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Authors
Kalia, Vrinda
Schuur, Stacy
Hobson, Keith
et al.

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Relationship between the Pacific Decadal Oscillation (PDO) and persistent organic pollutants in sympatric Alaskan seabird (Uria aalge and U. lomvia) eggs between 1999 and 2010

Vrinda Kalia[1,*,†], **Stacy S. Schuur**[2], **Keith A. Hobson**[3], **Howard H. Chang**[4], **Lance A. Waller**[4], **Steven Hare**[5], **Matthew O. Gribble**[1,6]

[1]Department of Environmental Health, Emory University Rollins School of Public Health, 1518 Clifton Road NE, Atlanta, Georgia 30322

[2]National Institute of Standards and Technology (NIST), Hollings Marine Laboratory, 331 Fort Johnson Road, Charleston, South Carolina 29412

[3]Environment and Climate Change Canada, Stable Isotope Hydrology and Ecology Research Laboratory, 11 Innovation Blvd. Saskatoon, SK 27N 3H5 Canada

[4]Department of Biostatistics, Emory University Rollins School of Public Health, 1518 Clifton Road NE, Atlanta, Georgia 30322

[5]The Pacific Community, BP D5, 98848 Noumea, New Caledonia

[6]Department of Epidemiology, Emory University Rollins School of Public Health, 1518 Clifton Road NE, Atlanta, Georgia 30322

Abstract

Although climate change occurs alongside other anthropogenic ecosystem impacts, little is known about how sea-surface temperature variability influences the ecotoxicology of persistent organic pollutants (POPs). We analyzed POP contaminant levels, and stable isotopes $\delta^{15}N$ and $\delta^{13}C$ as measures of trophic position in eggs collected from the Gulf of Alaska and Bering Sea

*Corresponding author: vk2316@cumc.columbia.edu Postal address: 650 W 168th street, Room 1602, New York, New York, 10032.
†Current affiliation: Department of Environmental Health Sciences, Columbia University Mailman School of Public Health, 722 W 168th St, New York, New York 1003

Credit author statement

Author contributions: S.S.S. and M.O.G. conceptualized the question, S.S.S. and K.A.H. performed the chemical analyses. V.K. and M.O.G. performed the spline regression, H.H.C. performed the Bayesian analyses. All authors contributed toward the writing and editing the manuscript.

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Declaration of interests

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between 1999 and 2010 from two similar avian species with different trophic positions: common murres (*Uria aalge*) and thick-billed murres (*Uria lomvia*). The ebb and flow of the Pacific Decadal Oscillation (PDO), a long-lived El Niño-like pattern of climate variability in the Pacific Ocean, predicted both trophic position and polychlorinated biphenyl (PCB) levels in thick-billed murres, but not in common murres. There was a similar pattern of association of the PDO with organochlorine pesticide levels in thick-billed murres, but not in common murres. The magnitude of association in thick-billed murres of PDO with the concentration of a specific PCB congener was a function of the number of chlorine groups on the PCB congener. Although this statistical analysis does not account for all factors contributing to climate variation, this contrast between the species suggests that facultative changes in foraging behavior, reflected in trophic position, can determine how POPs flow through and thereby alter ecosystems under climate change.

Keywords

Climate variability; ecotoxicology; behavioral ecology

1. Introduction

The health of marine ecosystems is influenced by factors including variations in climate and the presence of chemical contaminants in food webs (Bustnes et al., 2015). This is especially true at high latitudes where climate variability and change are pronounced, and contaminants deposit and concentrate in long food chains (Burkow and Kallenborn, 2000; Stocker et al., 2013). Climate variations affect the movement of environmental contaminants by altering biogeochemical cycles involving their transport and flux (Macdonald et al., 2005; Noyes et al., 2009). Climate variations also produce changes in the availability of prey (Ng and Gray, 2011) and biological processes involving primary producers (Macdonald et al., 2005), thus affecting trophic dynamics. Furthermore, climatic conditions are likely to affect average wind speed and associated patterns of ocean circulation, which are known to affect food availability (Mueter and Litzow, 2008; Oechel et al., 2012; Spear et al., 2019).

Variations in local climatic and oceanographic conditions can be driven by large-scale oscillations such as the El Niño Southern Oscillation (ENSO) and Pacific Decadal Oscillation (PDO), which include temporal trends in sea-surface temperature (SST) in the equatorial and north Pacific, respectively (Deser et al., 2010; Mantua et al., 1997). SST is correlated with a range of ecologically important climate variables (Mueter et al., 2002). Changes in SST affect competition between and among different marine species (Stenseth et al., 2015), normal biochemical processes of marine organisms (Hochachka and Somero, 2002) and ocean production rates (Gregg et al., 2003). The warm (or positive) phase of the PDO has been linked to changes in mortality, breeding and diet of a seabird (*Sula granti*) in the Pacific marine ecosystem (Champagnon et al., 2018).

Seabirds are useful indicators of the health of marine ecosystems and biomonitoring studies have suggested persistent organic pollutants (POPs), like polychlorinated biphenyls (PCBs) and legacy organochlorine pesticides (e.g., chlordanes and DDT), can adversely affect the health of these top predators (Bustnes et al., 2015). POPs have been associated with behavioural impairment, poor reproductive performance and lowered survival in seabirds (Gabrielsen, 2012).

Common and thick-billed murres are closely related alcids that often breed sympatrically in the Arctic. However, the birds have subtle differences in their feeding behavior. While primarily piscivorous, differences in feeding behavior and diving depths between these species have been identified. Common murres feed in the meso-pelagic closer to the colony while thick-billed murres dive deeper, also consuming benthic organisms, and forage farther from shore. This suggests that the birds have different trophic flexibility, i.e., thick-billed murres are more likely to swap food sources at different trophic levels (Ainley et al., 2002; Gaston and Hipfner, 2000). Thick-billed murres nesting in the Hudson Bay have shown altered trophic position (measured using stable isotopes of nitrogen) during 1993-2013, attributed to a change in diet, which altered contaminant temporal trends (Braune et al., 2015).

The role of trophic dynamics can be assessed using stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). Values of $\delta^{13}C$ and $\delta^{15}N$ in consumer tissues reflects that of their diet. A stepwise increase in $\delta^{15}N$ with each trophic level in marine ecosystems allows the use of this stable isotope as an indicator of trophic level (Sydeman et al., 1997). In marine systems, $\delta^{13}C$ values can provide insight into inshore or offshore foraging (Sydeman et al., 1997).

Using data obtained through the access policy of the Seabird Tissue Archival and Monitoring Project (STAMP – a multiple government agencies, academic institutions, non-governmental organizations, and Alaska Native communities program to use seabird tissues as a proxy for ocean and human health), we obtained levels of POPs: PCBs (36 congeners; 30 analytes with co-elutions), seven organochlorine pesticides, and stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes in eggs from common and thick-billed murres collected between 1999 and 2010 in Alaska (SI Data).

To study how contaminant levels correspond to changes in regional climate, we used the PDO index, which measures an east-west dipole pattern of SST variation in the North Pacific Ocean (20N-65N) (Mantua and Hare, 2002; Newman et al., 2016), as a marker of climate variation. The index has positive values when the SST within 1000 km of the west coast of North America are warmer than normal and the SST in the western and central North Pacific Ocean off Japan are colder than normal, and negative when these conditions are reversed (Mantua and Hare, 2002).

2. Materials and methods

2.1. Sample Information

Common and thick-billed murre eggs were collected, processed and archived for STAMP using standard protocols (Rust et al., 2010; York et al., 2001). Data obtained through the access policy for 198 eggs collected from four colonies in Alaska that were sampled in at least three years between 1999 and 2010 were used for this study (SI Data).

2.2. Persistent organic pollutant analysis

Earlier publications describe the sample analysis in specific detail (Vander Pol et al., 2012, 2009, 2004, 2003). Extraction of compounds of interest were accomplished by pressurized fluid extraction (PFE). Lipids were measured gravimetrically or directly determined separately using nuclear magnetic resonance (NMR) technology before removal by size exclusion chromatography (SEC). Further clean-up may have been by aminopropylsilane liquid chromatography (LC) or solid phase extraction (SPE). Analysis was completed by gas chromatography (GC) with electron capture detection (ECD) or mass-spectrometry (GC/MS) with electron impact (EI) ionization for PCBs and selected pesticides and negative chemical ionization (NCI) for selected pesticides with selected ion monitoring mode for mass to charge (m/z) ratios as given in SI Data. The results were obtained as internal standard ratios in samples and calibration solutions. Quality control included three procedural blanks per batch of samples and SRM 1946 and an in-house egg control material (Vander Pol et al., 2007).

Data was reduced to those analytes with less than 20 % of the values below the Limit of Detection/Quantitation (LOD/LOQ), resulting in 36 PCBs (30 analytes with co-elutions): PCB 28+31, 52, 56, 63, 66, 70, 74, 99, 101, 105, 106+118, 107, 128, 138, 146, 149, 153+132, 156+202+171, 157, 158, 163, 170, 180+193, 183, 187, 194, 195, 201, 206, and 209 and seven organochlorine pesticides: a-HCH (alpha–hexachlorocyclohexane), 4,4’-DDE (dichlorodiphenyltrichloroethylene), *cis*-nonachlor, HCB (hexachlorobenzene), heptacholor epoxide, mirex, and oxychlordane (SI Data).

2.3. Stable isotope analysis

Samples were analyzed at the Stable Isotope Hydrology and Ecology Research Laboratory facility (Environment and Climate Change Canada) for $\delta^{13}C$ and $\delta^{15}N$ analyses using methods previously described by Hobson et al (2003). After the samples were freeze dried, lipids were extracted and extraction filtrates were dried under a fume hood for 24 hours. Values of $\delta^{13}C$ and $\delta^{15}N$ were obtained using continuous-flow isotope ratio mass spectrometry (CFIRMS) on a Europa 20:20 IRMS interfaced with a Robo Prep combustion system. The ratios were expressed in delta (δ) notation relative to the Vienna Pee Dee Belemnite (VPDB) or AIR standards for $\delta^{13}C$ and $\delta^{15}N$, respectively.

2.4. The Pacific Decadal Oscillation (PDO)

National Oceanic and Atmospheric Administration (NOAA) and the University of Washington track the Pacific decadal oscillations, and data is openly available at http://research.jisao.washington.edu/pdo/. As the birds are most likely gathering energy stores at the colony for egg laying between February and May, the mean of these months was used for analysis (SI Data).

2.5. Data Management

All data management was done in Stata 15.1 S/E (StataCorp, College Station, Texas). Datasets obtained from STAMP and the University of Washington with different variables were merged, providing the final dataset with values for the years 1999 through 2010. Contaminant levels at LOD/LOQ were replaced with (LOD/LOQ)/√2. All contaminant levels were adjusted for lipid content, by dividing by lipid content, and were natural log-transformed.

2.6. Statistical analysis

2.6.1. Linear spline regression: We created a spline knot at PDO (February to May) = 0, to allow for possible heterogeneity in the association between the cool and warm phase of PDO. We used cluster-robust standard errors to account for the non-independence of eggs collected from the same colony.

Model specification for linear spline regression: For chemical (organochlorine pesticide, PCB congener, or stable isotope) *c* and for bird *j*, lognormal lipid-adjusted biomarker or the isotope ratio *b*, Pacific Decadal Oscillation (PDO) score *p*, indicator variables $I_{p<0}$ or $I_{p>0}$ for when PDO is negative or positive, year *t*, and location indicators (Gulf of Alaska or Bering Sea) I_{l_1}, I_{l_2}: for bird *j*,

$$\ln b_{j,c} = \alpha + (I_{p<0})\beta_{c,1}(p_j) + (I_{p>0})\beta_{c,2}(p_j) + \beta_{c,3}(t_j) + \beta_{c,4}(t_j^2) + \beta_{c,5}(I_{l_1,j}) + \beta_{c,6}(I_{l_2,j}) + \varepsilon_{j,c}$$
$$\varepsilon_{j,c} \sim N(0, \tau_c^2)$$

where τ_c^2 is the residual variance.

2.6.2. Bayesian meta-analysis and meta-regression: Second-stage Bayesian meta-analysis or meta-regression models of the coefficients from the first-stage spline regression models were implemented using just another Gibbs sampler (JAGS) in R (version 3.4.4) with packages *R2jags* (version 0.5-7 linked to JAG 4.3.0) and *coda* (version 0.19-1). We provide point estimates and their 95% credible intervals (statistical significance is determined by whether the posterior 95% credible intervals include the null value.)

Model specification for Bayesian meta-analysis: For PCB congener *c*, index *k* for whether pre/post knot association: (k= 1: $\hat{\beta}_{1,c}$, k=2: $\hat{\beta}_{2,c}$), and covariance matrix of estimates S_C,

$$\begin{bmatrix}\hat{\beta}_{1,c}\\ \hat{\beta}_{2,c}\end{bmatrix} \sim N\left(\begin{bmatrix}\theta_{1,c}\\ \theta_{2,c}\end{bmatrix}, S_C\right)$$
$$\theta_{1,c} \sim N(\mu_1, \sigma_1^2)$$
$$\theta_{2,c} \sim N(\mu_2, \sigma_2^2)$$

where $\theta_{k,c}$ is the unobserved true effect, μ_k is the average effect across PCB congeners, and σ_k^2 is the between-congener heterogeneity variance. Weakly-informative priors were assigned to parameters μ_k and σ_k^2 ($\mu_k = \sigma_k^2 = 1 \times 10^{-4}$)

Meta-regression in thick-billed murres conditioning on the number of chlorine groups: For PCB congener *c*, index *k* for whether pre/post knot association: (k= 1: $\hat{\beta}_{1,c}$,

k=2: $\hat{\beta}_{2,c}$), x the number of chlorine groups in the congener and covariance matrix of estimates Sc

$$\begin{bmatrix}\hat{\beta}_{1,c}+\hat{\alpha}_{1,c}(x_c)\\ \hat{\beta}_{2,c}+\hat{\alpha}_{2,c}(x_c)\end{bmatrix} \sim N\left(\begin{bmatrix}\theta_{1,c}\\ \theta_{2,c}\end{bmatrix}, S_C\right)$$
$$\theta_{1,c} \sim N(\mu_1, \sigma_1^2)$$
$$\theta_{2,c} \sim N(\mu_2, \sigma_2^2)$$

All other assumptions remain the same as in the meta-analysis above.

2.6.3. Sensitivity analysis: We obtained monthly ocean sea surface temperature anomalies for latitudes ranging from 20°N to 90°N from the NOAA Merged Land Ocean Global Surface Temperature Analysis Dataset (NOAAGlobalTemp) (Zhang et al., 2019). The linear spine regression model from 2.6.1. was additionally adjusted for the average sea surface temperature anomaly from February to May during 1999 – 2010, and its squared term.

3. Results

In thick-billed murres, when the PDO was in its cool phase, there was a positive association between the egg contamination by PCBs and the PDO index (Figure 1). When the PDO was in its warm phase, the levels of PCBs in the egg was negatively associated with the PDO index (Figure 1). Levels of chlorinated pesticides in thick-billed murres were also negatively associated with the PDO index when the PDO was in its positive phase. In common murres, the associations between all organochlorines and the PDO index were null, and did not differ by phase (Figure 1). The inflection point (point of the curve at which a change in the direction of curvature occurs) of contaminants around the neutral PDO in only thick-billed murres was not expected, but may be related to species-specific differences in trophic structure and dynamics that change from the cool to the warm phase.

The relationship between contaminant loads and the PDO index was consistent across almost all PCB congeners and chlorinated pesticides in thick-billed murres (Figure 2). A Bayesian meta-regression model (Figure 3) estimated that, on average across all PCB congeners, each unit increase in the PDO index in its cool phase was associated with a 0.26 increase in geometric mean ratio of PCBs (95% credible interval: 0.074 to 0.44). In the warm phase of PDO, each unit increase in the PDO index was associated with a 0.38 (95% credible interval: −0.46 to −0.306) decrease in geometric mean ratio of PCBs. Chlorinated pesticides showed a similar trend with the warm phase of PDO, a decrease of 0.19 in geometric mean ratio (95% credible interval: −0.452 to 0.031), and under cool phase PDO conditions, an increase of 0.47 (95% credible interval: −0.18 to 1.16). Further, in thick-billed murres, the mean association between PCB congener levels decreased with increasing number of chlorine groups in the congener (Figure 3).

In our study, both $\delta^{13}C$ and $\delta^{15}N$ values associated differently with the PDO index under warm and cool phases. The $\delta^{13}C$ values were positively associated with the cool phase of the PDO and negatively with the warm phase of PDO in common murres. In thick-billed murres, $\delta^{13}C$ values were positively associated with both the cool and warm phase of the PDO. Similarly, the patterns of association between $\delta^{15}N$ and the PDO index were found to be different between the two species. In common murres, $\delta^{15}N$ was positively associated with both, the cool phase and the warm phase of PDO, while in thick-billed murres, $\delta^{15}N$ values are positively associated with the cool phase of PDO and negatively associated with the warm phase of PDO (Figure 1).

Sensitivity analysis adjusting for the effect of average sea surface temperature anomaly from February through May changed the relationship between PCB congeners and PDO in thick-billed murres and the meta-analysis coefficient no longer showed a clear trend between the association of PCB congener levels and the two phases of the PDO (Figure S3).

Discussion

Persistent, organic pollutants (POPs) are resistant to degradation and persist in the atmosphere, land, and aquatic environment. While a large proportion of these chemicals are emitted in warmer parts of the globe, the chemicals travel long distances toward cold parts, like the arctic (Burkow and Kallenborn, 2000; Jiménez et al., 2015; Klecka et al., 2000). The degradation, fate, and transport of these chemicals is dependent on atmospheric conditions and their physicochemical properties (Hansen et al., 2015). Several studies have attempted to predict how changes in atmospheric conditions due to climate change will affect their behavior in the environment. It is predicted that a higher mean temperature will cause a shift in the mass of these chemicals from surface media to the atmosphere, however, increased mean temperature is also likely to increase the degradation of the chemicals. It remains unclear whether climate change will decrease or increase the environmental concentration of POPs (Hansen et al., 2015).

Studies have also considered the effect of changing SST on the bioaccumulation of POPs in the marine food web. The highly complex nature of biotic interactions and feedback processes makes this a difficult relationship to study (Walther, 2010). Changes in SST can have effects across all layers of the food web. It has been reported that the positive (warm) phase of ENSO (El Niño- Southern Oscillation) is associated with altered phytoplankton chlorophyll levels and primary production anomalies (Racault et al., 2017). A different study reported adverse effects on essential amino acid and fatty acid levels in a primary producer when artificially exposed to warmer and more acidic environments (Bermúdez et al., 2015). Altered health and biochemistry in these lower trophic level organisms can propagate changes across the food web as bottom-up effects through the ecological networks (Walther, 2010), thus also affecting the bioaccumulation of POPs in the network. Change in SST has also been associated with the ability of penguins, a high trophic-level organism, to capture prey (Carroll et al., 2016).

Our data suggest that the effect of the PDO index on the bioaccumulation of a POP depends on the physicochemical properties of the chemical and the trophic network. We find that in thick-billed murres, PCB congeners follow a similar pattern of association with the PDO index (Supplemental Figure 1) while this pattern was less pronounced in the organochlorine pesticides, some of which, like HCH, are more easily degraded than other members of this class. Common murres did not show a clear pattern of association with the PDO index in these two classes of chemicals (Supplemental Figure 2). We found that in thick-billed murres, during the cool phase of PDO, the estimated association between the PDO index and PCB levels decreased significantly as the number of chlorine groups increased, while during the warm phase of PDO, there was not significant variation in the PDO-PCB association according to the number of chlorine groups. The number of chlorine groups is a surrogate for the hydrophobicity and persistence of a PCB in the environment (Bruggeman et al., 1982).

We believe these overall findings are consistent with the idea that more environmentally persistent PCBs may be less sensitive to changing sea surface temperatures and vice versa as we observed different accumulation patterns in the eggs of thick-billed murres whose feeding habits may differ by sea surface temperature.

After adjusting for anomalous SST, we found that the relationship between the PDO index and chemical levels were changed. This suggests that SST anomaly is probably an important variable determining the pattern between PDO and chemical levels. However, the SST anomaly data were coarse and could make us susceptible to measurement error.

Findings from this study uncovered a complex relationship between climate variability and vulnerability to POPs in two sympatric Alaskan seabirds. The species-specific difference in relationship could be due to several reasons, including: differences in trophic structure and dynamics, changes in primary producers related to changes in sea-surface temperature, or changes in space use by the two species. The association of $\delta^{15}N$ and PDO revealed a pattern similar to the association between the PCB congeners and PDO seen in thick-billed murres. Thus, indicating that the observed pattern of bioaccumulation could be driven by changes in trophic dynamics. Feeding behavior in more extreme sea-surface temperature conditions were associated with lower levels of the contaminants in the eggs. The association of $\delta^{13}C$ with PDO revealed a pattern consistent with known differences in foraging behaviors of the two species and relate to pelagic vs. more inshore foraging (Ainley et al., 2002; Gaston and Hipfner, 2000).

We were unable to account for other variables that may be associated with PDO and with the environmental fate and transport of POPs such as storms, sediment resuspension, and precipitation (Trenberth and Shea, 2005). Thermal inertia can induce a decoupling of anomalously cold winter temperatures in deep oceanic layers from summer SSTs. This “memory” of temperature can reemerge the following winter through entrainment at deep layers (Newman et al., 2016). The effect of trapped and lagged temperature on trophic structure would provide more information in teasing apart this relationship. Other changes in atmospheric chemistry and properties could affect both, the amount of POPs entering the food web, the distribution of fish, and the PDO (Mueter et al., 2002; Nye et al., 2009). Furthermore, more mechanistic bioenergetics and food web modeling would enable a better understanding of factors affecting the observed trends.

A recent study found a similar negative association between sea surface temperature and accumulation of methylmercury in fish tissue measured from the Gulf of Maine (Schartup et al., 2019). When determining risk of exposure and adverse effects of POPs in seabirds, it will be important to account for differences in vulnerability that stem from changes in trophic structure, which are large enough to affect two sympatric birds in dissimilar ways. We would also expect this differential vulnerability to impact local populations that rely on their ecosystem for subsistence and their economy (Balbus et al., 2013; Lam et al., 2016). The stability of marine biospheres relies on many variables that can interact in multiple ways. Predicting their change under a changing climate is complicated by these interactions and by differently resilient behavioral ecology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability:

All data used in analysis are available in the supplementary materials. Aliquots of STAMP eggs are available for other research, please contact S.S.S. for details (Stacy.Schuur@nist.gov).

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Highlights:

- Climate variability measured as temporal oscillations in sea surface temperature has been associated with altered trophic dynamics in marine ecosystems.
- Using data from the seabird tissue archival and monitoring program (STAMP), we found differential effects on feeding behavior and accumulation of pollutants in two sympatric seabirds, *Uria aalge* and *Uria lomvia*.
- Differences in vulnerability that stem from changes in trophic structure may affect the risk of exposure and adverse effects of persistent, bioaccumulative and toxic chemicals in seabirds in light of changing climate variability.

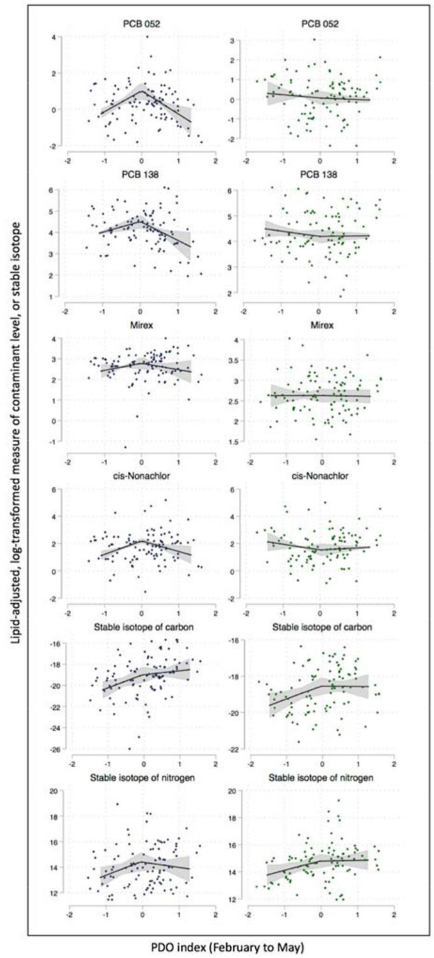

Figure 1. Relationships between the Pacific Decadal Oscillation (PDO) index and the natural log-transformed levels of lipid-adjusted persistent organic pollutants, and stable isotopes ratios ($\delta^{15}N$ and $\delta^{13}C$) in the eggs of thick-billed murres (*Uria lomvia*, in blue on the left) and common murres (*U. aalge*, in green on the right) collected from colonies in the Gulf of Alaska and Bering Sea between 1999 and 2010.

This plot illustrates the patterns for a few chemicals, results for additional chemicals are presented in Supplemental Figures S1 and S2. Associations were estimated using linear regression models with a spline knot at PDO index = 0 (separating the cool phase from the warm phase of the PDO), adjusted for year and for geographic region, with cluster-robust standard errors to account for the clustering of eggs within seabird colonies. Dots show observed data. The black line shows the expected value of a ln-transformed chemical at each value of the PDO index, adjusted for year and region, and the grey shaded region depicts the corresponding 95% confidence interval.

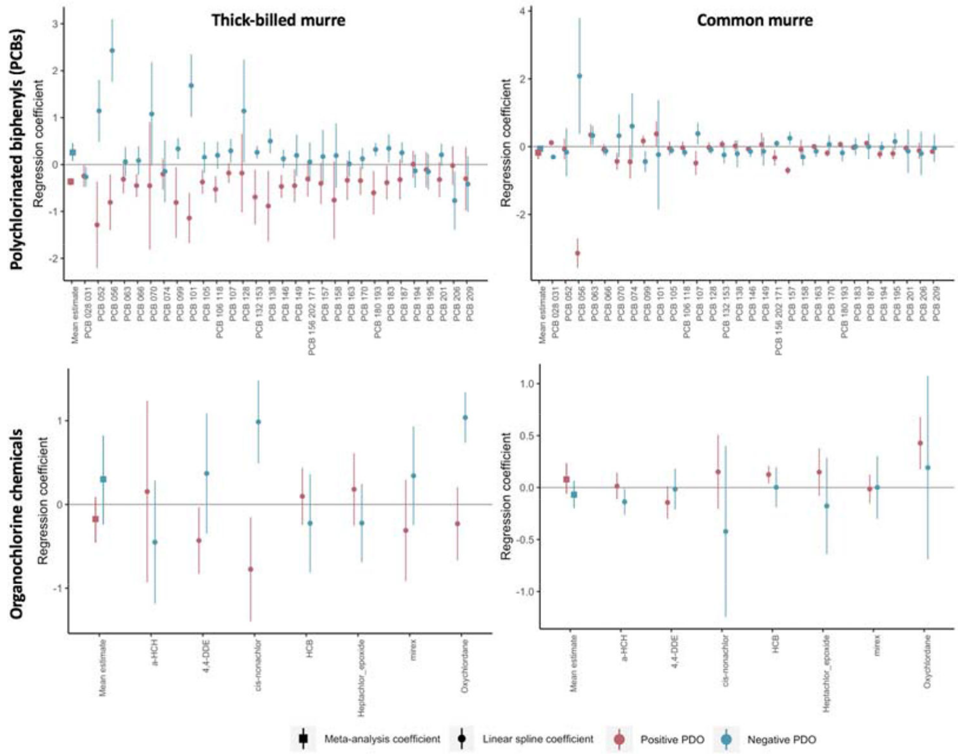

Figure 2. Associations between the PDO index and lipid-adjusted persistent organic pollutant biomarkers measured in bird eggs, adjusted for year and geographic region, differed according to bird species, chemical, and cool vs. warm phase of the PDO.

For thick-billed murres (*Uria lomvia*), an increase in the PDO index corresponded to higher levels of PCBs in eggs during the cool phase of PDO, and to lower levels of PCBs during the warm phase of the PDO, after adjusting for year and geographic region. Chlorinated pesticides showed a similar pattern in thick-billed murre. In contrast, there were not clear relationships of the PDO index to persistent organic pollutant levels in common murre (*U. aalge*) eggs, after adjusting for year and geographic region. The dots represent the estimate of association between levels of the transformed POP and PDO in the warm (pink) and cool (blue) phase. The lines around the points represent the 95% confidence interval. The mean estimate of association between each type of chemical (PCB or Organochlorine pesticide) and the warm (pink) and cool (blue) phase of the PDO determined by the Bayesian meta-regression are represented by the square points. The lines around the mean estimate represent the 95% credible interval.

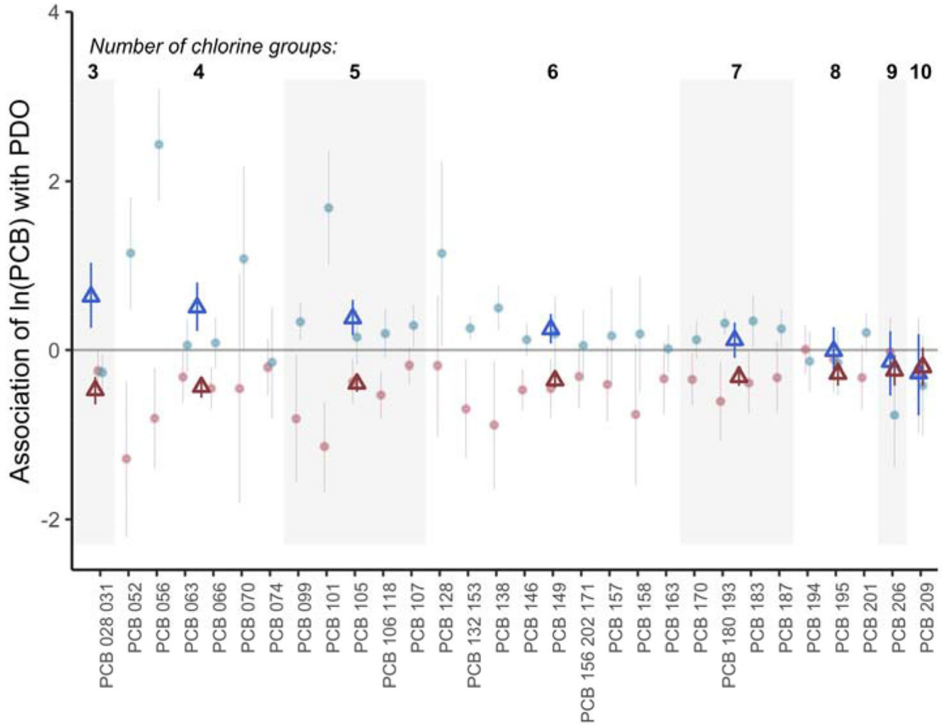

Figure 3. PCB congeners with fewer chlorine groups had a larger difference between the positive and negative phases of the PDO in the expected association after adjustment for age and geographic region of the PDO index with lipid-adjusted PCB biomarker levels in thick-billed murre (*Uria lomvia*) eggs.

Regression coefficients from the linear spline models of ln-transformed PCB levels on the PDO index, adjusted for year and geographic region, for thick-billed murre eggs, were entered as outcomes into a second-stage bivariate normal Bayesian meta-regression model that included the number of chlorine groups in the PCB congener as a linear continuous predictor: see Methods text in 2.8.2 for additional model specification details. The expected relationship (i.e., regression coefficient of the PDO index to the ln-transformed PCB level region, adjusted for year and geographic region) for a congener with a given number of chlorine groups is represented as a triangle; vertical lines around the triangle show the 95% credible interval. The dots and vertical lines around the dots are repeated from Figure 2 and show the point estimates and 95% confidence intervals of the multivariable-adjusted associations of PDO with each PCB congener. During the cool phase of the PDO (shown in blue), as the number of chlorine groups on the PCB congener increased, the year-and-region-adjusted association of PDO with PCB biomarker levels became less positive. During the warm phase of the PDO (shown in red), the number of chlorine groups had a negligible effect on the year-and-region-adjusted relationship of PDO index to PCB biomarker levels.