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Tracking variations in wetland use by breeding flamingos using stable isotope signatures of feather and blood

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ABSTRACT

This study tracks temporal variations in the habitat use of breeding adult greater flamingos (*Phoenicopterus roseus*) in the Camargue (southern France) using simultaneous sampling of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ from feather and blood of chicks at fledging. Camargue flamingos forage in a heterogeneous complex of saltpans, permanent and temporary brackish lagoons, freshwater and euryhaline marshes to provision their chicks over a two month period. Using Bayesian mixing models with diet- and tissue-specific discrimination factors and invertebrates collected from 23 locations, we investigated whether blood and feather isotopes indicated temporal variations in habitat use relative to salinity and hydroperiod. We also tested whether fledgling body condition could be explained by the isotopic signatures of their tissues. While $\delta^{13}C$ and $\delta^{15}N$ values did not differ significantly between blood and feather, marked differences were apparent in the $\delta^{34}S$ values obtained from these tissues. Saltpans (38%) and freshwater marshes (33%) appeared to be the main habitats visited by adult birds in the early phase of parental care with use of saline wetlands increasing later in the season (54%). This habitat shift may be related to the peak of resources in saltpans and the drying up of freshwater and brackish marshes by mid-summer. Habitat shift (as expressed in individual shifts in isotope values between feather and blood) was not correlated with offspring age, and differed between individuals. A negative relationship was observed between offspring body condition and feather $\delta^{15}N$, indicating that chicks fed from temporary flooded marshes fare better than those provisioned from permanent marshes. Foraging strategies of greater flamingos during parental care were heterogeneous, possibly tracking changes in resource availability as the season progressed and reflecting differences in the competitive ability of parent birds. Given that the Camargue saltpans face closure and the region's temporary wetlands remain threatened, our results emphasize the critical importance of conserving these two key habitats for greater flamingos. Dual tissue, triple-stable isotope analysis provides a useful and sensitive means of tracking localized environmental change in this threatened system.

1. Introduction

Habitat shifts during different life history stages are known to be commonplace among fish (Muñoz and Ojeda, 1998), including freshwater (e.g. Schleuter and Eckmann, 2008), estuarine (e.g. Jackson and Rundle, 2008) and reef (e.g. Wells et al., 2008) species. Such shifts are generally thought to be adaptive strategies to minimize intraspecific competition (Werner and Gilliam, 1984) or to support ontogenetic changes such as growth (Ward-Campbell and Beamish, 2005). Similar shifts in resource use are also observed in other species where offspring are supported by parental investment, including birds. After chicks have hatched, their increasing energy demands lead to greater food (nutritional) requirements. Parents that feed their offspring must respond to these changes either by increasing the quantity of food supplied (Winkler, 1987; Emms and Verbeek, 1991) or by shifting to more profitable foods (e.g. Cairns, 1987).

Identifying shifts in foraging behaviour during different stages in the reproductive process may have important consequences for delineating habitat use and prioritizing conservation areas (Ramirez et al., 2011; Brittain et al., 2012). However, direct estimation of habitat use by breeding birds is difficult. Tracking adult birds on their foraging trips usually requires either telemetric methods (Amat et al., 2005) or traditional and time-consuming approaches such as marking and resighting (e.g. Béchet et al., 2009).

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An alternative approach to tracking habitat use by breeding birds is to monitor stable isotope ratios of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulphur ($\delta^{34}S$) in multiple tissues obtained from dependent chicks (Quillfeldt et al., 2008). Carbon isotope ratios ($\delta^{13}C$) reflect the source of primary productivity and can be used as indicators of an animal's habitat utilization as well as distinguishing the nutritional composition of diet (e.g. Rubenstein and Hobson, 2004). Enrichment of $\delta^{15}N$ with increasing trophic levels provides a means of estimating an organism's relative trophic position (Peterson and Fry, 1987). Sulphur isotope ratios ($\delta^{34}S$) provide information on dietary protein source and geographical origin (Richards et al., 2003) and have been used to identify patterns relating to the salinity of feeding locations (e.g. Fry and Chumchal, 2011). As dietary nutrients are incorporated at different times depending on growth, metabolism and protein turnover of the tissue considered, different tissues from the same individual may provide information on dietary source at different phases of life history. In the case of birds, whole blood (hereafter blood) is an active tissue and its isotopic signature may represent diet integrated over weeks to about a month (Bauchinger and McWilliams, 2009), while feather is inert keratin tissue that reflects dietary sources used during feather formation. Analysis of simultaneously acquired samples can therefore reveal both spatial and temporal variations in diet.

This study aimed to investigate temporal variation in habitat use by breeding greater flamingos (*Phoenicopterus roseus*), a flagship species for the conservation of Mediterranean wetlands (Johnson and Cézilly, 2007; Béchet et al., 2012). During incubation and early chick rearing, breeding adults forage in heterogeneous and complex habitat patches with varying salinity gradients and hydroperiods (Béchet et al., 2009). Chicks are fed by parents for an extended period, being unable to feed by themselves for up to 75 days after hatching, as their bill (which is used as a filter apparatus for feeding) is not sufficiently developed (Jenkin, 1957). At this stage, parents do not regurgitate food for their young, but feed them with secretions from glands located in the digestive tract (Lang, 1963). Young birds fly and become independent 2–3 months (75–90 days) after hatching. Summer drying of marshes during the rearing season may enforce a change in habitat use to effectively exploit available resources. Furthermore, qualitative and quantitative changes in the dietary needs of developing chicks may trigger temporal variation in adult habitat use. Sex-specific habitat preferences in adults could also lead to temporal variation in chick dietary provenance, because as chicks get older, male parents typically increase the time spent feeding their offspring, while females do not (Cézilly et al., 1994).

The current study sought to test a hypothesis based on the above observations, that greater flamingos raising offspring exhibit habitat shift. Stable isotope signatures were obtained from samples of two tissue types with varying isotopic turnover rate, namely blood and feather, taken from chicks shortly before fledging. It was expected that feather and blood isotope signatures would allow for the comparison of early (~first month) versus *late* (~second month) habitat use by parent birds. However, differences in isotopic composition between diet and consumer tissues, also known as discrimination factors (or fractionation factors), are known to restrict direct interpretation of tissue stable isotopes. Usually, discrimination factors are tissue- and diet-specific (Caut et al., 2009), so applying them independent of tissue or diet isotopic values can bias the interpretation of the results and lead to erroneous conclusions. For this reason, diet- and tissue-specific discrimination factors were applied to the analyses, as described in Caut et al. (2009), allowing habitat use of breeding greater flamingos to be tracked using simultaneous sampling of feather and blood $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$.

The following predictions were made:

1 if parental habitat use tracks changes in resource availability such that offspring are provisioned from seasonally variable dietary sources, there should be a detectable shift (both in magnitude and direction) in most of the individuals examined, i.e. habitat shifts are adaptive responses to changes in resource availability;
2 if foraging habitat shifts are related to morphological changes in chicks (e.g. growth and physiological maturation), then a strong correlation between chicks age and tissue stable isotope signatures is to be expected, i.e. habitat shifts are adaptive responses to ontogenetic changes in offspring, such as growth.

This study used triple-isotopic models with 5 endpoints of salinity gradient and 3 hydroperiod to investigate whether variations in blood and feather stable isotopes indicate population-level variations in habitat use with varying salinity and hydroperiod.

2. Materials and methods

2.1. Study species and chick sampling

Greater flamingos have bred intermittently in the brackish lagoons of the Camargue for centuries (Johnson and Cézilly, 2007). Since 1974, successful breeding has taken place on a man-made island on the Fangassier lagoon (43°25′N, 4°37′E), part of the 11,000 ha commercial saltpans of Salin-de-Giraud (Fig. 1). A single egg is usually laid in April and the laying period is spread over 30–74 days. Both parents incubate and feed the chick and incubation lasts 29 days. When chicks are about three weeks old, they form a crèche (Tourenq et al., 1995) where they remain until fledging, 75–90 days after hatching.

In 2010, 45 flamingo chicks were caught just prior to fledging on day 79 (4th of August) after first egg-hatching. The following year, a further 12 chicks were captured on day 66 (27th of July 2011). Each bird was ringed with a band bearing a unique alphanumeric code. Tarsus length was measured and body weight determined using a 0–5000 g Pesola spring balance. From each chick, a few body feathers were collected and stored dry until required for stable isotope analyses. Blood (300–500 μl) was taken from 22 of the 45 birds captured in 2010 and 8 of the 12 birds taken in 2011. Samples were drawn from a vein in the leg soon after capture and kept frozen (−80 °C) until required for laboratory analysis.

2.2. Invertebrate sampling

In July 2011, samples of greater flamingo prey were collected from 23 adult flamingo foraging locations (Fig. 1; Béchet et al., 2009). In order to minimize bias that could be generated if a mixture of invertebrate prey species were to be sampled at each site, this study focused on one taxon, namely oligochaetes. Oligochaetes are abundant across the region's wetlands and their isotopic signature is believed to be broadly representative of the habitats they are collected from. Their signatures should therefore allow tracking of parental habitat use during feather and blood cell synthesis in flamingo chicks.

2.3. Salinity and hydroperiod

Salinity and hydroperiod are key factors shaping the invertebrate communities of the Camargue wetlands (Waterkeyn et al., 2008). The salinity (as the practical salinity scale) of each sampled wetland was measured *in situ* in July 2011 by electric conductivity, and varied from 3.6 in freshwater marshes to 114 in