



Original Articles

Trace element contamination in feather and tissue samples from Anna's hummingbirds



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ABSTRACT

Trace element contamination (17 elements; Be, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Ba, Hg, Tl, and Pb) of live (feather samples only) and deceased (feather and tissue samples) Anna's hummingbirds (*Calypte anna*) was evaluated. Samples were analyzed using inductively coupled plasma-mass spectrometry (ICP-MS; 17 elements) and atomic absorption spectrophotometry (Hg only). Mean plus one standard deviation (SD) was considered the benchmark, and concentrations above the mean + 1 SD were considered elevated above normal. Contour feathers were sampled from live birds of varying age, sex, and California locations. In order to reduce thermal impacts, minimal feathers were taken from live birds, therefore a novel method was developed for preparation of low mass feather samples for ICP-MS analysis. The study found that the novel feather preparation method enabled small mass feather samples to be analyzed for trace elements using ICP-MS. For feather samples from live birds, all trace elements, with the exception of beryllium, had concentrations above the mean + 1 SD. Important risk factors for elevated trace element concentrations in feathers of live birds were age for iron, zinc, and arsenic, and location for iron, manganese, zinc, and selenium. For samples from deceased birds, ICP-MS results from body and tail feathers were correlated for Fe, Zn, and Pb, and feather concentrations were correlated with renal (Fe, Zn, Pb) or hepatic (Hg) tissue concentrations. Results for AA spectrophotometry analyzed samples from deceased birds further supported the ICP-MS findings where a strong correlation between mercury concentrations in feather and tissue (pectoral muscle) samples was found. These study results support that sampling feathers from live free-ranging hummingbirds might be a useful, non-lethal sampling method for evaluating trace element exposure and provides a sampling alternative since their small body size limits traditional sampling of blood and tissues. The results from this study provide a benchmark for the distribution of trace element concentrations in feather and tissue samples from hummingbirds and suggests a reference mark for exceeding normal. Lastly, pollinating avian species are minimally represented in the literature as bioindicators for environmental trace element contamination. Given that trace elements can move through food chains by a variety of routes, our study indicates that hummingbirds are possible bioindicators of environmental trace element contamination.

1. Introduction

Hummingbirds are a widespread and abundant taxon in the Americas, and are under-studied both in terms of the effects of trace elements on their health as well as their potential as indicators of pollination services and urban ecosystem health (Godoy et al., 2014). Despite their importance as pollinators, the relationship between hummingbirds and environmental contamination is largely unknown

with the exception of one published study (Góngora et al., 2016). Hummingbirds could be useful bioindicators both for pollination services as well as urban ecosystems, as they are easily captured, widespread, abundant, and their ecology intersects with multiple habitat types and trophic levels. When evaluating hummingbird trace element exposure, a factor to consider is their insectivorous foraging behavior that varies by species, life stage, sex, season, location, and time of year (Powers et al., 2010; Remsen et al., 1986; Stiles, 1995;

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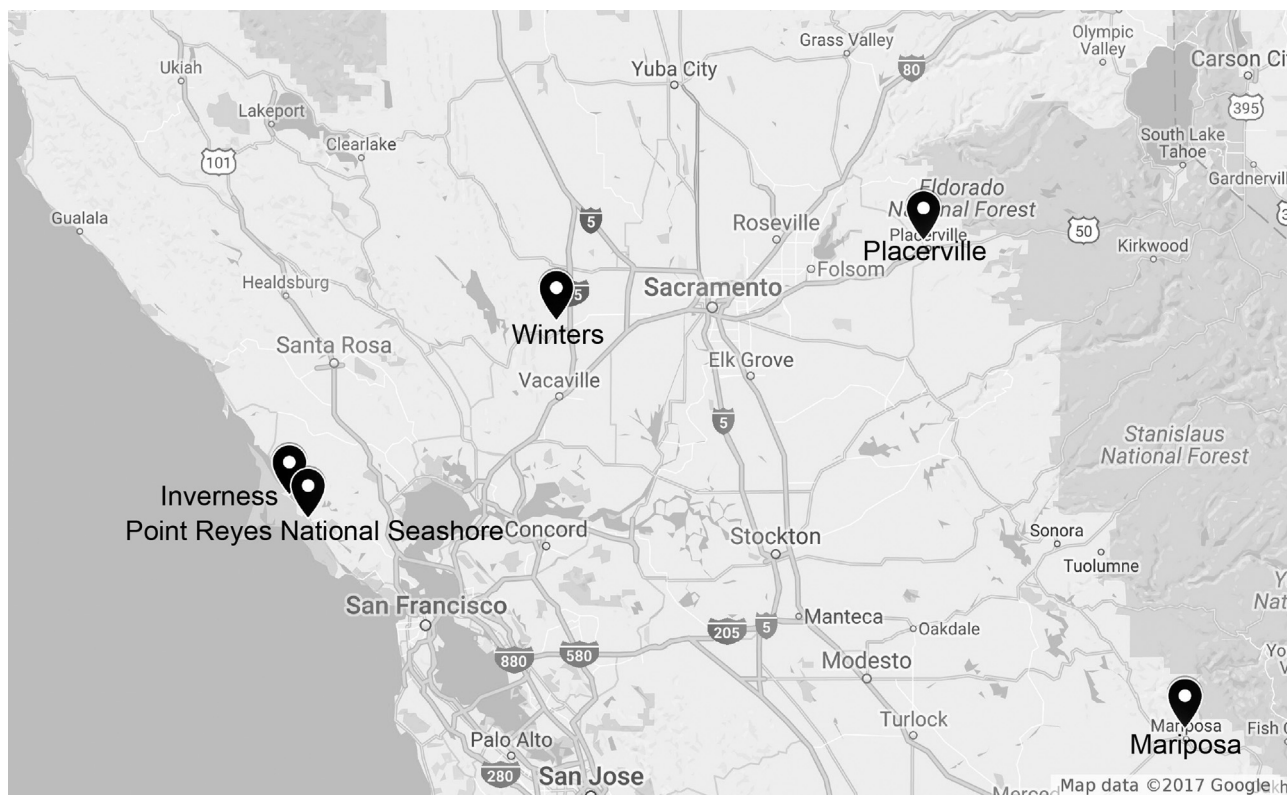


Fig. 1. Location sites for collection of feather samples from live Anna's hummingbirds. California areas sampled (from west to east) include Inverness, Point Reyes National Seashore, Winters, Placerville, and Mariposa.

Preest et al., 2003; Brice and Richard Grau, 1991). Therefore, when using hummingbirds as bioindicators, researchers should consider the potential for trace element burdens to be related to consumption of contaminated insects (Hare and Landis, 1992; Beck et al., 2013; Kidd et al., 2012).

The Anna's hummingbird (*Calypte anna*) is an important species for monitoring the health of the California Floristic Province (CFP), a biodiversity hotspot in western North America, due to this species' nectarivorous and insectivorous feeding habits and broad geographic range (Dobson et al., 1997; Myers et al., 2000). As a year-round resident of the CFP, the Anna's hummingbird may also serve as a sentinel species for other pollinators and pollination services that are vital to sustaining endemic plant diversity.

In general, avian pollinators are poorly represented in the literature as bioindicators for environmental trace element contamination. However, recent studies have suggested that trace elements move through food chains by a variety of routes, and can therefore potentially affect a broader diversity of animal groups and ecosystem services than has previously been investigated (Ahmadpor et al., 2016). For example, the majority of research evaluating bioindicators for methylmercury (MeHg) has been focused on piscivorous animal species (Evers et al., 2005; Ackerman et al., 2008) that typically have higher MeHg concentrations than other foraging groups of animals (Ackerman et al., 2016b). However, insectivorous species can also be a pathway for methylmercury from aquatic to terrestrial ecosystems (Cristol et al., 2017; Albert et al., 2013; Chaves-Ulloa et al., 2016). More research is needed to establish indicator species for trace element exposure representing alternative foraging animal groups, such as pollinators.

Avian overexposure to Pb, Hg, Se, Cd, Zn, and As has been linked to reproductive failure, impaired growth, and behavioral alterations (Beyer et al., 2004; Custer et al., 1986; Scheuhammer, 1987; Binkowski et al., 2016a; Álvarez et al., 2013; Govind and Madhuri, 2014; Martínez-López et al., 2005). Liver, kidney, and bone have been used to assess specific trace element concentrations in internal tissues

(Binkowski et al., 2016b; Binkowski et al., 2013a, 2013b) and feathers have been shown to reflect trace element burden (Dauwe et al., 2003; Burger and Gochfeld, 1997; Honda et al., 1986; Scheuhammer, 1987; Ahmadpour et al., 2016; Karimi et al., 2016). Feathers have been used to estimate bird exposure to lead, mercury, zinc, iron, copper, and arsenic and in some cases elemental exposure has been correlated with impaired physiological function in birds (Burger et al., 1994; Burger and Gochfeld, 1997; Burger et al., 1997; De Bruin and Goede, 1986; Honda et al., 1986; Borghesi et al., 2016).

The relevance of feathers for trace element biomonitoring depends on the trace element and context of exposure. Feathers differ as an index of trace element exposure compared to other tissue and blood samples because they can represent trace element blood concentrations at the time of feather molt (De Bruin and Goede, 1986; Eagles-Smith et al., 2008). Feathers have also been shown to sequester certain trace elements during feather growth that become biologically inert once molt is complete (Karimi et al., 2016). One study recommended blood as the biological sample of choice for mercury exposure in live birds (Ackerman et al., 2016a). However, for extremely small birds, such as hummingbirds, sampling enough blood volume from live birds is often not possible, as it exceeds their physiological limits. Therefore, despite their limitations, feathers might be the least invasive and most readily available material for evaluating trace element contamination in live hummingbirds.

In this study, we performed an evaluation of trace element contamination in Anna's hummingbirds sampled in California and assessed the feasibility of using small mass feather samples from live Anna's hummingbirds as a bioindicator for trace element contamination in pollinators. We had two main objectives: 1) to assess if trace element concentrations in small mass feather samples from live hummingbirds could be quantified and reliably reflect exposure to numerous trace elements and 2) to analyze feather and tissue samples from Anna's hummingbird carcasses to see what trace elements the birds are being exposed to and to evaluate how trace element concentrations in feather

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