater in the Tableland than Sixty Lake Basin was also found in the present study. Among 36 pesticide–medium–time combinations for these two areas in the present study, the mean concentrations for the two samples (i.e., two sites for sediment and tadpoles, two samples from one site for air) in the Tableland were greater than the mean for the two samples in Sixty Lake Basin for all but two of these combinations. However, the pattern of greater pesticide concentrations occurring in an area with low frequency of frog population occurrence that was evident in Fellers et al. [14] did not hold in the present study, when many more sites were evaluated (up to 28 sites in 14 areas). Moreover, frog populations in Sixty Lake Basin that were extant in 1997 have since been decimated by the amphibian disease chytridiomycosis [5]. Frog numbers declined in the Sixty Lake Basin from over 2000 in 2004 to less than 50 in 2008 [5].

The lack of association between ChE and *Rana* population status and between ChE and chlorpyrifos (the only ChE-inhibiting pesticide detected with >30% frequency in the study) is not surprising given that chlorpyrifos concentrations in tadpoles were extremely low (maximum = 1.4 ng/g dry wt [8]). A depression of 50% in ChE activity from a reference level has been considered a forensic threshold for pesticide-caused inhibition in other vertebrates [40], and a 56% reduction in ChE in the toad *Bufo arenarum* resulted in increased mortality [41]. Tissue concentrations of chlorpyrifos in recently metamorphosed *P. sierra* associated with 50% inhibition of ChE is predicted to be approximately 2,200 ng/g dry mass (based on equations in

Sparling and Fellers [21]), which is over three orders of magnitude greater than the maximum chlorpyrifos concentration found in tadpoles in the present study. Moreover, the maximum chlorpyrifos concentration tested for effects on ChE in *P. sierra* (200 μg/L [21]), which resulted in a reduction in ChE of approximately 25%, is at least five orders of magnitude greater than chlorpyrifos concentrations measured in surface water and snow at high elevation in the southern Sierra Nevada [9,10,14]. The finding of lower ChE levels associated with *Rana* population declines in central California by Sparling et al. [7] differs from results in the present study, perhaps because the Sparling et al. study spanned a much larger area with sites closer to areas of pesticide application and included few if any sites at the high elevations represented in the present study.

The cause for the over twofold variation in ChE level among sites in the present study is not evident. Tadpole developmental stage, which varied among sites, had minimal effects on ChE. Environmental temperature can affect ChE in *P. sierra* [32], but a large effect resulting from temperature differences seems unlikely given the narrow elevation range among sites and the finding that ChE showed no relationship with elevation.

Few data are available for toxicity of pesticide concentrations in tissue, sediment, and air for amphibians. However, the measured concentrations in *P. sierra* tadpoles for chlorpyrifos (0.7 ng/g dry mass [8]) and the three endosulfan compounds (total 5 ng/g dry mass [8]) are many orders of magnitude lower than the tissue concentrations known to be toxic for this species [21].

Geographic distribution of remaining Rana *populations*

The most striking finding of the present study is the significant positive association between *Rana* spp. population status in 1997 to 2002 and linear distance from the San Joaquin Valley (also evident in Fig. 1 in Davidson and Knapp [13]). The *Rana* spp. distribution described by the 1997 to 2002 sampling might have resulted from a generally west-to-east pattern of population declines across the high-elevation portions of Sequoia and Kings Canyon National Parks. The earliest reported declines occurred in 1978 and 1979 in area I on the western side of the Parks [38,39]. Declines reported by 1990 were prevalent throughout the western half of the parks [22] and declines reported in the 2000s occurred on the eastern side of the parks [4,5]. It is unclear how airborne pesticides could be responsible for a west-to-east sequence of population declines across the high-elevation portions of the Parks given that little support exists for any geographic pattern for pesticide concentrations in this area [8]. Moreover, pesticide use in general has not increased in California or become more toxic during this time [42]. Specifically, between 1992 and 2005 statistical analyses ''do not indicate a significant trend of increase or decrease in pesticide use'' in California [42].

As an alternative to pesticides, it is plausible that the recently discovered infectious amphibian disease chytridiomycosis has decimated *Rana* populations in a generally west-to-east pattern across Sequoia and Kings Canyon National Parks. The chytridiomycois pathogen, *Batrachochytrium dendrobatidis*, spread among other anurans across central California in a generally west-to-east pattern beginning in the early 1960s [43]. In the vicinity of the Parks, frog specimens collected from the western edge of the Parks in 1975 revealed infection by *B. dendrobatidis* [44]. This time predates the first reported frog population declines within the Parks, which occurred in the late 1970s [38,39]. Since the early 2000s chytridiomycosis in *R. muscosa* and *R. sierrae* has been extensively studied in the southern Sierra Nevada, including following frog populations from the uninfected state through infection and population extinction [4,5]. Thus, chytridiomycosis has been the proximate factor causing population die-offs in many sites in eastern Kings Canyon National Park since the early 2000s [4,5]. Nevertheless, it is possible that pesticide exposure contributed to these infections, because at least the pesticide carbaryl can reduce production of amphibian skin peptides that inhibit *B. dendrobatidis* growth in culture [45].

CONCLUSIONS

Analyses in the present study were limited to nine pesticide compounds, yet hundreds of pesticides have been used in California that potentially could have been transported to the southern Sierra Nevada. Nevertheless, the nine compounds represent a diverse set of pesticides, the study analyzed for many historical- and current-use pesticide compounds, the method detection limits were extremely low, and sampling was conducted in multiple media, at multiple sites, and at multiple times during a period of intensive pesticide application in the adjacent San Joaquin Valley. Results do not support the hypothesis that pesticides have contributed to the population declines of *R. muscosa* and *R. sierrae* in the alpine zone of the southern Sierra Nevada. In particular, no association was found between any pesticide-related metric and population declines. By contrast, the amphibian disease chytridiomycosis has been demonstrated in other studies as the cause for dramatic population declines of many populations of these species in recent years. Moreover, linear distance from the Valley was strongly related to frog population status in the present study, a finding that is consistent with the apparent pattern of spread of chytridiomycosis.

It seems much more likely that effects of windborne pesticides on amphibian populations would be manifested in areas closer to pesticide sources in the San Joaquin Valley than at high elevation in the Sierra Nevada. Studies summarized by Bradford et al. [8] show a general pattern for pesticide concentrations to decrease substantially with distance from the San Joaquin Valley up to approximately 40 km, beyond which elevation is high (i.e., >2750 m) and pesticide concentrations are very low and remain constant or decrease only slightly. Most of the habitat for *R. muscosa* and *R. sierrae* in the southern Sierra Nevada is at high elevation and more than 40 km away from the San Joaquin Valley. For several other amphibian species occurring closer to sources of pesticides, however, the associations between upwind pesticide use and population declines reported by others [12] may indeed be real. Consistent with this idea, water samples from lower elevation in the Sierra Nevada contained endosulfan concentrations within the range of lethality for *Rana boylii*, a frog that has experienced substantial population declines in the Sierra Nevada [21].

SUPPLEMENTAL DATA

Supplemental Data. Analysis of covariance for effects of tadpole developmental stage on cholinesterase activity. (59 KB PDF)

Acknowledgement—We are very grateful to Joanna Christion and Amanda Marusich of Sequoia and Kings Canyon National Parks (SEKI) for meticulously conducting much of the field sampling and to Harold Werner, Danny Boiano, and Annie Esperanza also of SEKI for facilitating the project in many ways. We also thank Hassan Basagic, Chad Cross, Rebecca Rising, Krystal Ward, Carrie Vernon, and other members of the SEKI helicopter crew for considerable help. We are also grateful to Carlos Davidson for discussing the project, Carlos Davidson and Vance Vredenburg for sharing data, and Deborah Chaloud for reviewing the manuscript. James Seiber, Thomas

Cahill, Walter Jarman, and Lara Hansen kindly reviewed the research plan for the project. The research described herein was funded by the U.S. Environmental Protection Agency (U.S. EPA) through Interagency Agreement DW14989008 with the National Park Service and by the U.S. National Institute of Environmental Health Sciences (grant P30ES00210). The article has been approved for publication by the U.S. EPA.

REFERENCES

1. Lannoom E, ed. 2005. *Amphibian Declines: The Conservation Status of United States Species*. University of California Press, Berkeley, CA, USA.
2. Vredenburg VT, Bingham R, Knapp R, Morgan JAT, Moritz C, Wake D. 2007. Concordant molecular and phenotypic data delineate new taxonomy and conservation priorities for the endangered mountain yellow-legged frog. *J Zool* 271:361–374.
3. Knapp RA, Matthews KR. 2000. Non-native fish introductions and the decline of the mountain yellow-legged frog from within protected areas. *Conserv Biol* 14:428–438.
4. Rachowicz LJ, Knapp RA, Morgan JAT, Stice MJ, Vredenburg VT, Parker JM, Briggs CJ. 2006. Emerging infectious disease as a proximate cause of amphibian mass mortality. *Ecology* 87:1671–1683.
5. Vredenburg VT, Knapp RA, Tunstall TS, Briggs CJ. 2010. Dynamics of an emerging disease drive large-scale amphibian population extinctions. *Proc Natl Acad Sci U S A* 107:9689–9694.
6. LeNoir JS, McConnell LL, Fellers GM, Cahill TM, Seiber JN. 1999. Summertime transport of current-use pesticides from California's Central Valley to the Sierra Nevada mountain range, USA. *Environ Toxicol Chem* 18:2715–2722.
7. Sparling DW, Fellers GM, McConnell LL. 2001. Pesticides and amphibian population declines in California, USA. *Environ Toxicol Chem* 20:1591–1595.
8. Bradford DF, Stanley K, McConnell LL, Tallent-Halsell NG, Nash MS, Simonich SM. 2010. Spatial patterns of atmospherically deposited organic contaminants at high-elevation in the southern Sierra Nevada mountains, California. *Environ Toxicol Chem* 29:1056–1066.
9. Hageman KJ, Hafner WD, Campbell DH, Jaffe DA, Landers DH, Simonich SLM. 2010. Variability in pesticide deposition and source contributions to snowpack in western U.S. national parks. *Environ Sci Technol* 44:4452–4458.
10. Landers DH, Simonich SL, Jaffe DA, Geiser LH, Campbell DH, Schwindt AR, Schreck CB, Kent ML, Hafner WD, Taylor HE, Hageman KJ, Usenko S, Ackerman LK, Schrlau JE, Rose NL, Blett TF, Erwaym M. 2008. The fate, transport, and ecological impacts of airborne contaminants in western national parks (USA). EPA/600/R-07/138. U.S. Environmental Protection Agency, Corvallis, OR.
11. Bradford DF, Heithmar EM, Tallent-Halsell NG, Momplaisir GM, Rosal CG, Varner KE, Nash MS, Riddick LA. 2010. Temporal patterns and sources of atmospherically deposited pesticides in alpine lakes of the Sierra Nevada, California, USA. *Environ Sci Technol* 44:4609–4614.
12. Davidson C. 2004. Declining downwind: Amphibian declines in California and historic pesticide use. *Ecol Appl* 14:1892–1902.
13. Davidson C, Knapp RA. 2007. Multiple stressors and amphibian declines: dual impacts of pesticides and fish on yellow-legged frogs. *Ecol Appl* 17:587–597.
14. Fellers GM, McConnell LL, Pratt D, Datta S. 2004. Pesticides in mountain yellow-legged frogs (*Rana muscosa*) from the Sierra Nevada mountains of California, USA. *Environ Toxicol Chem* 23:2170–2177.
15. Daly GL, Wania F. 2005. Organic contaminants in mountains. *Environ Sci Technol* 39:385–398.
16. Relyea RA, Diecks N. 2008. An unforeseen chain of events: lethal effects of pesticides at sublethal concentrations. *Ecol Appl* 18:1728–1742.
17. Taylor SK, Williams ES, Mills KW. 1999. Effects of malathion on disease susceptibility in Woodhouse's toad. *J Wildl Dis* 35:536–541.
18. Hayes TB, Case P, Chui S, Chung D, Haefele C, Haston K, Lee M, Pheng Mai V, Marjuoa Y, Parker J, Tsui M. 2006. Pesticide mixtures, endocrine disruption, and amphibian declines: are we underestimating the impact? *Environ Health Perspect* 114:40–50.
19. Rohr JR, Sager T, Sesterhenn TM, Palmer BD. 2006. Exposure, postexposure, and density-mediated effects of atrazine on amphibians: breaking down net effects into their parts. *Environ Health Perspect* 114:46–50.
20. Sparling DW, Fellers GM. 2007. Comparative toxicity of chlorpyrifos, diazinon, malathion and their oxon derivatives to larval *Rana boylii*. *Environ Pollut* 147:535–539.
21. Sparling DW, Fellers GM. 2009. Toxicity of two insecticides to California, USA, anurans and its relevance to declining amphibian populations. *Environ Toxicol Chem* 28:1696–1703.
22. Bradford DF, Graber DM, Tabatabai F. 1994. Population declines of the native frog, *Rana muscosa*, in Sequoia and Kings Canyon National Parks, California. *Southwest Nat* 39:323–327.
23. Bradford DF, Cooper SD, Jenkins TM, Kratz K Jr, Sarnelle O, Brown AD. 1998. Influences of natural acidity and introduced fish on faunal assemblages in California alpine lakes. *Can J Fish Aquat Sci* 55:2478–2491.
24. Adamsm J, Hosack BR, Knapp RA, Corn PS, Diamond SA, Trenham PC, Fagre DB. 2005. Distribution patterns of lentic-breeding amphibians in relation to ultraviolet radiation exposure in western North America. *Ecosystems* 8:488–500.
25. Fellers GM, Bradford DF, Pratt D, Wood LL. 2007. Demise of repatriated populations of mountain yellow-legged frogs (*Rana muscosa*) in the Sierra Nevada of California. *Herpetol Conserv Biol* 2:5–21.
26. Cahill TC, Carroll JJ, Campbell D, Gill TE. 1996. Air quality. Sierra Nevada Ecosystem Project: Final report to Congress, Vol II—Assessments and scientific basis for management options. Centers for Water and Wildland Resources. University of California, Davis, CA, USA, pp 1227–1261.
27. Knapp RA, Matthews KR, Preisler HK, Jellison R. 2003. Developing probabilistic models to predict amphibian site occupancy in a patchy landscape. *Ecol Appl* 13:1069–1082.
28. Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183–190.
29. Antweiler RC, Taylor HE. 2008. Evaluation of statistical treatments of left-censored environmental data using coincident uncensored data sets: I. Summary statistics. *Environ Sci Technol* 42:3732–3738.
30. Ellmann GL, Courtney KD, Andres V Jr, Featherstone RM. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 7:88–95.
31. Ewell DM, Flocchini RG, Myrup LO, Cahill TM. 1989. Aerosol transport in the southern Sierra Nevada. *J Applmeteorol* 28:112–125.
32. Johnson CS, Schwarzbach SE, Henderson JD, Wilson BW, Tjeerdema RS. 2005. Influence of water temperature on acetylcholinesterase activity in the Pacific tree frog (*Hyla regilla*). *Environ Toxicol Chem* 24:2074–2077.
33. Berry WD, Felman S. 1985. *Multiple Regression in Practice*. Sage Publications, Beverly Hills, CA, USA.
34. Allison PD. 1999. *Logistic Regression Using the SAS System: Theory and Application*. SAS, Carey, NC, USA.
35. Racke KD. 1993. Environmental fate of chlorpyrifos. *Rev Environ Contam Toxicol* 131:1–150.
36. Caballo-Lopez A, Luque de Catro MD. 2006. Gas chromatography-electron capture detection determination of dacthal and its di-acid metabolite in soil after ultrasound-assisted extraction and in situ focused microwave-assisted derivatization. *Anal Bioanal Chem* 386: 341–345.
37. Wan MT, Kuo J-N, Buday C, Schroeder G, Aggelen GV, Pasternak J. 2005. Toxicity of α-, β-, and (α+β)-endosulfan and their formulated and degradation products to *Daphnia magna, Hyalella azteca, Onchorhynchusmykiss, Onchorhynchus kisutch*, and biological implications in streams. *Environ Toxicol Chem* 24:1146–1154.
38. Bradford DF. 1983. Winterkill, oxygen relations and energy metabolism of a submerged dormant amphibian, *Rana muscosa*. *Ecology* 64:1171–1183.
39. Bradford DF. 1991. Mass mortality and extinction in a high-elevation population of *Rana muscosa*. *J Herpetol* 25:174–177.
40. Hill EF, Fleming WJ. 1982. Anticholinesterase poisoning of birds: Field monitoring and diagnosis of acute poisoning. *Environ Toxicol Chem* 1:27–38.
41. Rosenbaum EA, Caballero de Castro, Gauna A, Pechen L, de D'Angelo AM. 1988. Early biochemical changes produced by malathion on toad embryos. *Arch Environ Contam Toxicol* 17:831–835.
42. California Department of Pesticide Regulation. 2006. Summary of pesticide use report data; 2005; indexed by chemical. California Department of Pesticide Regulation, Sacramento, CA, USA.
43. Padgett-Flohr GE, Hopkins RLI. 2009. *Batrachochytrium dendrobatidis*, a novel pathogen approaching endemism in central California. *Dis Aquat Org* 83:1–9.
44. Wake DB, Vredenburg VT. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proc Natl Acad Sci U S A* 105:11466–11473.
45. Davidson C, Benard MF, Shaffer HB, Parker JM, O'Leary C, Conlon JM, Rollins-Smith LA. 2007. Effects of chytrid and carbaryl exposure on survival, growth and skin peptide defenses in foothill yellow-legged frogs. *Environ Sci Technol* 41:1771–1776.