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Commentary

Relationships between stable isotopes and metal contaminants in feathers are spurious and biologically uninformative

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Relationships between metals and stable isotopes in feathers are biologically uninformative because of the differences in integration times.

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ABSTRACT

Stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are used frequently in ecotoxicological investigations of birds to relate contaminant levels to trophic position ($\delta^{15}N$) or foraging location ($\delta^{13}C$) and many researchers using avian feathers in such investigations use $\delta^{13}C$ or $\delta^{15}N$ as a predictor of contaminant concentrations. Contaminants, especially mercury, however, are integrated into feathers over different time periods than are stable isotopes, resulting in spurious relationships that have no biological meaning. I show the fundamental principles behind the conclusion that relating $\delta^{13}C$ or $\delta^{15}N$ to contaminant concentrations in feathers is not appropriate in light of the number of recent studies that have employed this approach, and make recommendations for those wishing to investigate the relationship between contaminants and stable isotope ratios.

1. Introduction

Since landmark papers by Keith Hobson and colleagues in the 1990s (Hobson and Clark, 1992a,b, 1993; Hobson, 1995), stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) have become widespread in avian ecology (Inger and Bearhop, 2008; Bond and Jones, in press). Combined with an increased understanding of feather moulting patterns (Pyle, 1997, 2008), stable isotopes have allowed researchers to investigate aspects of avian biology that were previously challenging (e.g., during migration or non-breeding season). This is because tissues reflect the stable isotope ratios of the individual's diet at the time of synthesis (Hobson and Clark, 1992a, 1993). While there remains much to be learned about how stable isotopes behave physiologically (Martínez del Rio et al., 2009), their use is frequently touted as a cutting-edge technique that is often employed (e.g., Barrett et al., 2007), yet there remain challenges in interpreting stable isotope values in both a physiological and ecological context (Bond and Jones, in press).

One field that has made particular use of stable isotopes is ecotoxicology (Jardine et al., 2006), especially studies concerning marine birds (e.g., Bearhop et al., 2000; Nisbet et al., 2002; Braune et al., 2005; Ricca et al., 2008; Bond and Diamond, 2009a). This is because $\delta^{15}N$ increases from prey to consumer (Minagawa and Wada, 1984), meaning that $\delta^{15}N$ can act as a continuous measure of trophic position. Additionally, $\delta^{13}C$ varies in the oceanic environment with inshore carbon sources enriched in ^{13}C as compared with offshore sources (Peterson and Fry, 1987; Kelly, 2000), but also at much larger scales (Quillfeldt et al., 2005), allowing an investigation of the relationship between foraging area and contaminant concentrations (e.g., Ricca et al., 2008; Bond and Diamond, 2009a; Anderson et al., 2010). In this regard, advances in the application stable isotope analysis to ecotoxicological studies have granted greater insight into patterns of contamination among sites, species, and individuals.

Implicit, yet seldom acknowledged, in studies that relate $\delta^{13}C$ or $\delta^{15}N$ to contaminant concentrations is the assumption that both measures are comparable. It makes little biological sense to examine contaminant levels from Species A and relate it to the isotope ratios from Species B, and just as little sense to compare the contaminant concentrations accumulated over Time Period X with the stable isotope values obtained during Time Period Y. In other words, the integration times of each measure of interest (contaminant and stable isotope) must not only be similar, but must also reflect the same ecological or ecotoxicological process for them to be comparable directly. Such is not often the case when feathers are considered.

Feathers are used widely in contaminant studies (Burger, 1993), and were adopted early in the development of stable isotope applications (Hobson and Clark, 1992a; Thompson et al., 1995; Hobson et al., 2002), yet almost immediately the differences in integration times between contaminants and isotopes was discussed (Thompson et al., 1998; Bearhop et al., 2000). It is therefore

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concerning to find that authors analysing stable isotopes and contaminant concentrations in feathers are still attempting to make inferences using the relationship between $\delta^{13}C$ or $\delta^{15}N$ and contaminant concentrations (e.g., Nisbet et al., 2002; Anderson et al., 2009, 2010; Ramos et al., 2009).

The most commonly-studied metal contaminant in marine birds is mercury (Hg, Burger, 1993), so I will now restrict the discussion to the relationship between Hg and stable isotopes, although similar processes are almost certainly involved with other metalloids and heavy metals. Hg is obtained via ingestion of contaminated prey and is eliminated through guano, eggs, and feathers (Braune and Gaskin, 1987b; Monteiro and Furness, 1995, 2001). Once ingested, Hg travels through the blood (Kahle and Becker, 1999) to the liver, where some of the more toxic methylmercury (MeHg) is likely converted to inorganic Hg (Spalding et al., 2000; Burger and Gochfeld, 2002). The remaining MeHg is maintained in a body reservoir until it can be eliminated through moulting feathers or eggs (Braune and Gaskin, 1987a; Monteiro and Furness, 2001; Rumbold et al., 2001; Bond and Diamond, 2009b). The Hg in feathers is therefore the difference between Hg ingestion and the sum of Hg detoxification and Hg elimination in other feathers or via eggs. On an annual basis, feather Hg concentrations depend on not only dietary intake, but also the progress of moult, timing of sampling in relation to egg laying (for females), and the previously accumulated, but not yet eliminated Hg in the body pool or reservoir.

Feathers reflect the isotopic composition of the bird's diet while the feathers are growing, and once fully grown, they are inert and do not receive any additional nutrient input (Hobson and Clark, 1992a). The duration of flight feather moult scales allometrically with body size and growth rates can range from 1.7 to 11.0 mm d^{-1} (Rohwer et al., 2009). And since only a small amount of each feather is required for stable isotope analysis (approximately 0.25 mg, Bond and Diamond, 2009a), there could potentially be anywhere from 5 to 1000 possible analytes for stable isotopes within individual feathers (Murphy, 1996). Feathers are inherently heterogeneous as compared with tissues such as blood, yolk, albumen, or internal tissues often used in either contaminant or isotopic investigations for this very reason. The amount selected for stable isotope analysis is likely 1–4 days' growth (Grubb, 2006; Rohwer et al., 2009), and although nutrients continue to flow into the growing feather, the period over which feathers integrate stable isotope ratios from a birds' diet is on the order of a few weeks at most.

Herein lies the disconnect: comparing Hg accumulated over a protracted period, including, possibly, some stored in body reservoirs for lengthy periods of time with the dietary information integrated over a few weeks during feather synthesis does not provide any biologically meaningful information. At best, the result is a poorly formulated point of discussion in an article that perpetuates the utility of this spurious relationship, while at worst several thousands of dollars in analytical costs could be wasted depending on the biological question of interest. Tissues that reflect local acquisition of both contaminants and diet (e.g., blood, or egg components if the species uses exogenous nutrients for egg production) are the only ones that can be used to examine the relationship between contaminants and trophic ecology using stable isotopes.

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