Polybrominated Diphenyl Ether (PBDE) Levels in Peregrine Falcon (Falco peregrinus) Eggs from California Correlate with Diet and Human Population Density

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Peregrine falcons are now considered a conservation success story due in part to the phasing out of harmful contaminants that adversely affected reproduction. Recent studies have shown that peregrine eggs collected from California cities, however, have high levels of the higher-brominated polybrominated diphenyl ethers (ΣPBDE183–209), a class of industrial flame retardants, in comparison to published data for other wildlife. Sources of these high PBDE levels and unusual PBDE profiles are unknown. Here we analyzed the stable carbon (δ13C), hydrogen (δD), and nitrogen (δ15N) isotope composition of peregrine eggs collected from urban and nonurban habitats. We found that δ13C values were significantly higher in urban versus nonurban eggs, suggesting that urban peregrines indirectly receive anthropogenic subsidies via their consumption of prey reliant on corn-based anthropogenic foods. δ15N and δD values were significantly lower in urban versus nonurban eggs, reflecting differences in dietary diversity and food/water sources available to peregrines in each habitat. These patterns suggest a link between an anthropogenic diet and high levels of ΣPBDE183–209 in California peregrines, and identify anthropogenic food as a potentially important PBDE exposure pathway for urban wildlife. If diet is an important PBDE exposure pathway for peregrines, continued high body burdens of ΣPBDE183–209 may be a potential risk to ongoing peregrine conservation efforts in California.

Introduction

Peregrine falcons (Falco peregrinus) are top predators in coastal and inland habitats in California and are known to consume a wide variety of marine and terrestrial bird species (1). In the early 1960s, high concentrations of organochlorine pesticides, industrial compounds, and heavy metals threatened peregrine populations with near extinction by adversely affecting reproduction (2). Over the past 30 years, however, peregrines have made a successful recovery due in part to the phasing out of harmful contaminants. Some of the hotspots of peregrine recovery in California are in major urban centers—San Francisco, Los Angeles, San Diego—where ~80% of the state’s human population resides (3, 4). In these densely populated human-dominated environments, the diet of peregrines largely contains rock doves (Columba livia), European starlings (Sturnus vulgaris), and mourning doves (Zenaida macroura). In contrast to this urban diet, nonurban peregrines in California consume a more diverse assemblage of prey species (1). Upon reaching sexual maturity and finding a mate, peregrines remain monogamous, and are long-lived (12–15 years). These ecological traits, as well as the peregrine’s role as a top predator, make the peregrine a useful sentinel species for monitoring and contrasting the fates of organic contaminants in a variety of biotic environments.

Polybrominated diphenyl ethers (PBDEs) are widely used industrial flame retardants that are commonly added to plastics, polyurethane foam, synthetic textiles, and electronics found in a variety of consumer products worldwide. Global PBDE production totaled more than 67,000 tons in 2001 (5). PBDEs were available in three commercial mixtures called the penta-, octa-, and deca-mixtures corresponding to their average bromine content. PBDEs found in the two less-brominated mixtures (penta and octa) are widely dispersed in abiotic and biotic environments (6). These mixtures have been banned in the European Union (7) and Canada, and voluntarily phased-out of production in the United States, likely because of their extensive contamination of aquatic (marine and freshwater) wildlife and their activity in laboratory studies as carcinogens, endocrine disruptors, and neurodevelopmental inhibitors (6, 8, 9).

The deca commercial mixture, however, remains in wide use. Manufacturers consider the deca mixture to be environmentally stable, and one that does not bioaccumulate in wildlife or debrominate into the lower, more toxic penta-, hexa-, and hepta-BDEs. Recent studies, however, have reported measurable levels of BDE-209, the fully brominated PBDE, in birds (10–15) and terrestrial wildlife (16–18). Moreover, several studies of peregrine falcons found higher levels and proportions of BDE-209 and the higher brominated PBDEs (hepta- to nona-BDEs) in eggs from urban versus nonurban habitats (11, 14, 15, but see 19).

Our recent study found large differences in levels and profiles of PBDEs between eggs from peregrine falcons that nested in urban versus nonurban environments in California, with urban eggs having much higher levels and proportions of the higher brominated PBDEs, including BDE-209, as well as the nona-, octa-, and hepta-PBDEs (14). The source(s) of these unusual PBDE profiles and high levels remain unknown. Likely explanations include a combination of two sources: (1) direct consumption of contaminated prey, and/or (2) inhalation of dust particles laden with contaminants during...
preening. To examine this question, we measured the stable isotope composition of the same peregrine eggs analyzed in ref 14. We compared PBDE profiles in these eggs with their stable carbon ($\delta^{13}C$), hydrogen ($\delta^D$), and nitrogen ($\delta^{15}N$) isotopic compositions to assess the relationships between PBDE levels and dietary preferences in peregrine populations residing in urban versus nonurban environments.

Materials and Methods

Sample Information. Peregrine falcon eggs were collected and archived in California as part of the Peregrine Recovery Program. Some samples were collected as addled eggs in nests while others were archived after they were brought in for captive incubation but did not hatch. Eggs were frozen and stored at $-20^\circ C$ until PBDE or isotopic analyses. Of the 82 eggs examined here, 41 eggs were collected from 13 urban nest locations and 41 eggs were collected from 14 nonurban (mostly coastal) nest locations over the period 1986–2007; nest locations are shown in Figure 1. Therefore, some nests are represented by several eggs, each one collected during a different year. Bird eggs are typically formed in a relatively short amount of time (several weeks) and calculations of energy requirements suggest that only a small increase in the daily energy budget of the adult may be necessary for egg formation (20). Thus, the isotopic composition of eggs collected from the same nest but in different years represent independent sampling units for dietary analysis.

PBDE and Stable Isotope Analysis. PBDEs were extracted with the lipid fraction from lyophilized eggs and measured by an Agilent 6890 gas chromatograph coupled to a Thermo-Finnigan MAT95 mass spectrometer. Detailed information about PBDE analysis and quality assurance is provided in the Supporting Information (SI).

For stable isotope analysis of egg samples, homogenized and lyophilized material was treated with petroleum ether in a Dionex ASE 200 to remove lipids. Our lipid extraction method included two cycles of a 5 min preheat, 5 min heat, 5 min static, 60 mL flush, and 60 s purge at 1500 psi and 40 °C. Lipid-extracted samples were then lyophilized and $\sim 0.5$ mg was sealed in tin or silver boats for isotopic analysis. $\delta^{13}C$ and $\delta^{15}N$ isotope values were determined using a Carlo–Erba elemental analyzer (NC 2500) interfaced with a ThermoFinnigan Delta V mass spectrometer at the Carnegie Institution of Washington (Washington, DC). $\delta^D$ values were determined using a Finnigan TCEA coupled to a Thermo-Finnigan Delta Plus XL mass spectrometer in the same laboratory. Isotopic results are expressed as $\delta$ values, $\delta^{13}C$, $\delta^{15}N$, or $\delta^D = 1000 \times [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}]$, where $R_{\text{sample}}$ and $R_{\text{standard}}$ are the $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^2H/^1H$ of the sample and standard, respectively. The standards are Vienna-Pee Dee Belemnite (V-PDB) for carbon, atmospheric N$_2$ for nitrogen, and Vienna Standard Mean Ocean Water (V-SMOW) for hydrogen. The units are expressed as parts per thousand, or per mil (%o). The within-run SD of an acetalinide standard was $\pm 0.2%o$ for both $\delta^{13}C$ and $\delta^{15}N$ values. The within-run SD of two organic (keratin) and two inorganic (oil and mineral) $\delta^D$ standards was $<3%o$. As a control for the lipid content we also measured the $[C]/[N]$ ratios of each homogenized egg sample.

Identification of Nest Prey Remains. Bird feathers were collected from 10 of 13 urban and 11 of 14 nonurban nests from which eggs were collected. Feathers were annually...
removed from nests as part of the Peregrine Recovery Program from 1986 to 2007. Feathers were identified to the lowest possible taxonomic unit using comparative collections, which typically resulted in the identification of a feather to a general bird type (e.g., gull) instead of specific identity. Urban and nonurban nests contained feathers from 44 and 77 prey types respectively, but we only present data for prey types represented by ≥10 feathers. Because it is impossible to quantify the exact number of individual prey represented by feather remains collected from nests, this method is considered to provide a coarse qualitative measure of prey abundance and peregrine diet. This data, however, can be used to quantify differences in the diversity of prey among nests or habitats.

GIS and Statistical Methods. Geographic analyses were performed in ARCGIS version 9.3.0.1770, ESRI Corporation. We used the Census 2000 Block Group Data; released in 2003 and available online at http://www.atlas.ca.gov/download.html as a base layer to extract human population size from a 25-km diameter buffer surrounding each nest location. We then compared human population density within a 25-km radius (buffer) of each nest with egg isotopic and PBDE data. We chose a 25-km radius because this area represents the approximate home range for a breeding pair of peregrine falcons. For eggs collected on bridges, GIS-based estimates for human population density represent minimum densities because they incorporate a significant portion of uninhabited water (e.g., San Francisco Bay, San Diego Bay).

Isotopic differences among urban and nonurban peregrines were assessed using a one-way analysis of variance (ANOVA) and posthoc Tukey-HSD test with significance assigned at an α-level of 0.05. Differences in isotopic variation among peregrine groups were assessed using a two-sided F-test with significance also assigned at an α-level of 0.05. Correlations between ΣPBDE163–209 and human population density were assessed with a linear model. The program JMP 7.0.1 (SAS Institute) was used for all statistical comparisons.

Results and Discussion
Comparison of PBDE Concentrations/Patterns in Urban vs Nonurban Peregrine Eggs. Peregrine eggs from urban versus nonurban California environments differed markedly in their PBDE concentrations and congener profiles (Table S1 and Figures 1 and 2 (14)). Eggs from urban habitats have significantly higher concentrations of PBDEs, particularly the higher-brominated PBDEs (Figure 1), and strikingly different PBDE congener patterns than nonurban eggs (Figure 2). Higher-brominated congeners (BDE-183 to BDE-209) comprised a larger proportion of total PBDEs in eggs from urban versus nonurban birds (Figures 1 and 2), while lower-brominated congeners (BDE-47 to BDE-100) dominated the nonurban profile (Figure 2).

Peregrine eggs collected from urban nest locations in California had much higher levels of mean (±SE) BDE-209 (990.9 ± 155.8 ng/g lipid weight (lw)) than did peregrine eggs collected from other North American and European localities. Urban and nonurban eggs from the northeastern United States had median BDE-209 = 480 ng/g lw (11); Chesapeake Bay eggs from eastern U.S. had median BDE-209 = 6.3 ng/g wet weight (15); and nonurban eggs from southern Sweden had mean (±SE) BDE-209 = 130 ± 28.6 ng/g lw (10). California’s urban peregrine eggs also had higher mean BDE-209 levels in comparison to other wildlife species: red fox (Vulpes vulpes) livers from Belgium had median BDE-209 of 9.1 ng/g lw (maximum =760 ng/g lw (18), and other rural avian and mammalian wildlife had a maximum of ~190 ng/g lw (16, 17).

Ecological and Environmental Isotopic Gradients in California. Stable isotope analysis (SIA) can be used to characterize the diet and habitat preferences of elusive and highly mobile animals such as peregrine falcons (21, 22). The isotopic composition of an animal’s tissues mirrors that of its diet, offset by predictable trophic discrimination factors. Consumers are enriched in the rare heavy carbon (13C) or nitrogen (15N) isotope relative to their diets and for comparison of similar tissue types among consumers and their prey, the enrichment is 3‰ for δ13C and +3–5‰ for δ15N for each increase in trophic level (22–24).

The isotopic composition of food webs and their constituents also varies predictably across environmental gradients. In California, primary productivity in terrestrial ecosystems is dominated by C3 photosynthesis (25), resulting in...
C3 vegetation in California. Terrestrial environments (25, 26) for nitrogen, coastal marine ecosystems typically have higher $\delta^{15}N$ values (+10–16%) than their terrestrial counterparts (+4–10%) because they contain a greater number of trophic levels (21). For hydrogen, the $\delta D$ value of ocean water (0‰) is higher than the $\delta D$ value of precipitation falling on inland terrestrial environments (29–31). These baseline differences in the $\delta D$ of water are transferred up food webs to label their components (32). This coastal to inland $\delta D$ gradient is potentially enhanced in many California urban centers—San Francisco, Los Angeles, San Diego—because their water and food supplies incorporate snowmelt from the Sierra Nevada or Rocky Mountains, which has significantly lower $\delta D$ values than coastal rainfall along the western United States (31, 33).

While less studied than gradients from marine to terrestrial ecosystems, significant isotopic gradients have been identified along urban to nonurban transects in California (34). Recent studies have shown that commercially produced foods commonly consumed by people living in the United States typically have higher $\delta^{13}C$ values than natural ecosystems in the western United States (25, 35). The principal factor driving this trend is that many foods common in the diet of North Americans contain corn (Zea mays), or its common industrial derivative corn syrup, and many domesticated animals (cattle, pigs, or poultry) reared for meat are fed corn during maturation prior to slaughter (36, 37). Corn utilizes the C₄ photosynthetic pathway characterized by higher $\delta^{13}C$ value of about −12‰ to −14‰ (26, 27) in comparison to native C₃ vegetation in California.

$\delta^{13}C$, $\delta D$, and $\delta^{15}N$ in Urban versus Nonurban Peregrine Nests. We found significant differences in mean $\delta^{13}C$, $\delta^{15}N$, and $\delta D$ values of peregrine eggs from urban and nonurban nests (ANOVA, $P < 0.05$; Table 1). We used a combination of $\delta^{13}C$ and $\delta D$ values to identify anthropogenic food sources in the diet of urban peregrines. We used $\delta^{15}N$ values to assess the presence of marine versus terrestrial food sources (i.e., dietary diversity) for both urban and nonurban peregrines. Significantly higher $\delta^{13}C$ values were found in eggs from urban versus nonurban habitats ($P < 0.05$; Table 1 and Figures 1 and 3A), indicating that urban peregrines consume prey with correspondingly high $\delta^{13}C$ values. Common prey species for peregrines in these urban areas are rock doves, mourning doves, and starlings, as evidenced by prey remains found in peregrine nests (Figure 4). High $\delta^{13}C$ values in urban peregrines indicate that their urban avian prey consume anthropogenic food sources that contain corn or corn syrup. Our $\delta^{13}C$ data therefore indicate that peregrines living in California’s cities indirectly receive anthropogenic subsidies via their consumption of prey reliant on C₄-based anthropogenic foods.

As with $\delta^{13}C$, our results with $\delta D$ values point to dietary differences between urban and nonurban peregrines. Significant differences between mean $\delta D$ values of urban versus nonurban eggs (Table 1, Figure 3B) relate to differences in the hydrogen isotope composition of food and water sources available to prey in the respective urban and nonurban peregrine habitats. Controlled feeding studies have shown that ~70–80% of the hydrogen in bird tissues is derived from food, with the remainder coming from drinking water (38). The hydrogen isotope composition of terrestrial plants, and by extension species at higher trophic levels in a terrestrial food chain, are largely determined by the $\delta D$ values of local rainfall, which varies spatially on continental scales in a predictable fashion (31). $\delta D$ values in rainfall are known to decrease in the western United States with increasing elevation and with distance from the Pacific Ocean (31). Food for California’s coastal cities is not typically locally grown, but is produced where precipitation $\delta D$ values are significantly lower, such as California’s Central Valley and the Midwestern United States. Furthermore, tap water in California’s cities is sourced from snowmelt from the Sierra Nevada (San Francisco, Oakland, Los Angeles) and Rocky Mountains (San Diego). Snowmelt has significantly lower $\delta D$ values than does California’s coastal rainfall (31, 33). As a consequence, anthropogenic food and water sources (e.g., fountains) in California’s cities likely have lower $\delta D$ values than nonurban coastal habitats.

### Table 1. Mean Sum of the Higher-Brominated PBDE Congener Concentrations ($\Sigma$-PBDE$_{183-209}$). Ratio of the Sum of the Higher-Brominated (HB, BDE-183 to PDE-209) to Lower Brominated (LB, BDE-47 to PDE-100) Congener Concentrations, $\delta^{13}C$, $\delta^{15}N$, $\delta D$ Values, and [C]/[N] Ratios of the Urban and Nonurban Peregrines (Numbers in Parentheses Represent Standard Error)

<table>
<thead>
<tr>
<th>Group</th>
<th>$\Sigma$-HB (183–209)</th>
<th>HB/LB</th>
<th>$\delta^{13}C$</th>
<th>$\delta^{15}N$</th>
<th>$\delta D$</th>
<th>C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td>2874.5</td>
<td>(364.4)</td>
<td>(0.4)</td>
<td>(0.3)</td>
<td>(1.4)</td>
<td>3.9</td>
</tr>
<tr>
<td>Nonurban</td>
<td>409.1</td>
<td>(95.1)</td>
<td>(0.1)</td>
<td>(0.3)</td>
<td>(2.1)</td>
<td>3.9</td>
</tr>
</tbody>
</table>

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Our isotopic, contaminant, and nest prey remain data show that nonurban peregrines have a more diverse prey base than their urban counterparts. Nonurban peregrines have a wider range of $\delta^{15}$N values (Figure 5D), higher proportions of lower-brominated PBDEs (Figure 2), and a greater diversity of prey species in their nests in comparison to urban birds (Figure 4). Whereas peregrine eggs from nonurban sites had significantly higher mean $\delta^{15}$N values than their urban counterparts (Table 1 and Figure 3), these values were lower than those expected for a raptor solely consuming marine prey, even though most of the nonurban nests are located in coastal habitats (Figure 1). Overall, these data are consistent with a mixed diet of terrestrial and aquatic (marine and/or freshwater) prey. A similar correlation between PBDE congener patterns and isotopic data has been observed in grizzly bears from British Columbia known to consume prey from a mixture of aquatic and terrestrial habitats (16).

Correlations Among $\Sigma$PBDE$_{183-209}$, Isotope Values, and Human Population Density. The concentration of higher-brominated PBDE congeners (hepta- to nona-BDEs) was positively and significantly correlated with GIS-based estimates of human population density within a 25-km radius of each nest examined (Figure 5A). $\Sigma$PBDE$_{183-209}$ varied widely in eggs from densely populated areas containing $\sim$5–6 million people within a 25-km radius of the nest (Figure 5A). Because $\Sigma$PBDE$_{183-209}$ did not significantly change over the ~20-year period eggs were collected (14), the observed variation in PBDE levels suggests that exposure potential to these chemicals may be heterogeneous across the urban.
FIGURE 5. Relationship between human population density and
the ΣPBDE_{183-209} (A), δ^{13}C (B), δD (C), and δ^{15}N (D) of urban
white circles) and nonurban (black circles) peregrine falcon
eggs. ΣPBDE_{183-209} was positively and significantly correlated
with human population density within a 25-km radius of each
nest examined (A).

environment. Particular urban areas may be associated with
high baseline ΣPBDE_{183-209} loads, while other areas have
inherently lower exposure potential. For both urban and
nonurban areas, however, the potential degree of exposure
correlates with human population density, with the exception
of a single egg. This outlier, with exceptionally high ΣPBDE_{183-209}, was collected from the western section of the Bay
Bridge that spans San Francisco Bay. The 25-km radius for
this nest encompasses a large portion of uninhabited water,
so our GIS approach underestimated the effective human
population density for this nest.

As described above, several independent lines of evidence
support the contention that nonurban peregrines have a more
diverse prey base than their urban counterparts. Within the
urban environment, however, dietary diversity as measured
by variability in δ^{13}C values also correlates with human
population density. Eggs from moderately populated urban
areas that contain ∼1–4 million people within 25 km of the
nest have higher δ^{13}C variation than eggs from the most
densely populated urban areas containing ∼5–6 million
people within 25 km of the nest (Figure 5D; F-test, P < 0.05).
In contrast, peregrine egg δ^{13}C and δD values show a
"stepped" response with increasing population density
(Figures 5B and C), with a significant change in mean values
but no significant difference in variance with increasing
density (F-test, P > 0.05). This is a pattern suggestive of a
change in baseline δ^{13}C and δD of available food sources. As
discussed above, the trend in δ^{13}C is likely driven by a baseline
change from a C_{3}-based food web in the nonurban setting
to an anthropogenic C_{4}-based food web in the urban
environment. For δD, the baseline change reflects a shift
from a food chain fueled by coastal meteoric waters in
nonurban settings to an anthropogenic food chain based on
inland water sources source from the California Sierra
Nevada, Rocky Mountain West, and/or Midwestern U.S.

Implications for Peregrine and other Urban Wildlife
Conservation. Our isotopic data suggest a strong link between
an anthropogenic diet and high levels of the higher bromi-
nated PBDEs (ΣPBDE_{183-209}) in California peregrines, and
identify anthropogenic food as a potentially important
exposure pathway for urban wildlife in general. The high
ΣPBDE_{183-209} levels found in urban peregrines have been
linked with various toxicological effects in other avian species,
including impaired growth, reduced clutch size, and de-
creases in reproductive fitness (13, 39, 40). Our previously
published study of urban California peregrines is consistent
with BDE-209 undergoing metabolic debromination to the
biologically harmful and commercially banned lower-bro-
ninated PBDEs (e.g., BDE-153, BDE-183) (12, 40, 41). Recent
studies have shown that dietary inputs contribute between
∼70–90% of the total PBDE loads in humans (42, 43). If diet
is also an important PBDE exposure pathway for peregrines,
which our data suggest that it is, continued high body burdens
of ΣPBDE_{183-209}—burdens that are associated with develop-
mental effects in other species—may represent a risk to urban
peregrine populations in California. Although there are
reports of possible PBDE effects on reproduction and
development in raptors (44, 45), these costs may be offset by
short-term benefits of abundant prey in urban versus
nonurban habitats, since peregrines in urban habitats have
significantly higher fecundity than birds in nonurban habitats
(3). The long-term costs associated with high body burdens
of ΣPBDE_{183-209} in long-lived peregrines are unknown. Future
comparative work on diet, survivorship, and lifetime repro-
ductive output of urban and nonurban peregrines and their
offspring in California is warranted to determine the effects
of high ΣPBDE_{183-209} concentrations in urban peregrines (e.g.,
ref 46). Our study also shows that δ^{13}C and δD analysis may
be an effective tool for examining the indirect exploitation
of anthropogenic foods by urban wildlife populations in the
western United States. Ultimately, the coupling of isoto-
pically derived dietary information and PBDE data can be used
to identify challenges to urban wildlife populations from the
continued production and use of the commercial deca-BDE
mixture, and to support legislative measures that reduce
human and wildlife exposure to these potentially harmful flame retardants.

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**Supporting Information Available**
Materials for PBDE analysis, sample analysis and quality assurance, effects of dessication on the lipid content of added eggs, and Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

**Literature Cited**


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