A long-term temporal analysis of heavy metal concentrations in seabird feathers with implications for overgeneralized trophic dynamics

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Executive Summary

Anthropogenic deposition and natural cycling of heavy metals can impact ecosystem function; dense metals accumulate in marine sediment layers and remain there for long periods of time. These metals integrate into the biological environment and move through the ecosystem to higher trophic level organisms. High trophic species present higher concentrations, which have known toxic effects including decreased reproductive success and compromised immune systems. Seabird feather levels may be representative of broader ecosystem signals and heavy metal cycling. They are long-lived, migratory species that eat a variety of prey species across large landscapes. While seabirds are still considered high trophic organisms, their trophic position has declined over time due to changes in feeding strategies. This truncation in trophic range may have an effect on the heavy metal biodynamics.

This study utilizes heavy metal concentration data from a historic collection of seabird feathers and builds on the results of previous studies that presents the declines found from tracking trophic levels of top predators across time. This analysis pairs these trophic changes with heavy metal concentrations from the 1880s to 2016, as well as carbon and nitrogen stable isotopes. The main questions to be answered are 1) to determine if changing trophic levels will alter ecosystem dynamics, and 2) the accuracy of modeling baseline heavy metal concentrations using trophic transfer factors.

For this study, the feathers were collected from a museum collection stored at the Bishop Museum located in Honolulu, Hawaii. We looked eight different seabird species, including: Laysan albatross, wedge-tailed shearwater, brown booby, Brown Noddy, Bulwer Petrel, Sooty Tern, White Tern, and White-tailed Tropicbird. We analyzed nine different heavy metals and trace elements, including arsenic, cadmium, copper, iron, lead, manganese, mercury, molybdenum, and zinc. We conducted our statistical analysis using R software. We first modelled concentration over time, interpolating for years that did not have data. To find a trophic transfer factor, we tried to fit different regression models to a concentration-trophic level plots using data from a food web analysis. To adjust our feather concentrations to a baseline concentration, we created a TTF look up table using the trophic information from previously published research by our team. We then took our feather concentration data that had trophic levels for each data point, and found the value on our chosen model, a smooth spline. The point on the curve became the factor by which we divided the value and found what we assume to indicative of the baseline of the food web, 1 pp. We then model that against time to determine differences from the original.

The concentration trend across the entire timeframe of study is on average persistently declining for lead, arsenic, and mercury. Levels appear consistent for zinc, manganese, and copper in any of the species under study. However, there are conflicting patterns for cadmium, iron, and molybdenum and directional trends are difficult to determine. When metals were modeled against trophic position, different trophic dynamics emerged for specific metals. Specifically, Mercury displayed an exponential relationship. Arsenic displayed a log-linear relationship most commonly. The relationship was unable to be determined for lead, copper, and others. Declining trophic levels caused the metal concentrations to shift downward in absolute value but have only subtle changes in directionality.

Looking at the previous research: This is one of the first studies to look at seabird contaminant concentrations over such a long time scale. Additionally, a literature review revealed inconsistencies in the present studies that look at trophic dynamics of heavy metals in coastal ecosystems. We introduce the possibility that certain metals do not accumulate up trophic levels, differing from the previous view

that all heavy metals bioaccumulate. The data is consistent with previous studies on the intense accumulation effects of mercury and arsenic, but there has been little research on the trophic dynamics of other trace elements.

The conflicting studies could mean that metals accumulate differently at different trophic levels, so the conventional log-linear model that that assumes equal change across steps lacks the ability to account for this property. Most studies also assume that all trace elements magnify, and our lack of clear declines across time and clear trophic relationships introduce the idea that some elements do not magnify through aquatic systems, and relationships would more accurately by describing by using the term “trophic transfer” instead. Seabirds are appropriate indicators for directionality in concentration trends, but extrapolation to ecosystem magnitudes should be made cautiously. Absolute values may be influenced by metal-specific affinities and site-specific conditions that must be included to create more accurate concentrations. If we want to extrapolate to the base of the food web to assess bio-available heavy metal levels, then more controlled studies in a diversity of lab and wild systems are needed to model trophic transfer of a range of metals beyond the well-established work of mercury, arsenic, and lead.

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Introduction

It is important to document heavy metal concentrations in high trophic level animals

Anthropogenic deposition and natural cycling of heavy metals can impact ecosystem function. They can accumulate in marine sediment layers and persist there for long periods of time. This means harmful effects can be seen far into the future. Their rate of dispersal across ecosystems also makes them a monitoring priority (Page et al 2014). As these metals accumulate and move through the ecosystem to higher trophic level organisms, these metals have known toxic effects including decreased reproductive success and compromised immune systems (SOURCE). Humans can experience these detrimental effects as we perform recreational activities or consume high trophic seafood species.

Heavy metals can enter aquatic ecosystems through both human actions and natural processes. Human actions can include direct industrial activity, as well as non-point pollution from agricultural production (Morais et al 2012). The biggest anthropogenic sources of heavy metals include commercial products such as batteries, paint, and plastic products. Fossil fuel mining and production is a large contributor of mercury, lead, and arsenic pollution. Cadmium, copper, manganese, and lead enter primarily through runoff from agricultural fertilizers. Lead and copper are also common ingredients in ship anti-fouling paints. Increased urbanization and population growth leads to more sewage discharge and runoff, and production of lead, chromium, and zinc have increased in the last 50 years (Nriagu, 1995). These increases precipitate the need for better understanding of how to analyze their risk using environmental monitoring.

These metals have inconsistent monitoring protocols historically, but are increasing in use due to population growth and more common use in industrial activities. Therefore, setting up processes to analyze metal concentrations in different trophic levels is of interest to many environmental managers. Water quality testing programs are becoming more common, and refined monitoring systems need to be developed.

High concentrations of heavy metal in seabirds has often been attributed to their persistent tendencies in tissues and high trophic position: heavy metals concentrations accumulate in birds throughout their lifetime through a process known as bioaccumulation. Due to the high trophic level position that most seabirds inhabit, they are exposed to increased concentrations of metal through their prey diet. This process is called trophic magnification and leads to increased body burdens and decreased rates of survival and recruitment. (Bisi 2012.) Table 1 lists our definitions of several of the terms used interchangeably in trophic dynamics studies, even though they are non-equivalent. Most of these definitions come from a paper highly cited in recent trophic studies.

Seabird feather levels may be representative of broader ecosystem signals and heavy metal cycling. They are long-lived, migratory species that eat a variety of prey species across large landscapes (Burger 2004, Keller 2009). This gives researchers an indirect look at the toxicological status of the environment as a whole, as opposed to point sampling studies that only give the water quality conditions of local geographies. It has also been shown that seabirds adjust their feeding strategies in response to climate change and fishery-driven prey availability, making them a good parallel of the changes occurring over time (Gagne 2018, Furness 1997). Other advantages to using seabird feathers is the cost-effective sampling where researchers can collect large numbers of feathers from nesting colonies that represent a larger sample area than can be easily or quickly accessed. Feathers are also a non-disruptive, non-lethal biological sample that can give both spatial and temporal information.

Trophic position of seabirds is declining; this can affect temporal trends of metal biomonitoring

Previous research by this research team has determined that many pelagic seabirds have decreased in trophic position over long time periods. While they are still considered top trophic level organisms in their ecosystems, their absolute trophic level is decreasing in value. Most time-dependent contaminant studies analyze their data under the assumption that their organisms have the same trophic level throughout the study. But figure 1, from a recent paper by my research collaborators at the Monterey Bay Aquarium, analyzed carbon and nitrogen concentrations and found a decrease in trophic position over the same time period of this analysis. While useful for documenting food web change, trophic position decline has not been considered when monitoring contaminant trends and may need to be included in future analyses given potential alteration of heavy metal concentration dynamics. This study aims to start to characterize those alterations.

In biomonitoring of heavy metal with seabirds, we rely on the notion that they predictably accumulate or dilute with trophic position, though exposure pathways via diet, living medium, and metal specific solubility can introduce ambiguity. This variability is demonstrated in the lack of consensus across a multi-study literature review across different geographic locations and taxa. We introduce the possibility that no major accumulation occurs, especially with the essential metals.

Ultimately, we want to understand trend patterns and persistence of availability of heavy metal in ecosystems, especially those known to be toxic at even minute levels. The toxicological effects of Mercury have been widely studied, but there has been much less investigation into other metals (Cardwell 2013).

Both the absolute level of metals in tissues and the nature of the relationships with those metals and trophic position is critical to understanding broader scale repercussions. Ecosystem vulnerability is also an important function to understand when it comes to prioritizing locations for management or restoration.

Species' movement through trophic levels can perpetuate uncertainty in temporal trend and magnitude

Metal specific dynamics impact results and may be unduly generalized. The different requirements of essential versus non-essential metals has limited research and should be further investigated (Cardwell 2013). Some trace elements, including Manganese, Iron, Nickel, Copper, and Zinc are more bioactive than other species, which contributes to different transportation pathways. Previous studies have concluded that the trophic level of birds have an effect on their metal accumulation (Abbissi 2014).

If metal concentrations in high trophic position species across time is important as an indicator, then what is the extent of impact of declining trophic position over time? This study aims to determine whether declining trophic positions causes a shift in how metals accumulate in seabirds; this becomes important when using seabirds as an ecological health indicator. Baseline concentration data and knowledge of dynamics are necessary to recognize changes in those relationships. Comparing baseline concentrations to known health thresholds is how environmental managers determine the risk of certain areas from heavy metals in the environment.

The purpose of our project was to 1) document historical change in tissue metal concentration and 2) begin to characterize the implications of this uncertainty by assessing the ability to transform seabird concentrations of each metal to those indicative of ecosystem baselines by developing a trophic transfer factor, and 3) determine if declining trophic levels clouds this transformation. I also offer suggest where this information may be applied to regulatory protocols put in place by federal law. We do this with the acknowledgement that this is done with the best data available and may need to be revisited once further ecological data is collected.

Materials and Methods

Specimen collection, processing, and lab analysis

We sampled eight seabird species: Laysan albatross (*Phoebastria immutabilis*), Black-Footed Albatross (*Phoebastria nigripes*), wedge-tailed shearwater (*Puffinus pacificus*), brown booby (*Sula leucaster*), Brown Noddy (*Anous stolidus*), Bulwer Petrel (*Bulweria bulwerii*), Sooty Tern (*Sterna fuscata*), White Tern (*Gygis alba*), and White-tailed Tropicbird *(Phaethon lepturus*). The specimens date from 1891 to 2015, covering almost 13 decades. The specimens are located in a museum collection at the Bernice Pauahi Bishop Museum, located on Honolulu, Hawaii. The specimens all originated from the Northwestern Hawaiian Islands, which are considered a relatively pristine environment. We collected body contour feathers to limit aesthetic impact to the specimens. Fully-developed flight feathers were obtained from the more recent specimens. These feathers were collected by Gagne (2018) for a trophic position study published in 2018. Mr. Gagne and Dr. Van Houtan are contributing authors on this study and gave permission for the use of trophic position data found in that paper.

Collected feathers were cleaned with compressed air to remove debris and were stored in polyethylene bags. We homogenized the individual feathers and sent the samples to the UC Davis Stable Isotope Facility (SIF) for CSIA-AA stable isotope and heavy metal contaminant analysis. Gagne (2018) had assays conducted for nine elements: Arsenic, Cadmium, Copper, Iron, Lead, Manganese, Mercury, Molybdenum, and Zinc. For values below the detection limit, the reference limit of the metal halved was used for statistical analysis. This was based on the procedure used by Lucia (2012).

Raw data manipulation

On our heavy metal data, we used general linear modeling using R (v3.4.3, R Development Core Team 2017) to show changing trends in concentration across time in each seabird species. We interpolated for years in which we did not have data. We also used a winsoring technique to exclude measurements outside the 90% confidence levels to limit skewing of trend lines. While all transformations were done on each seabird species individually, the average of the ensemble was used in the final figures for clarity. Figure 2 displays the initial findings of concentrations across time.

Trophic Transfer Factor (TTF) approximation

We fit a three-degree smooth spline model with a constrained origin to a concentration-trophic level plot using data from Campbell's 2005 food web analysis. This is a polynomial regression that allows for both exponential and potentially non log-linear relationships to be approximated. The curves were fitted using data points that come from concentration measurements and trophic position data found from Campbell's publication, using carbon and nitrogen concentrations. The point along the curves where the trophic position of our seabirds lay are interpreted as a multiplicative corrective factor for our own concentration data. The location of our code is identified in the Supplements. This model is organized by metal in figure 3, to demonstrate the element-specific relationships that emerged.

Metal concentration correction

To adjust the concentration found in the feathers to a baseline concentration, we created applied the TTF model to the known trophic position of species taken from Gagne et al. 2018a. For every concentration measurement, there is corresponding trophic position data taken at the same time. We found the trophic position on our TTC model, chosen from amongst our supporting literature, and thus found the appropriate corrective factor. We divided our concentration by this corrective factor to determine the concentration of the base level of the food web, which we determined as 1 ppm.

RESULTS

Apparent long term declines in lead, arsenic, mercury

The corrected trend across the entire time course of study is on average persistently declining for lead, arsenic, and mercury. This compares to long term declines observed for worldwide Mercury emissions. (Pacyna and Pacyna 2001). Declines are also reasonable when the introduction of the EPA in 1988 is considered, which started limiting the emissions of several heavy metals and trace elements used in this study.

Figure 4 show an adjustment in absolute concentration between our feather concentrations with the concentrations manipulated using the trophic transfer model, but a relatively similar directional trend.

Corrections do introduce some short periods of possible increases depending on species and metal. This could be due to local activities, but further information would be required for an accurate explanation. The delayed date of decline beyond the implementation of supposed legislation may be a function of environmental persistence due to the long half-life of the metals we are studying.

Trends suggest consistent levels in essential trace metals zinc, manganese, copper

There is no significant trend for zinc, manganese, and copper in any of the species under study. The concentration trend did not display any linear or logistic relationships but rather maintained a roughly constant concentration.

Inconclusive patterns in Molybdenum, Cadmium, and Iron

For molybdenum, cadmium, and iron, there are unclear trends across the time of the study. For these metals, more than one species displayed conflicting trend patterns, and the average trend of all species did not display a significant directional pattern.

TTC approximation yields varied trophic transfer dynamics of specific metals

We chose Campbell's study as the TTC model the lookup because it contained the longest range of trophic positions in one study, and also studied a pelagic ecosystem. We conducted an in-depth literature review prior to our analysis, and when compared to the same analyses applied to other studies, we observed different trophic transfer dynamics for several metals. This comparison is displayed in figure 2. Mercury had a similar exponential relationship across all 3 studies. Arsenic displayed a log-linear relationship in two of the three studies. However, the relationship is not resolved for elements such as lead, copper and others, as there was no significant relationship. Figure 3 presents the log-linear relationships with TP for mercury and arsenic, but less clear for other metals, especially the semi-essential trace elements

TTF correction doesn't appear to effect trend assessment particularly strongly given the range of tissue concentrations observed and the range of TP decline, though, it can have a large impact on the magnitude when estimating ecosystem baseline concentrations.

Seabirds have demonstrated to be relatively robust indexes for trend direction, but extrapolation to ecosystems magnitudes of parts per million availability is tenuous.

Discussion

Feather tissue and baseline corrected declines suggest broader scale declines in lead, arsenic, and mercury in seabird tissues

This is one of the most comprehensive analyses of metal concentrations in seabirds across long time scales, and allows us to observe long term shifts. Both the uncorrected feather concentrations and the corrected baseline concentrations displayed general declines across the time period of the study. Function of historical high impact legislation? This corresponds to national legislation in the 1960s and 1970s, including the Clean Air and Clean Water Act of 1972, and the Oil Pollution Act of 1990 (NOAA). These pieces of legislature make direct deposit of pollutants into waterways illegal without a permit, and also created water quality standards with which companies must remain in compliance (EPA website). The decreasing concentrations in the seabird feathers demonstrate that these laws and consequences for non-compliance have been effective in decreasing pollutant discharge.

The lack of consisting evidence for the other trace elements demonstrate that uptake of these metals differs greatly amongst even highly related species and it is much harder to utilize multiple species without comparative corrective factors.

Conventional TTF calculation may oversimplify the dynamics of metals in food webs

Regressions are used as a predictive model based on a curve fit through a dataset, with several different kinds available. Linear regressions are the most simple equation, but assume a linear relationship between the data and can miss fine-scale shifts. To predict non-linear fits, polynomial regressions are used. These take the original predicted points to a power and create a polynomial function for the fit line. The most commonly used are cubic and quadratic, taking more points and forcing the curve to pass through more points. However, higher power functions can lead to overfitting.

To avoid overfitting, regression splines can be used. Splines are piece-wise regressions used on continuous data where the dataset is divided and regressions are done on each section of the data. For the highly variable data used for our calculations, a cubic spline was found to fit the data without curving so much that overfitting occurred. We chose a smooth spline to determine the trophic transfer factor because it allows for error and is based off the relationship between two points, rather than the entire data set.

Log of concentration as a linear relationship with trophic level is often used with methylmercury and pollutants like DDT/PCBS appear to hold true in mercury, arsenic, and often lead, though does not appear as generalizable in situations of non-pollutant, or bio-soluble, or semi-essential compounds.

Conventional log-linear regressions utilize the trophic transfer factor as an average of the entire sampled system (Mackay 2016). This approach constrains the models from incorporating trophic level-specific relationships. The studies in our literature view all use this approach, but their variability in the tropic level range studied led to the appearance of different trophic relationships for the same metal. Species at different trophic levels have different assimilation capabilities for heavy metals. These differences are especially dramatic when comparing species such as shellfish, detritivores, and apex predators. Since metals accumulate differently at different trophic levels, a regression that that assumes a linear change across steps lacks the ability to account for this property.

Other tests also have potential, but non-parametric tests like the Mann-Kendell test can't be used when there are alternating trends in the data, which was observed in the data.

We chose our selection of contaminants because there has been identified hazardous limits and pathologies to humans and people or have been linked to anthropogenic activities. Most conventions define heavy metals as metals with a specific density > 5g cm^{-3}. Future contaminant studies might do well to assess metals based on their biological properties, rather than generalizing across all contaminants. Similar studies have determined that the human health risk of certain metals such as Cr, Cd, and Hg may be less than previously thought.

*Seabirds appear to be still reliable indices for change in heavy metals, but not suitable approximates of ecosystem baseline concentrations***.**

For this study, we assume that trophic changes are based primarily due to diet shifts (Borga 2012). The plots of both the raw concentration data and the adjusted concentration data displayed the same directional trends across time. However, the values of the concentrations are not within the published standard limits published by the Environmental Protection Agency (EPA.). Without laboratory diet studies, it is difficult to parse out the source of the exposure.

Absolute values may be influenced by metal-specific affinities and site-specific conditions that must be included to create more accurate concentrations. There has not been enough cross-environment studies completed to have robust adjustment measures currently. Therefore, if we want to extrapolate to the base of the food web to assess bio-available heavy metal levels, then more controlled studies in a diversity of lab and wild systems are needed to model trophic transfer of a range of metals beyond the well-established work of mercury, arsenic, and lead.

Contaminant Monitoring and Environmental Management

Concentrations at the base of the food web and those available in the abiotic environment is a valuable piece of information for chemical management (Borga 2012). Understanding the process of using high trophic organism data to look at trophic levels can also be used to determine historical concentrations in ecosystems before benthic sampling began.

Future studies might take field-based benthic sampling data and use it to determine how accurately this trophic transfer model quantifies concentrations at the base of the food web. This will determine whether expensive and labor-intensive studies that sample the intermediate trophic stages are necessary to create adequate trophic transfer factors. Other useful tools might be a database of data based on ecosystem types to help standardize trophic analysis in management while still encompassing site-specific qualities.

Relevant Marine Pollution Laws and Regulations and Ecosystem System Monitoring

Pollution control policies can be addressed using governmental regulation, as well as scientific assessments (Gray JS 2009). There are global and national regulations currently in place for monitoring and restricting emissions for several specific metals. However, there are uncertainties surrounding some of the trace elements and are currently used without as strict restrictions.

The first big law concerning water pollution was the Water Pollution Control Act of 1948, to reduce pollution in interstate waters. This program was expanded through the Clean Water Act and Clean Air Act, both enacted in 1972. This requires The Environmental Protection Agency (EPA) to set standards for chemicals considered hazardous. They also currently conduct annual water quality sampling to determine ecosystem health and risk to human health for heavy metals. (Wen 2016.) Other current pollution monitoring techniques include NOAA's Coastal Zone Management Plan, which focuses on non-point pollution control and requires states and territories to create non-point pollution control programs. U.S. Geological Surveys National Water Quality Assessment program does long-term monitoring. NOAA's Mussel Watch Project uses bi-annual mussel collection to assess pollution trends and areas at risk. Using seabirds as monitors might lend information for years without collection or areas that don't have large populations of mussels, but have large numbers of seabirds (NOAA).

Some global measures include the Minamata Convention on Mercury in 2013 to reduce world-wide emissions of mercury.

On the domestic scale, The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), enacted in 1980, makes government agencies responsible for responding to waters contaminated with hazardous wastes. This project highlights the need for more research on trace elements to ascertain whether they should be considered hazardous and require agency response.

Other federally managed databases that this information could be stored in and utilized from include the EPA Center for Exposure Assessment Modeling (CEAM) and the EPA Environmental Monitoring Methods Index, which contains a list of pesticides and other substances routinely monitored by the EPA.

The EPA, USGS, and the National Water Quality Monitoring Council (NWQMC) all manage the Water Quality Portal, which may benefit from the inclusion of analyses from historical samples to supplement years with less monitoring data. Regional Natural History collections may also offer information to complete ecosystem dynamics in specific areas and allow for more specific modeling.

Acknowledgements

I begin by thanking the Monterey Bay Aquarium for allowing this opportunity to collaborate, and the Bishop Museum in Honolulu for providing the samples for this analysis. I sincerely thank my research partners at the Aquarium, especially my research partner Tyler Gagne for contributing significantly to the coding analysis, figure creation and preliminary framework of the discussion. I thank my advisor Dr. Stuart Pimm for ensuring the creation of clear results and a robust project, and my fellow Masters students who also spent the summer in California for sharing this learning experience with me.

Figures

Figure 1

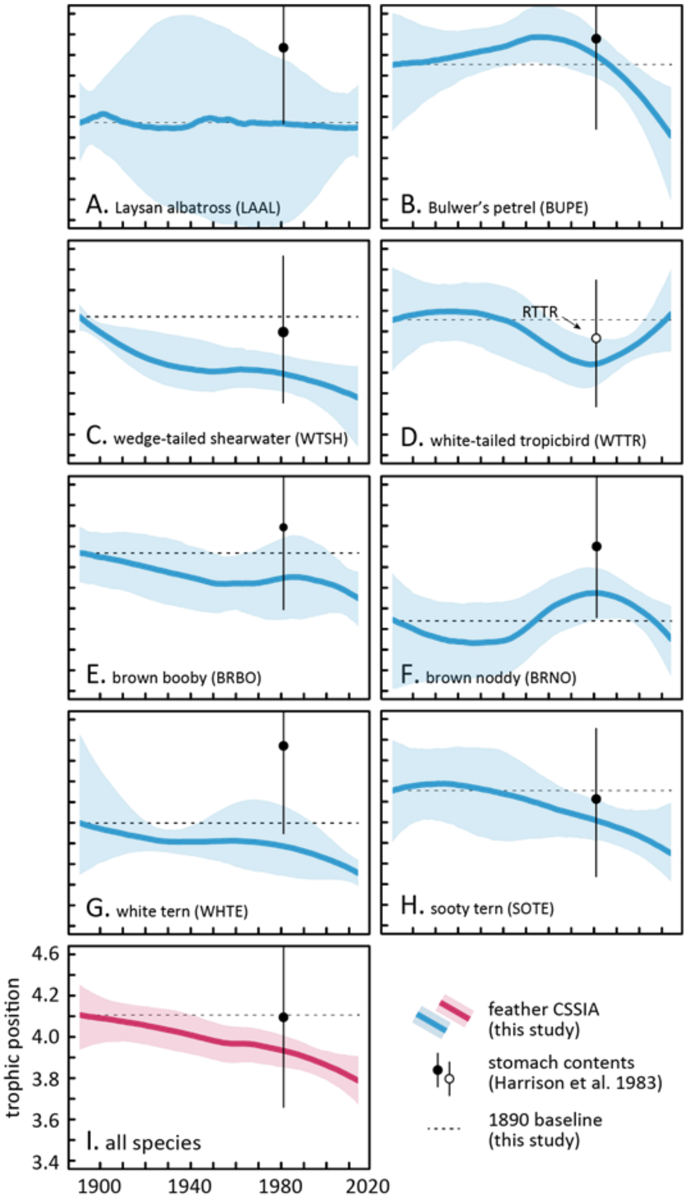

Figure 1 depicts the decreases in trophic position of seven species of pelagic seabirds between 1900 and 2016, previously determined and published by members of this research team using the same stable isotope data used in this analysis. This data was used in this study as the most accurate trophic position of each seabird we studies. Each plot is separated by species. This figure is taken from recent publication Gagne et al 2017, who is a co-author on this study.

Figure 2

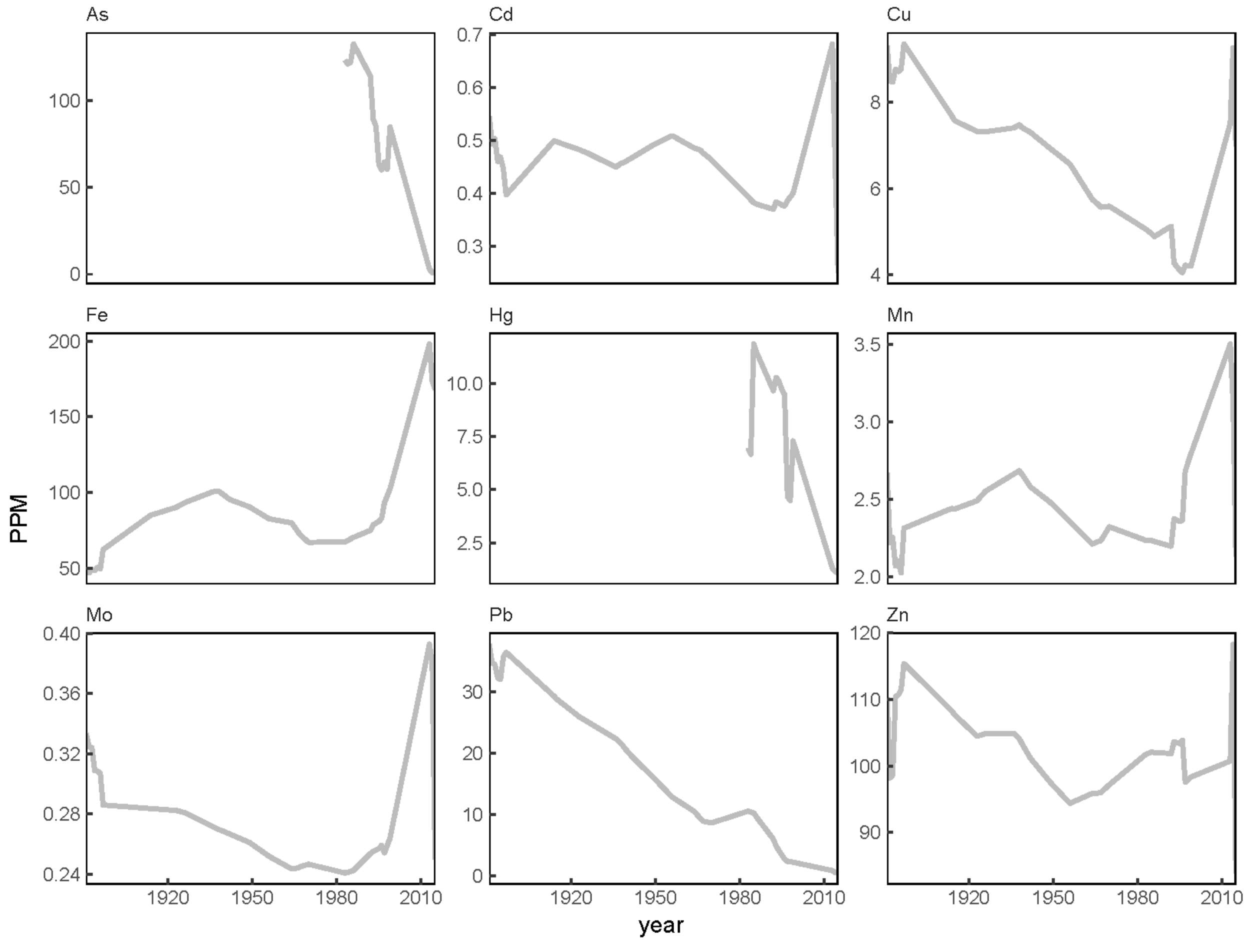

Figure 2: These plots display the interpolated concentrations of each of our elements of interest over the time scale of the study. Arsenic and Mercury only have concentration data modeled for 1980 onward due to preservative techniques prior to this that contained them. Since each species varies in how dramatically their concentrations decrease, these plots display the average of the ensemble of all species for each metal.

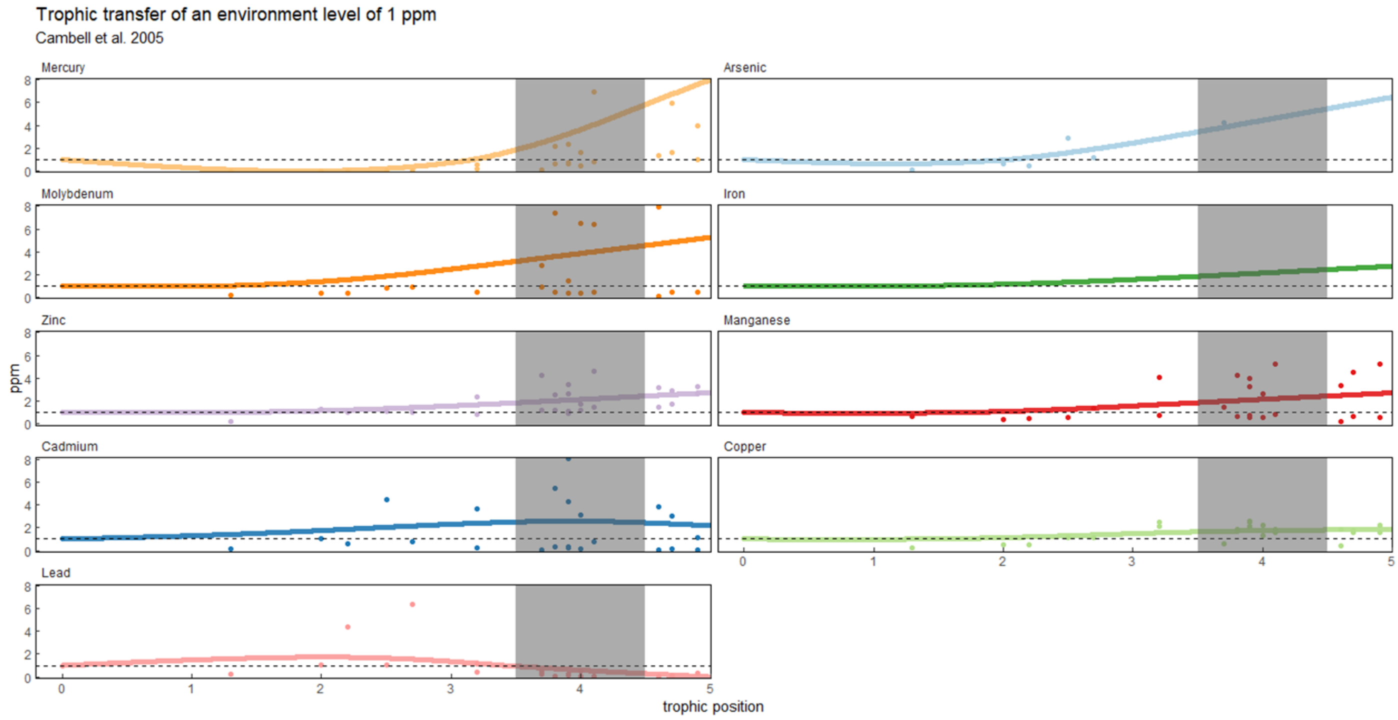

Figure 3: This picture depicts the trophic transfer quantification for each metal, based on Campbell's 2005 ecosystem study. The fitted line was created using a logistic spline, with the concentration data and the decimal trophic position data found from the same publication. Each metal is plotted separately to account for metal-specific absorbance in tissue. Iron has no data points since it was not included in Campbell's study and was modeling using the most similar metal included.

Figure 4

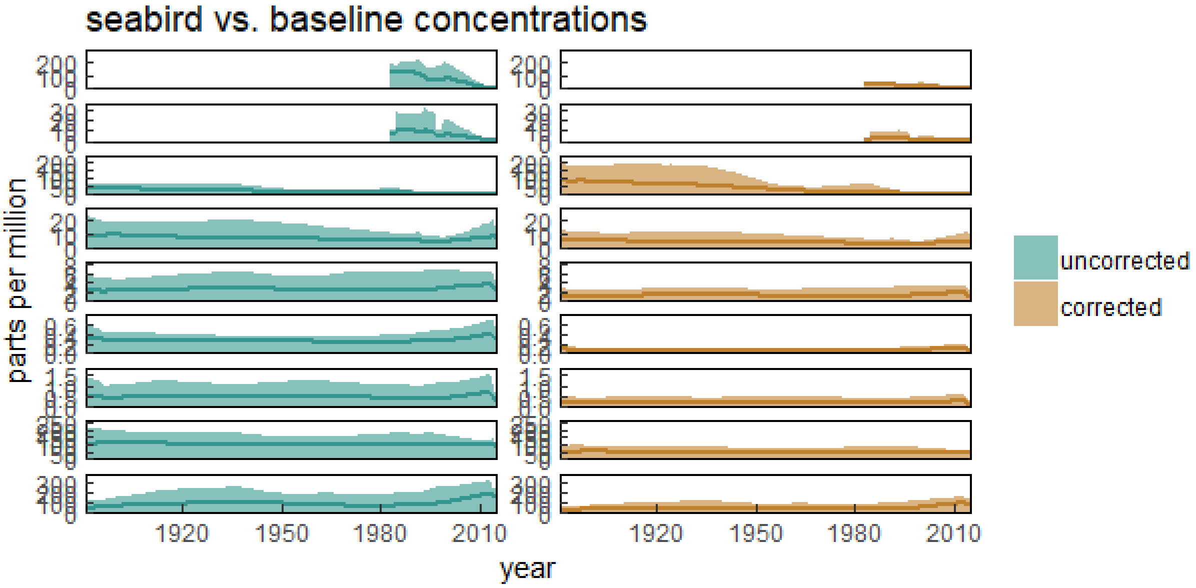

Figure 4 compares the initial concentrations found from the feather analysis and the corrected concentrations found after applying the trophic transfer factor to demonstrate concentrations at trophic level 1, assumed to be 1 ppm. Each row represents a different metal. The major finding here is that by changing the trophic level, the magnitude of concentration changes dramatically. The colored bands surrounding each trend line represented the confidence interval, which is significantly larger in the plots for the baseline trophic level.

Table 1.

Term	Definition
Bioconcentration	The uptake of a contaminant by an aquatic organism where water is the sole contaminant source
Bioaccumulation	The uptake of a contaminant from both water and sources
Biomagnification	The process of both bioconcentration and bioaccumulation that result in increased tissue concentrations of a contaminant as it passes through two or more trophic levels
Biodilution	The decrease in concentration of a pollutant with an increase in trophic level
Trophic Transfer Coefficient	Defined herein as the concentration of a contaminant in consumer tissue divided by the concentration contaminant in preceding trophic level. Simply, a measure of the potential for a contaminant to biomagnify or dilute

All definitions taken from Macek et al. 1979 and Campbell et al. 2005

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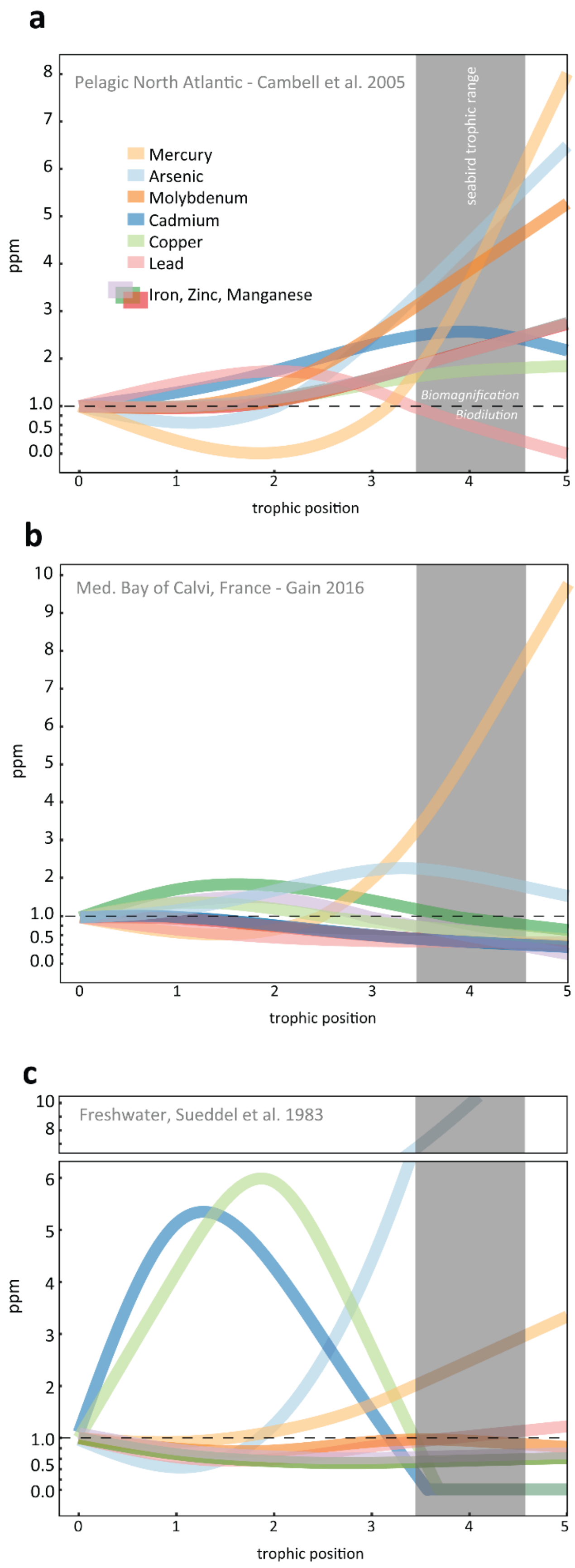
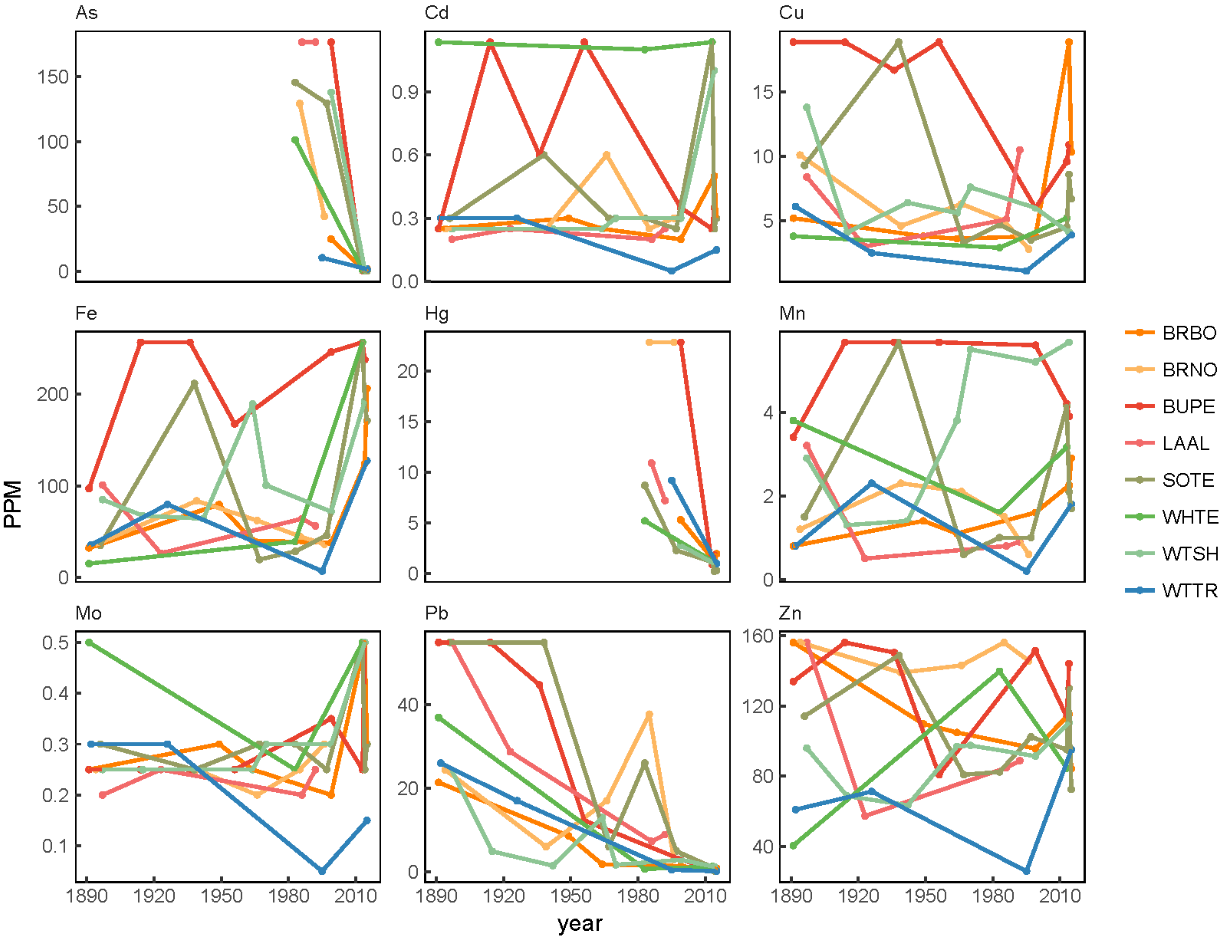
All Code for the statistical analysis can be accessed at the GitHub Repository found at this url:

https://github.com/lizmjohnson/heavy_metal_birds

Supplemental Figures



Supplement 1: This compares a few of the ecosystem studies we included in our literature review. A) Cambell et al 2005 is the model ecosystem we used. B) Suedel et al 1995 was a comprehensive trophic transfer review at the time of its publication, but included both freshwater, marine, laboratory, and field studies. C) Gain et al 2016 was also a pelagic bay environment and created similar curves to the Cambell model. All metals are graphed on the same plot for each study. Due to the differences in ecosystem type and trophic range included, they produce different curves when the same metals are modeled using the same spline.



Supplement 2: This plots the unaltered concentrations of each metal from 1890-2016 for each of the 9 seabird species included in the study. As and Hg have data from 1980 onward to avoid artificially high concentrations due to the use of these metals in museum specimen preservation protocols prior to 1980.