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POLYCHLORINATED BIPHENYLS AND TOXAPHENE IN PACIFIC TREE FROG TADPOLES (*HYLA REGILLA*) FROM THE CALIFORNIA SIERRA NEVADA, USAJEFFREY E. ANGERMANN,[†] GARY M. FELLERS,[‡] and FUMIO MATSUMURA*[†][†]Department of Environmental Toxicology, University of California, Davis, California 95616, USA[‡]U.S. Geological Survey, Western Ecological Research Center, Point Reyes National Seashore, Point Reyes, California 94956

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Abstract—Pacific tree frog (*Hyla regilla*) tadpoles were collected throughout the Sierra Nevada mountain range, California, USA, in 1996 and 1997 and analyzed for the presence of polychlorinated biphenyls (PCBs) and toxaphene. Whole-tadpole Σ PCB levels ranged from 244 ng/g (wet wt) at lower elevations on the western slope to 1.6 ng/g high on the eastern slope, whereas Σ toxaphene levels ranged from 15.6 to 1.5 ng/g. Linear regression of PCB and toxaphene residue levels versus elevation indicated a significant relationship, with an r^2 value of 0.33 for PCB and 0.45 for toxaphene indicating a significant elevation effect on PCB and toxaphene bioaccumulation in Sierra Nevada *H. regilla*. Tadpole samples from sites in east-facing versus west-facing drainage basins showed significant differences in PCB and toxaphene residue levels, suggesting the possibility of a rain-shadow effect in the long-range atmospheric transport of these contaminants to the Sierra Nevada Mountains.

Keywords—Polychlorinated biphenyls Toxaphene Sierra Nevada Pacific tree frog Principal components analysis

INTRODUCTION

Nine species of anuran frogs and toads are indigenous to the foothills and higher elevations of California's Sierra Nevada mountain range (USA) [1]. All but two of these nine species are currently listed as threatened, endangered, or of special concern [2]. One of these species, the mountain yellow-legged frog (*Rana muscosa*), is indigenous at moderate to high elevations throughout the Sierra Nevada and the transverse southern California ranges, and it has been in decline throughout the last 20 to 30 years [3,4]. Much of its historic habitat lies within remote regions of the John Muir Wilderness and Kings Canyon and Sequoia National Parks in the central and southern portions of California's Sierra Nevada range. Various potential causes have been advanced in an attempt to explain these declines [3,5–8], including organophosphate insecticides [9], but the potential effects of pesticides and other anthropogenic contaminants on high-elevation species have not been fully addressed.

Several studies have established the atmospheric transport of anthropogenic compounds from California's Central Valley and subsequent deposition of these pollutants in Sierra Nevada watersheds [10–16]. This avenue of transport arises from a unique geographical attribute of northern California, in which offshore winds are channeled through gaps in California's Coastal range and flow eastward through the San Francisco Bay area and other major metropolitan centers, picking up pollutants en route. When these air masses reach the Central Valley, they are enriched with pollutants of agricultural origin and, ultimately, disseminate these wind-borne residues throughout the Central Valley as well as into the Sierra Nevada Mountains [17,18].

From an ecotoxicological viewpoint, assessing the atmospheric transport of organochlorine insecticides and polychlorinated biphenyls (PCBs) to the Sierra Nevada range is partic-

ularly interesting because of the persistence of these compounds in environmental media and their demonstrated biological effects. Toxaphene is a multicomponent organochlorine insecticide that was applied heavily in the Central Valley during the 1960s and 1970s (before its ban in 1982), whereas PCBs continue to be released to the environment via leakage and disposal of electrical transformers and capacitors as well as by volatilization from contaminated water bodies and soil. The U.S. Environmental Protection Agency (EPA) estimates that 52,779 kg of PCBs were released to land in California during 1990 alone [19].

To investigate the potential for PCB bioaccumulation in native Sierra Nevada amphibians, we analyzed tadpoles of the Pacific tree frog (*Hyla regilla*) as a surrogate for species in decline throughout the Sierras. *Hyla regilla* is still widely distributed throughout the Sierra Nevada range and, at present, appears to be stable or declining [2,4]. Here, we report the analysis of tadpoles collected from sites throughout the Sierra Nevada range for the presence of toxaphene and PCBs. A variety of sites were chosen, ranging from 610 m above sea level on the western slope to 3353 m above sea level on the eastern slope, to investigate the effect of increasing elevation in the Sierra Nevada range on PCB and toxaphene distribution and subsequent bioaccumulation in tadpoles.

MATERIALS AND METHODS

Sampling

Hyla regilla tadpoles (Gosner stages 25–41 [20]) were collected by dip net from various locations throughout the Sierra Nevada Mountains. A total of 21 sites were sampled and analyzed, with an average pool number of three tadpoles per site. Tadpoles collected from backcountry sites were transported (travel time <3 h) live to the trailhead in Nalgene bottles (Nalgene, Milwaukee, WI, USA), where they were transferred to Nalgene cryovials and then frozen and maintained in liquid nitrogen. Tadpoles collected at car-accessible sites were trans-

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ferred to cryovials and directly frozen in liquid nitrogen before they were able to void gut contents; the time interval between tadpole collection and freezing in nitrogen did not vary by more than 4 h among all samples. On returning to the laboratory, the samples were stored at -80°C until analysis.

Residue analysis

Whole tadpoles from each site were pooled (in triplicate) to achieve a sample weight of 2.5 to 3.5 g, digested in 2 ml of acetic:perchloric acid (1:1 v/v) for 2 h in a 65°C water bath (sufficient to homogenize the tissue), and then extracted with 2 ml of *n*-hexane. The homogenate was re-extracted, and the combined extracts were digested with 2 ml of sulfuric acid to precipitate lipids. The hexane fraction was washed with 2 ml of NaCl and dried with sodium sulfate before silica gel column chromatography. A 200- μl subsample of each pool was reduced to dryness under nitrogen for gravimetric determination of lipids (mean = $0.58 \pm 0.33\%$, range = 0.21–1.72, $n = 63$).

The hexane fraction was applied to a preconditioned, 0.6- \times 5-cm silica gel column (60 Å, 200–425 mesh, activated at 180°C and rinsed with 3 ml of *n*-hexane; Fisher Scientific, Pittsburgh, PA, USA) and eluted first with 3.2 ml of 0.3% (v/v) benzene/*n*-hexane to yield the PCB-containing fraction and then with 3.0 ml of 25% (v/v) ethyl ether/*n*-hexane to yield the toxaphene-containing fraction. Each silica gel fraction was redried with sodium sulfate, reduced in volume to approximately 0.5 ml under a gentle stream of nitrogen, and transferred to amber, 1-ml sample vials (National Scientific, Lawrenceville, GA, USA). The fractions were then reduced to dryness and brought up in 100 μl of iso-octane containing 25 ppb of decachlorobiphenyl (Axact Standards, Commack, NY, USA) as the internal standard. All chemicals were supplied by Fisher Scientific.

Two microliters of each final extract were quantified by capillary gas chromatography on a Hewlett-Packard 6890 II (Avondale, PA, USA) equipped with a Restek RTX-5MS column (0.25 mm \times 0.25 μm \times 60 m; Restek, Bellefonte, PA, USA), splitless injection, and an electron-capture detector. The gas chromatographic conditions were as follows: Injection port, 250°C ; detector, 310°C ; helium carrier gas, 1.4 ml/min; and nitrogen makeup gas, 60.0 ml/min. The oven temperature was held at 75°C for 1 min, then increased to 200°C at $30^{\circ}\text{C}/\text{min}$ and held for 30 min; the second temperature ramp increased the temperature at $2^{\circ}\text{C}/\text{min}$ to 270°C and held it there for 10 min. A maximum of 101 PCB congeners were quantified using the Lake Michigan Mass Balance study (U.S. EPA Large Lakes Research Station, Grosse Ile, MI) three-Aroclor® (Monsanto, St. Louis, MO, USA) reference mixture (1232:1248:1262, 75:54:54 $\mu\text{g}/\text{ml}$) at a total concentration of 183 $\mu\text{g}/\text{ml}$. Toxaphene was quantified using the technical standard (U.S. EPA Research Center, Triangle Park, NC). Coeluting organochlorines in the toxaphene fraction were matched to separate standards and subtracted from the peak area total. After these coelutants were subtracted, an average of 16 congeners per sample (range = 10–23) matched to the toxaphene technical standard. Matrix spike-recoveries were performed for each extraction batch. Method recovery of PCBs, using Aroclor 1260 as a surrogate, was 93.8%, whereas method recovery of toxaphene technical standard was 94.7%. Method blanks were analyzed and subtracted for each sample batch and incorporated transported field water from Nalgene bottles where appropriate. Method detection limit, reported as the sum of components in each standard mixture and defined as three times

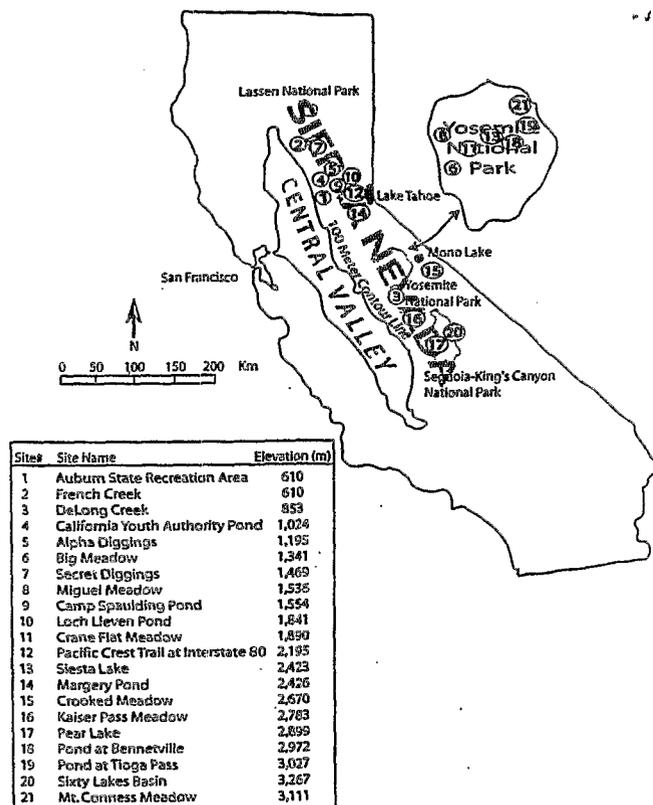


Fig. 1. Locations of sampling sites in the California's Sierra Nevada range (USA).

the matrix spike-background value for the least responsive component in the standard mixture, was 2 pg for Aroclor 1260 and 4 pg for the toxaphene technical standard. Data were not recovery-corrected or lipid-corrected.

Statistics/data analysis

To determine linear distances from each collection site to California's Central Valley, site coordinates were mapped using digitized 7.5' and 15' U.S. Geological Society topographic quads (National Geographic TOPO! Maps, San Francisco, CA, USA). For the purposes of the present study, the perimeter of the Central Valley was defined as the nearest 100-m contour line to the center of the valley, and the line-of-sight distance from each site to this contour was calculated at constant latitude.

Results were analyzed by linear stepwise multiple regression and analysis of variance to assess the effects of elevation, latitude, Central Valley-to-site (CVTS) distance, and site drainage aspect on PCB and toxaphene residue levels. Collinearity among continuous variables was tested by calculating Pearson's correlation coefficient. The PCB congener profile differences among sites were compared by principal components analysis (PCA) using initial factor solutions, and results were represented in the form of unrotated factor plots. All statistical analyses were performed with Statview software (SAS® Institute, Cary, NC, USA).

RESULTS

Locations of tadpole collection sites are depicted in Figure 1, and results of residue analyses are presented in Table 1. The Σ toxaphene residue levels in tadpoles from study sites ranged from 1.5 to 15.6 ng/g, whereas Σ PCB residue levels ranged from 1.6 to 243.8 ng/g (Table 1). Both toxaphene and PCB

Table 1. Σ Polychlorinated biphenyl and Σ toxaphene concentrations in *Hyla regilla* tadpoles from the Sierra Nevada (CA, USA)

Site	Site location ^a	Site designator	Latitude (DD) ^b	Longitude (DD) ^b	Elevation (m)	Dis- tance to valley (km) ^c	Drain- age aspect	Date collected	Mean (SE) ^d	
									Σ PCB (ng/g)	Σ toxaphene (ng/g)
1	Auburn State Recreation Area	ASR	38.9469	120.9761	610	22.0	West	6/26/1997	243.75 (36.37)	15.62 (6.79)
2	French Creek	FC	39.7289	121.3508	610	36.3	West	7/11/1996	12.26 (0.62)	3.88 (0.58)
3	DeLong Creek	DLC	37.4675	119.7972	853	56.1	West	6/23/1997	33.91 (1.84)	10.03 (0.67)
4	California Youth Authority Pond	CYA	39.3211	120.9275	1,024	44.2	West	5/29/1997	12.02 (5.78)	7.96 (0.79)
5	Alpha Diggings	AD	39.3339	120.7783	1,195	56.3	West	6/1/1997	14.51 (7.42)	8.60 (0.56)
6	Big Meadow	BM	37.7022	119.7408	1,341	66.7	West	6/22/1997	22.15 (0.21)	2.37 (0.61)
7	Secret Diggings	SD	39.6628	120.9767	1,469	62.6	West	7/24/1996	22.97 (1.18)	5.76 (0.47)
8	Miguel Meadow	MM	37.9542	119.8378	1,536	84.7	West	7/10/1997	6.75 (0.57)	3.81 (0.32)
9	Camp Spaulding Pond	CSP	39.2358	120.7092	1,554	62.9	West	6/27/1997	35.28 (2.07)	9.15 (4.98)
10	Loch Lleven Pond	LLP	39.2994	120.4969	1,841	83.0	West	6/27/1997	18.98 (2.53)	ND ^e
11	Crane Flat Meadow	CFM	37.7575	119.8022	1,890	79.2	West	6/24/1997	10.31 (1.95)	2.05 (0.46)
12	Pacific Crest Trail at I-80	PCT	39.3414	120.3264	2,195	96.9	East	7/14/1997	ND	4.72 (0.62)
13	Siesta Lake	SL	37.8503	119.6597	2,423	93.2	West	7/4/1997	22.77 (4.41)	ND
14	Margery Pond	MP	38.8622	120.1269	2,426	99.2	West	8/1/1997	20.89 (1.89)	ND
15	Crooked Meadow	CM	37.8244	118.8456	2,670	170.0	East	7/13/1997	14.70 (2.57)	ND
16	Kaiser Pass Meadow	KPM	37.2958	119.1050	2,783	97.6	West	9/8/1996	1.57 (0.81)	2.88 (0.47)
17	Pear Lake	TBL	36.6031	118.6622	2,899	82.7	West	8/18/1996	5.69 (0.64)	2.02 (0.25)
18	Pond at Bennettville	PB	37.9308	119.2503	2,972	134.7	East	7/21/1997	7.97 (1.45)	3.35 (0.57)
19	Pond at Tioga Pass	TPR	37.9081	119.2550	3,027	134.2	East	7/20/1997	4.36 (1.44)	2.28 (0.18)
20	Sixty Lakes Basin	SLB	36.8138	118.4199	3,267	120.0	East	9/24/1996	2.55 (0.35)	1.47 (0.09)
21	Mt. Conness Meadow	MCM	37.9601	119.2964	3,111	132.9	East	7/18/1996	4.21 (0.28)	2.23 (0.15)

^a All locations are within the California Sierra Nevada range.

^b DD = decimal degrees.

^c Measured from site to nearest equidistant 100-m contour line in Central Valley.

^d Concentrations expressed on a wet-weight basis. SE = standard error.

^e ND = no data.

levels generally diminished with increasing elevation; in stepwise linear regression, site elevation emerged as a significant independent variable for predicting both Σ PCB (standard coefficient = -0.571 , $r^2 = 0.34$) and Σ toxaphene (standard coefficient = -0.671 , $r^2 = 0.48$) residue levels (Table 2).

Site 1 (Auburn State Recreation Area), which is located near a large metropolitan area, was excluded from the regression and statistical analyses because of the abnormally high PCB residue values encountered in tadpoles collected from that site. Neither latitude nor CVTS distance correlated with PCB or toxaphene residues in multiple stepwise regression analysis, yet both were significant when regressed independently against Σ toxaphene residue levels (Table 2).

Tadpole collection sites were grouped by assignment to either east-facing or west-facing catchment basins, derived by

inspection of contour features and drainage patterns on U.S. Geological Survey 7.5' topographic quadrangles. A comparison of sites designated within east-facing catchment basins with sites designated within west-facing catchment basins (Table 2) yielded significantly different means by analysis of variance for both Σ PCB ($p = 0.050$) and Σ toxaphene ($p = 0.040$).

Principal components analyses of congener distribution differences among sites are depicted in Figures 2 and 3. A plot of factors 1 and 2 demonstrates the association of western-slope sites Miguel Meadow (MM), DeLong Creek (DLC), and Secret Diggings (SD), whereas east-facing sites Sixty Lakes Basin (SLB) and Pond at Bennettville (PB) (CA, USA) are distinctly separated (Fig. 2a). A separate plot of factors 2 and 3 also demonstrates high loadings for PB and SLB (Fig. 2b),

Table 2. Analysis of variance (ANOVA) and regression summary for polychlorinated biphenyl (PCB) and toxaphene residues

Source of variation	Contaminant	df ^a	Univariate regression		Multiple regression	
			p	r ²	SC ^b	r ²
ANOVA: east-west drainage aspect ^c	Σ PCB	18	0.050 ^d	—	—	—
	Σ Toxaphene	15	0.040 ^d	—	—	—
Elevation gradient	Σ PCB	18	0.0065 ^d	0.33	-0.571	0.34
	Σ Toxaphene	15	0.0047 ^d	0.45	-0.671	0.48
Latitude (UTME) ^e	Σ PCB	18	0.24	0.08	—	0.34
	Σ Toxaphene	15	0.011 ^d	0.38	—	0.48
Central Valley-to-site distance (km)	Σ PCB	18	0.059	0.19	—	0.34
	Σ Toxaphene	15	0.011 ^d	0.38	—	0.48

^a df = Degrees of freedom.

^b SC = standard coefficient.

^c Each site assigned to either a west-facing or east-facing drainage basin.

^d Significant at $p \leq 0.05$.

^e UTME = universal transverse mercator.

^f Variables did not meet model-entry criteria.

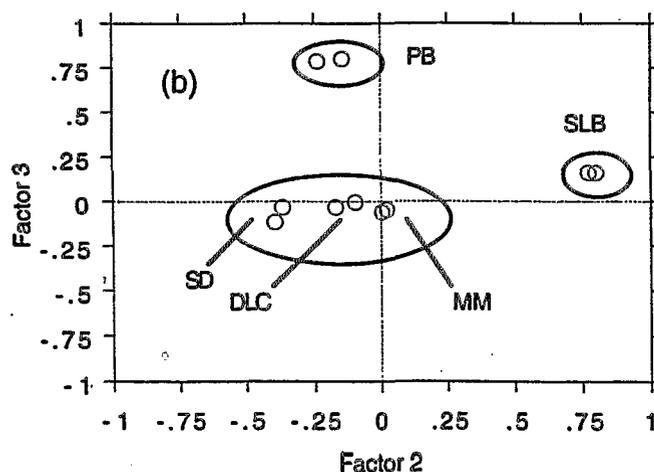
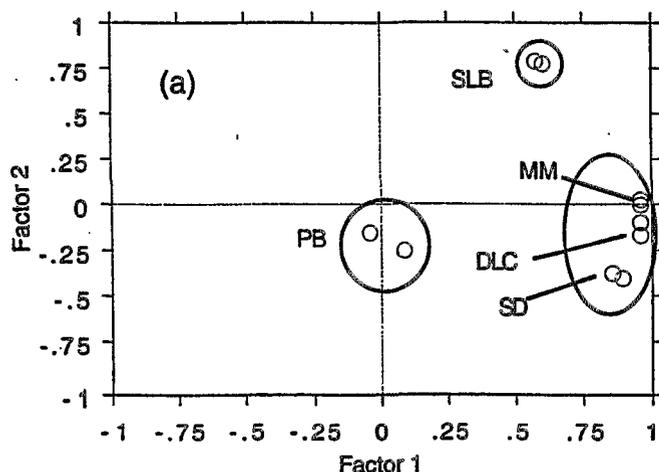


Fig. 2. Principal components unrotated factor plots showing differences in polychlorinated biphenyl congener profiles between east-facing (SLB and PB) and west-facing (SD, DLC, and MM) tadpole collection sites using (a) the first and second extracted factors and (b) the second and third factors. Ellipses represent interpreted site groupings. See Table 1 for definitions.

indicating that these factor solutions repeatedly identify characteristics that differentiate the western-slope from the eastern-slope sites. In Figure 3a, southern sites SLB and Pear Lake (TBL) are highly associated with factor 2, whereas PB is more closely associated with factor 1. Figure 3b further shows the distinction between SLB/TBL and other sites in the analysis, which are not closely associated with either factor 2 or factor 4.

The PCB congener-specific data for certain sites were organized by PCB homologue class (PCBs in a given homologue class all have the same number of chlorine substituents), and groupings of homologue classes 3+4, 5+6, and 7+8 were compared between sites (Fig. 4). Because of the paucity of data, homologue classes 0, 1, 2, and 9 were not included in the comparison groupings. West-facing sites SD, California Youth Authority Pond (CYA), and Alpha Diggings (AD) at the northern end of the sampling range, in the northern Sierra Nevada mountains, had relatively high proportions of low and moderately chlorinated congeners compared to sites in the central Sierra Nevada mountains and at the southern end of the sampling range: SLB, Mount Conness Meadow (MCM), Big Meadow (BM), and Crane Flat Meadow (CFM) (CA, USA).

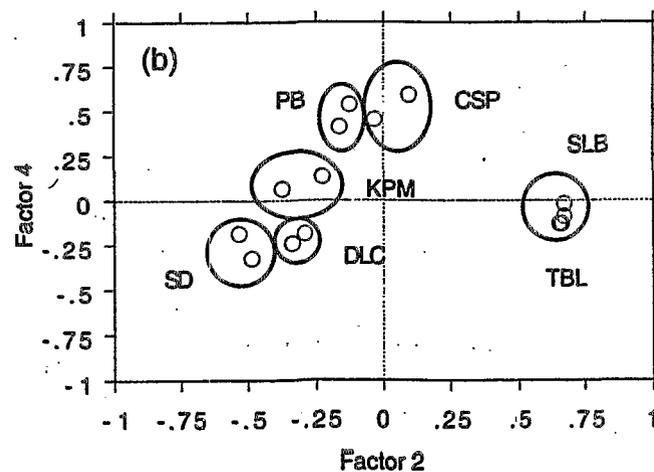
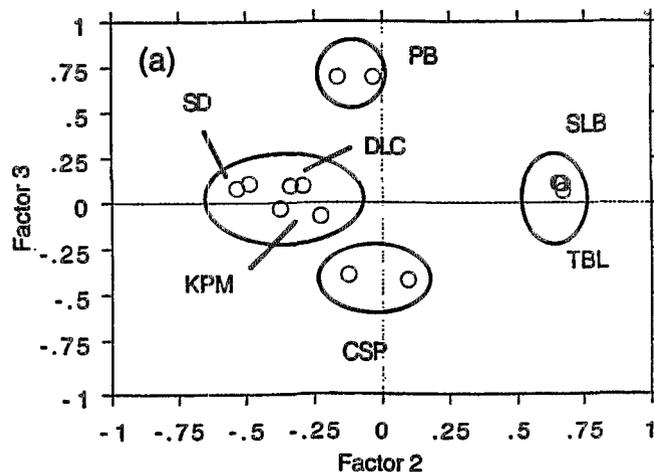


Fig. 3. Principal components unrotated factor plots showing differences in polychlorinated biphenyl congener profiles between northern (SD, DLC, and CSP) and southern (SLB and TBL) tadpole collection sites using (a) the second and third extracted factors and (b) the second and fourth factors. See Table 1 for definitions.

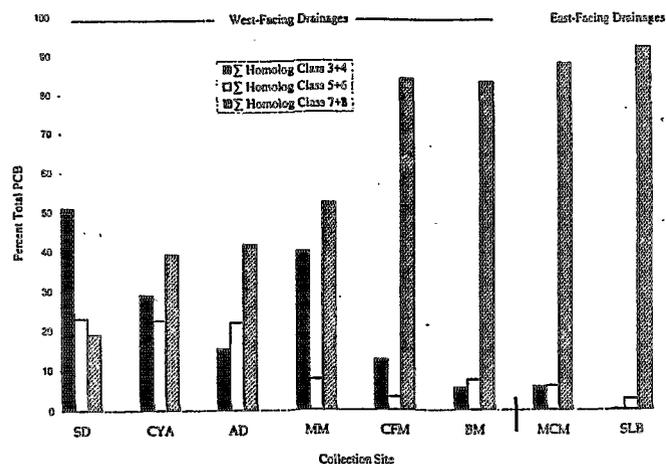


Fig. 4. Polychlorinated biphenyl (PCB) congener homologue class distributions, emphasizing differences in distributions among tadpoles collected from east-facing versus west-facing sites. Homologue classes 0, 1, 2, and 9 were omitted from the analysis because of marginal concentrations of congeners from these classes. See Table 1 for definitions.

Two of the sites located in east-facing drainage basins (SLB and MCM) also had congener distributions that were skewed toward higher-molecular-weight PCBs when compared to sites in west-facing drainage basins (SD, CYA, AD, BM, CFM, and MM).

DISCUSSION

The presence of toxaphene and PCB at high elevations combined with the lack of discernible sources of these pollutants within the Sierra Nevada range suggests air transport from the Central Valley, as well as from other developed regions in California, as a likely source of these contaminants. The diminution of total toxaphene and PCB residue levels with increasing elevation on the western slope (Table 2) is consistent with the findings of Zabik and Seiber [14], who established that levels of organophosphate insecticides in the Sierra Nevada foothills and mountains diminished with increasing elevation and distance from the Central Valley, and also with the findings of Datta et al. [11] who noted a 10-fold decrease in PCB levels between tadpoles collected at sea level in the Central Valley and those collected at 610-m elevation in Sequoia National Park in the Sierra Nevada range. Cory et al. [10] examined 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethene (*p,p'*-DDE) residue levels in fat bodies of adult *R. muscosa* collected from various locations in the Sierra Nevada range and found that *p,p'*-DDE levels in frogs collected from sites lying to the east of the Sierra crest (i.e., the line connecting the highest peaks in the range) were significantly lower than levels in frogs collected from west of the divide. The PCB and toxaphene residue level comparisons between east-facing and west-facing drainage basin-associated sites (Table 2) are in agreement with these findings and support a rain-shadow model of residue deposition in mountainous zones: As air masses travel eastward up the western slope of the Sierra Nevada range, orographic cooling and condensation results in larger levels of rainfall at higher elevations, leaving air masses depleted of moisture (and contaminants sorbed to raindrop-nucleating particulates) by the time these air masses reach the Sierra crest [21]. This model, however, does not account for dry deposition, which may be a seasonally significant route of contaminant deposition. During the summer and fall, dry deposition may also result in the preferential fallout of higher-molecular-weight PCBs, which are more likely to adsorb to particulate matter than lower-molecular-weight PCBs because of their lower equilibrium vapor pressures [22].

Toxaphene residue levels exhibited significant latitudinal and longitudinal variability, whereas PCB residue levels did not (Table 2). The variation in toxaphene levels with respect to latitude can be seen in higher average residue levels at sites in the northern portion of the sampling range compared to sites in the southern Sierra Nevada range. Even though historical application records [23] show that toxaphene was applied much more heavily in the (southern) San Joaquin Valley than in the (northern) Sacramento Valley, the incidence of down-valley or downslope/transitional airflows is much higher throughout the year in the San Joaquin Valley compared to the Sacramento Valley [17], potentially mitigating the relative extent of upslope transport of pollutants in the southern Sierra Nevada range.

The inverse correlation between CVTS distance and toxaphene levels (Table 2) tends to support the contention that toxaphene residues originated in California's Central Valley. On the other hand, the lack of correlation between CVTS

distance and PCB burdens suggests that sources of environmental PCBs in the Sierra Nevada mountains are more diffuse and may be contributed to by larger-scale circulation of PCBs in addition to regional sources in California's metropolitan regions.

Blais et al. [24] report that the concentrations of organochlorines in snowpack samples taken from between 0- and 1,000-m elevation in western Canada were positively correlated with site elevation for more volatile pesticides and lower-molecular-weight PCB homologue classes, but not for higher-molecular-weight PCB homologues. As the study addressed only organochlorines found in an abiotic matrix (i.e., snow), the observed correlations between concentrations and site elevation are consistent with the global fractionation/cold-condensation hypothesis espoused by Wania and Mackay [25]. The inverse relationship between organochlorine vapor pressure and deposition concentration noted by Blais et al. [24] does not seem to hold for the present study, however, as evidenced by the predominance of PCB homologue classes 7+8 at most sites, including those at high elevation (Fig. 4). Several reasons for this discrepancy are possible. First, the temperature regimes at the higher latitudes of the Canadian Rockies may be significantly colder, on average, than those encountered at similar elevations in the Sierra Nevadas, resulting in enhanced atmospheric deposition of low-molecular-weight congeners relative to equivalent elevations in the Sierra Nevadas. Because the given temperature regime at a particular elevation in the Sierra Nevadas would be found at a lower elevation in the Canadian Rockies, the atmospheric deposition of higher-molecular-weight PCBs would be limited to lower elevations in high-latitude locations, such as the Canadian Rockies. Second, the proximity of sampling sites to (regional) industrial and agricultural sources of organochlorines may differ greatly between the Canadian Rockies and the Sierra Nevadas. Any such source-to-site distance differences would affect the potential transport of the more heavily chlorinated PCBs, because these congeners have shorter atmospheric residence times than lesser-chlorinated PCBs because of lower vapor pressures and an increased degree of association with particulate matter that is subject to both wet and dry deposition [26]. Third, PCB congener distributions in fish are skewed toward higher-molecular-weight congeners compared to water, snow, and air congener distributions from an equivalent elevation [12] because of the enhanced relative bioaccumulation potential and degradation/biotransformation resistance of high-molecular-weight congeners [27,28]. Therefore, it may be difficult or misleading to compare PCB congener profiles in aquatic biota with profiles from wet-deposition samples.

Principal components analysis aids in identifying similarities in congener distribution patterns among sampling sites. The number of factors identified in a PCA is roughly proportional to the number of variables that bear on PCB concentration and congener distribution, whereas the factor score is proportional to the degree of association between the sample and the variable. Samples (i.e., sites) that group together on a factor plot have roughly the same degree of association with the variable represented by that factor. In Figure 2, the factor groupings for MM, DLC, and SD indicate similarities in congener profiles among these sites. Both SLB and PB, however, are less closely identified with factors 1 and 3, which may indicate that these factor solutions describe one or more variables particular to MM, DLC, and SD. Possibilities for these variables include drainage basin aspect, elevation, and CVTS

distance. Figure 3a shows that factors 2, 3, and 4 all describe similarities between SLB and TBL, which may be related to the southern location of these sites relative to SD, PB, Camp Spaulding Pond (CSP), DLC, and Kaiser Pass-Meadow (KPM). Factor 3 also describes some variability among the latter grouping of sites and, in particular, distinguishes PB and CSP from SD, KPM, and DLC (Fig. 2a). This distinction may be related to variability in PCB burdens, because PCB concentrations in tadpoles from CSP were relatively high (35.28 ± 2.07 ng/g) (Table 1) and those from PB were relatively low (7.97 ± 1.45 ng/g).

The consequences of chronic PCB exposure to western ranid and hylid frogs are unknown. A limited number of studies have investigated the acute toxicities and monooxygenase-inducing abilities of PCBs in ranid and bufonid frogs [29,30], whereas a larger number of studies have addressed the acute toxicity of toxaphene in various frog species [31–33]. Because toxicity endpoints in these studies are often limited to lethality and/or behavioral anomalies arising from acute laboratory treatments at ambient temperature, they almost certainly underestimate potential toxicities to amphibians inhabiting colder, high-elevation climates. Research detailing the effect of cold acclimation or exposure on amphibian health is also quite limited, yet studies investigating changes in immune function as a result of cold exposure have determined that certain markers of immune function are depressed on cold exposure, even in species that are adapted to seasonal cold conditions [34,35]. The discovery of pathogens associated with red-legged disease (*Aeromonas hydrophila*) [36] as well as chytrid fungi in natural populations of *R. muscosa* may suggest predisposition to infection by an unknown factor or factors (G.M. Fellers, unpublished data). As organochlorines and polyaromatic hydrocarbons cause immunosuppression in fish [37,38], the potential interaction of cold and contaminant exposure in high-elevation amphibians is an issue that should be addressed.

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