



# Mercury contamination and stable isotopes reveal variability in foraging ecology of generalist California gulls



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## ABSTRACT

Environmental contaminants are a concern for animal health, but contaminant exposure can also be used as a tracer of foraging ecology. In particular, mercury (Hg) concentrations are highly variable among aquatic and terrestrial food webs as a result of habitat- and site-specific biogeochemical processes that produce the bioaccumulative form, methylmercury (MeHg). We used stable isotopes and total Hg (THg) concentrations of a generalist consumer, the California gull (*Larus californicus*), to examine foraging ecology and illustrate the utility of using Hg contamination as an ecological tracer under certain conditions. We identified four main foraging clusters of gulls during pre-breeding and breeding, using a traditional approach based on light stable isotopes. The foraging cluster with the highest  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  values in gulls (cluster 4) had mean blood THg concentrations 614% (pre-breeding) and 250% (breeding) higher than gulls with the lowest isotope values (cluster 1). Using a traditional approach of stable-isotope mixing models, we showed that breeding birds with a higher proportion of garbage in their diet (cluster 2: 63–82% garbage) corresponded to lower THg concentrations and lower  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  values. In contrast, gull clusters with higher THg concentrations, which were more enriched in  $^{15}\text{N}$  and  $^{34}\text{S}$  isotopes, consumed a higher proportion of more natural, estuarine prey.  $\delta^{34}\text{S}$  values, which change markedly across the terrestrial to marine habitat gradient, were positively correlated with blood THg concentrations in gulls. The linkage we observed between stable isotopes and THg concentrations suggests that Hg contamination can be used as an additional tool for understanding animal foraging across coastal habitat gradients.

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## 1. Introduction

Animals integrate unique signatures of habitat use, geography, and diet into body tissues from their prey, and therefore animal tissues can reveal elements of foraging ecology that can be unobtainable from direct observation or use of electronic tracking instruments (Ramos and González-Solís, 2012). Common ecological tracers used to study animal foraging ecology include light stable isotopes (e.g. C, N, H, and S) and fatty acids (Budge et al., 2006; Hobson, 1999; Inger and Bearhop, 2008; Peterson and Fry, 1987), and less commonly used tracers include environmental contaminants (Adams and Paperno, 2012; Calambokidis and Barlow, 1991; Catry et al., 2008). Contaminants, such as heavy metals and persistent organic pollutants, are often studied because of their potential impact on organism and ecosystem health (Tanabe, 2002; Wiener et al., 2003). However, some contaminants bioaccumulate in

organisms and biomagnify in upper trophic level predators, which can allow these contaminants to serve as an ecological tracer and reveal elements of animal foraging ecology such as habitat type or location. Contaminants such as heavy metals and persistent organic pollutants are often non-uniformly distributed in the environment (Chasar et al., 2009; Meijer et al., 2003; Roscales et al., 2010), which may enable them to be used as tracers of habitat use at a regional or local scale.

Mercury (Hg) contamination in particular has many characteristics that may make it a useful ecological tracer. The unique processes that control methylmercury (MeHg) production from inorganic Hg are highly localized and vary substantially among habitat types (Eagles-Smith et al., 2016; Marvin-DiPasquale et al., 2003). As such, the biogeochemical processes influencing MeHg bioavailability in the environment can result in high variability in MeHg concentrations across aquatic and terrestrial habitats (Ullrich et al., 2001). Localized production of MeHg directly influences MeHg bioaccumulation in upper-trophic level predators because MeHg is the form of Hg that bioaccumulates in organisms and biomagnifies with increasing trophic level (Ullrich et al.,

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2001). Consequently, different habitat types in close proximity can contain markedly different MeHg concentrations in similar organisms (Chen et al., 2005; Eagles-Smith and Ackerman, 2014). For example, fish and bird MeHg concentrations varied up to 4-fold and 11-fold, respectively, in adjacent wetlands with different biogeochemistry (Ackerman et al., 2014a; Eagles-Smith and Ackerman, 2014). Additionally, MeHg concentrations can vary among similar habitats but geographically disjunct sites based on the availability of MeHg at the base of the food web and localized bioaccumulation processes (Eagles-Smith and Ackerman, 2014; Evers et al., 2011; Scudder et al., 2009). Furthermore, as a result of biogeochemical processes and differences between aquatic and terrestrial food webs, MeHg concentrations often are much higher in animals deriving their diet from aquatic, rather than terrestrial, habitats (Ackerman et al., 2016b; McGrew et al., 2014; Ochoa-Acuña et al., 2002; Post, 2002).

MeHg and stable isotopes in consumer tissues represent an integrated diet over varying lengths of time, depending on the tissue (Lewis and Furness, 1991; Vander Zanden et al., 2015; Wang et al., 2014), and variability in these tracers can reveal different components of animal foraging ecology (Caron-Beaudoin et al., 2013; Moreno et al., 2010; Ramos et al., 2009). Trophic position within a habitat can be established using  $\delta^{15}\text{N}$  values and MeHg concentrations (Anderson et al., 2009; Campbell et al., 2005). Habitat type and sources of primary productivity to a food web can be discriminated using carbon isotope ratios, although  $\delta^{13}\text{C}$  values also increase with trophic level but to a lesser degree than  $\delta^{15}\text{N}$  values (Inger and Bearhop, 2008; Peterson and Fry, 1987). Sulfur isotope ratios, considered to have low or undetectable levels of fractionation with trophic position, have been effectively used to reveal habitat use along a terrestrial to marine gradient for multiple taxonomic groups (Barros et al., 2010; Cotin et al., 2011; Fry and Chumchal, 2011; Lott et al., 2003; Ramos et al., 2009; Zazzo et al., 2011). MeHg concentrations can also relate to habitat use along a terrestrial to marine gradient, likely as a result of sulfate reduction and increased methylation in specific habitat types (Gabriel et al., 2014; Gilmour et al., 1992). Moreover, MeHg concentrations in organisms may reveal additional aspects of an animal's foraging ecology, including separation of foraging locations or specific habitats, than traditional ecological tracers (Adams and Paperno, 2012; Catry et al., 2008). For example, in a study where stable isotopes were inconclusive in differentiating foraging ecology of tropical seabirds, the addition of MeHg resulted in the ability to differentiate foraging locations (Catry et al., 2008). Therefore, MeHg may be a useful tracer to differentiate diet and foraging strategies for generalist species that forage across a range of diverse habitats, and complement traditional ecological tracers like light stable isotopes.

We used a combination of total Hg (THg) concentrations and light stable isotopes (nitrogen, carbon, and sulfur) of a generalist predator, the California gull (*Larus californicus*), to examine variability in foraging strategies and to illustrate the utility of using Hg contamination as an ecological tracer of foraging ecology. While in the San Francisco Bay Estuary (California, USA), California gulls can access marine and estuarine prey resources, as well as terrestrial anthropogenic diet sources associated with several large landfills along the bay margins. We used a traditional approach of using light stable isotopes to identify clusters of birds with similar foraging ecology during the pre-breeding and breeding time periods, and then examined if foraging clusters of gulls could be differentiated based upon their Hg contamination. We hypothesized that gulls from different foraging clusters would vary in their use of terrestrially-derived prey (from landfills), and that gulls with higher proportions of diet derived from landfills would result in lower Hg contamination than gulls with a higher proportion of diet derived from aquatic, estuarine prey. Additionally, because sulfur isotopes are strongly reflective of foraging along a terrestrial to

marine gradient, we examined whether  $\delta^{34}\text{S}$  values directly related to THg concentrations to demonstrate if Hg concentrations could link to animal foraging ecology.

## 2. Methods

### 2.1. Sample collection

We captured adult California gulls in 2007 and 2008 using rocket nets (Dill and Thornsberry, 1950), remotely detonated net launchers (Coda Enterprises, Mesa, Arizona, USA), and bow nets at three breeding colonies in south San Francisco Bay, California, USA (A6, Coyote Hills, and Mowry colonies; Ackerman et al., 2014b) from 6-March to 26-April, prior to the breeding season, and from 15-May to 30-May, during the breeding season. We captured chicks by hand from 16-June to 3-July, late in the breeding season. We collected approximately 2 mL of whole blood from the brachial vein using 23–25 gauge needles with a 1 or 3 cc syringe from all birds for both total mercury (THg) and stable isotope analyses, and held blood samples on ice while in the field. Additionally, we collected a drop of blood from the majority of adult birds for sex determination using the chromo-helicase-DNA binding protein gene (Zoogen Services, Inc., Davis, California, USA). We measured culmen length, bill depth at the gonys, head-to-bill length, and flattened wing length to the nearest 0.01 mm using digital calipers. Birds were also weighed to the nearest 1.0 g using a 1-kg Pesola spring scale (Pesola AG, Baar, Switzerland), which we used as a proxy for body condition (Labocha and Hayes, 2012). We used a discriminant function, based on gull morphometric measurements (Herring et al., 2010), for sex determination of adults in the cases where we did not have genetic results. All birds were temporarily held in screen-lined and shaded poultry cages (model 5KTC, Murray McMurray Hatchery, Webster City, Iowa, USA) at the capture site until they were sampled, banded with a U.S. Geological Survey (USGS) aluminum leg band, and subsequently released. Birds were captured and marked under a California State Scientific Collection permit, Federal Bird Banding permits, Federal Fish and Wildlife permits, and research was conducted under the guidelines of the USGS Western Ecological Research Center Animal Care and Use Committee.

To examine potential dietary sources for breeding adult California gulls, we obtained reference prey samples from April to August in 2007 and 2008 from estuarine habitats adjacent to breeding colonies and from terrestrial, human-sources including local markets and the Newby Island landfill. Gull use of sampling sites was confirmed with concurrent radio telemetry tracking (Ackerman et al., 2016c). We collected potential prey samples ( $n = 409$ ) from salt ponds, including brine shrimp (*Artemia franciscana*) and two fish species, three-spined stickleback (*Gasterosteus aculeatus*) and long-jawed mudsucker (*Gillichthys mirabilis*). We collected American avocet (*Recurvirostra Americana*) and Forster's tern (*Sterna forsteri*) eggs ( $n = 51$  and  $n = 26$ , respectively) and muscle samples from pre-fledged chicks ( $n = 17$  and  $n = 17$ , respectively), similar to methods described in Ackerman et al. (2011). Consumption of avocet and tern eggs and chicks in San Francisco Bay is well documented for California gulls (Ackerman et al., 2014b,c). To represent small mammals living in the terrestrial environment surrounding the wetland habitats frequented by gulls, we collected house mice (*Mus musculus*;  $n = 6$ ). From the local landfill and food markets, we collected samples representing common foods, including chicken, turkey, pig, cow, bread, potatoes, rice, and vegetables ( $n = 74$ ).

### 2.2. Mercury determination

We analyzed approximately 100  $\mu\text{L}$  of liquid whole blood (hereafter blood) for THg using a Milestone DMA-80 Direct Mercury

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