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POPULATION ECOLOGY - ORIGINAL RESEARCH

Unexpected hydrogen isotope variation in oceanic pelagic seabirds

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Abstract Hydrogen isotopes have significantly enhanced our understanding of the biogeography of migratory animals. The basis for this methodology lies in predictable, continental patterns of precipitation δD values that are often reflected in an organism's tissues. δD variation is not expected for oceanic pelagic organisms whose dietary hydrogen (water and organic hydrogen in prey) is transferred up the food web from an isotopically homogeneous water source. We report a 142 ‰ range in the δD values of flight feathers from the Hawaiian petrel (*Pterodroma sandwichensis*), an oceanic pelagic North Pacific species, and inquire about the source of that variation. We show δD variation between and within four other oceanic pelagic species: Newell's shearwater (*Puffinus auricularis newelli*), Black-footed albatross (*Phoebastria nigripes*), Laysan albatross (*Phoebastria immutabilis*) and Buller's shearwater (*Puffinus bulleri*). The similarity between muscle δD values of hatch-year Hawaiian petrels and their prey suggests that trophic fractionation does not influence δD values of muscle. We hypothesize that isotopic discrimination is associated with water loss during salt excretion through salt glands. Salt load differs between seabirds that consume isosmotic squid and crustaceans and those that feed on hyposmotic teleost fish. In support of the salt gland hypothesis, we show an inverse relationship between δD and percent teleost fish in diet for three seabird species. Our results demonstrate the utility of δD in the study of oceanic consumers, while also contributing to a better understanding of δD systematics, the basis for one of the most commonly utilized isotope tools in avian ecology.

Keywords Hydrogen isotope · Seabird · Salt gland · Salt load · Diet analysis

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Introduction

While hydrogen isotopes are used extensively in ecological studies of terrestrial environments, most frequently to decipher the biogeography of migration, far less attention has been given to applying δD measurements to address the ecology of consumers in oceanic pelagic ecosystems (Hobson and Wassenaar 1997, 2008; Hobson et al. 1999; Wassenaar and Hobson 2000; Lott et al. 2003). This is because phototrophs that form the base of marine food webs derive their hydrogen from an isotopically homogenous water source of ca. 0 ‰, and thus, variation in δD values of oceanic pelagic consumers is not expected (Craig 1961; Lecuyer et al. 1997). Yet, we previously observed a range of 123 ‰ in the δD values of flight feathers of the

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Hawaiian petrel, (*Pterodroma sandwichensis*), a seabird of the oceanic pelagic North Pacific that does not have access to fresh water (Simons 1985; Wiley et al. 2012). For comparison, a difference in average feather δD values of 45 ‰ or less is considered informative for identifying distinct latitudinal breeding and wintering locations for terrestrial birds (Chamberlain et al. 1997; Hobson and Wassenaar 1997; Mazerolle et al. 2005; Hobson et al. 2006). We postulated that, similar to evaporative water loss, this isotopic variability in the Hawaiian petrel largely results from isotopic fractionation associated with water loss through salt glands (large, salt-excreting Glandulae nasalis: McKechnie et al. 2004; Wiley et al. 2012). Functional nasal salt glands occur in at least 13 different orders of birds and principally function to concentrate and excrete excess salt in the form of saline water (Shoemaker 1972; Peaker and Linzell 1975; Goldstein 2001). Petrels, albatrosses and shearwaters are of particular interest because they are among the species that have the most highly developed nasal salt glands and they do not have access to fresh water for most or all of their lives (Shoemaker 1972; Skadhauge 1981; Warham 1996).

In the current study, we took several approaches to investigate the "salt gland hypothesis": the idea that isotopic discrimination during water loss through salt glands results in δD variation in seabird tissues. We asked if the large variation in δD values of feathers was restricted to the Hawaiian petrel, or if it existed within and among other procellariiform seabirds with well-developed nasal salt glands: Newell's shearwater (*Puffinus auricularis newelli*), Black-footed albatross (*Phoebastria nigripes*), Laysan albatross (*Phoebastria immutabilis*) and Buller's shearwater (*Puffinus bulleri*). We also wanted to know if the large variation in δD observed in Hawaiian petrel feathers also existed in their muscle and bone collagen. Lastly, we used data from Hawaiian petrels and their prey to test an alternative explanation for δD variation, trophic fractionation (difference in δD values between muscle tissue of consumer and diet). This inquiry into isotopic variation is critical to future applications of δD in the study of oceanic consumers. More broadly, it contributes to an understanding of hydrogen isotope dynamics in consumer tissues, which forms the basis for one of the most commonly utilized isotope tools in the field of avian ecology.

Methods

Sampling

We sampled muscle, bone and flight feathers from salvaged carcasses of 80 Hawaiian petrels and 26 Newell's shearwaters. Hawaiian petrels feathers were from the islands of Lanai ($n = 10$ adults), Maui ($n = 12$ adults and 10 hatch-year birds), Hawaii ($n = 14$ adults and 10 hatch-year birds) and Kauai ($n = 12$ adults and 12 hatch-year birds) and from Newell's shearwaters from Kauai ($n = 13$ adults and 13 hatch-year birds). From Hawaiian petrel carcasses, we also retrieved samples of bone (Kauai, $n = 3$ adults, 1 hatch-year bird; Lanai, $n = 3$ adults; Maui, $n = 7$ adult, 6 hatch-year birds) and muscle (Kauai, $n = 3$ adults, 11 hatch-year birds; Lanai, $n = 7$ adults; Maui, $n = 1$ adult, 8 hatch-year birds). From Newell's shearwater carcasses, we obtained muscle from 17 adults from Kauai. All of our samples derive from birds that were depredated by introduced mammals, or were grounded by attraction to lights or collision with human-constructed structures between 1989 and 2009. Prey regurgitated by adult Hawaiian petrels from Maui during capture and release work (July and August of 2007) were provided to us by the Hawaii Department of Land and Natural Resources; these included 21 cephalopods and eight teleost fish. We also obtained samples of flight feathers from museum specimens of ten breeding age adult Black-footed albatrosses, six breeding age adult Laysan albatrosses, and eight adult Buller's shearwaters that foraged in the transition zone of the North Pacific (the region bordered by the subarctic and subtropical frontal zones), but were killed in the Pacific Driftnet Fishery in 1991 (Gould et al. 1997, 1998). These individuals were a subset of the birds studied by Gould et al. (1997, 1998), which died between September and November and had likely spent considerable time in the transition zone. We sampled the innermost secondary from Black-footed albatross and Laysan albatross, and primary 1 from Buller's shearwaters, because these feathers had a high probability of being grown during the months preceding death, while birds were at sea and not frequenting land, as was the case during the period of time studied by Gould et al. (1997, 1998) (Edwards and Rohwer 2005; Pyle 2008).

Stable isotope analyses

Feathers were washed in solvent (87:13 chloroform:methanol by volume), rinsed with ultrapure distilled water (E-Pure, Barnstead), and dried at 25 °C in a vacuum oven. For Hawaiian petrel, Newell's shearwater, and Buller's shearwater feathers, samples representative of the entire primary 1 feather vanes were taken using the barb or three-section protocols described by Wiley et al. (2010). The innermost secondary was sampled from Black-footed and Laysan albatross by homogenizing barbs taken down the length of the vanes (ca. seven sets of barbs: four from either side of the rachis taken at 2 cm intervals). Collagen was isolated and purified via the method described in Wiley et al. (2013). For δD analysis, 0.5 mg aliquots of feather vane, muscle, or collagen were weighed into 4 mm × 6 mm silver capsules and allowed to air-equilibrate with ambient

laboratory conditions for at least 2 weeks prior to analysis (Wassenaar and Hobson 2003). Following equilibration, samples were pyrolyzed at 1,425 °C in a high temperature elemental analyzer (TC/EA, Thermo-Finnigan) interfaced to an isotope ratio mass spectrometer (Thermo-Finnigan DeltaPlus XL).

Stable isotope values are expressed in per mil (‰) as:

$$\delta D = \left(\left[R_{\text{sample}}/R_{\text{standard}}\right]-1\right) \times 1,000 \quad (1)$$

where R is the D/H abundance ratio and R_{standard} is with respect to Vienna Standard Mean Ocean Water (V-SMOW). Non-exchangeable δD values were normalized to keratin standards (LA bear hair, −78.1 ‰, AK bear hair, −171.5 ‰) calibrated with respect to the standards (BWB (bowhead whale baleen), CFS (chicken feather standard), and CHS (cow hoof standard) (−106, −142 and −185 ‰, respectively; Wassenaar and Hobson 2003). Analytical precision and accuracy were ≤ 4 ‰. Since many of our samples were outside of the calibration range, several experiments were conducted to constrain extrapolation error (Kelly et al. 2009). Wiley et al. (2012) confirmed linearity when δD values were extrapolated 100 ‰ beyond the calibration range and showed an increase in error to ±8 ‰. Matrix differences may confound the interpretation of these experiments, but complex organic standards with high δD values currently do not exist (Kelly et al. 2009). Further, hydrogen exchangeability of muscle and collagen appears to be comparable to that of keratin (Wassenaar and Hobson 2000; Chesson et al. 2009; Sauer et al. 2009; Soto et al. 2011). Thus, we analyzed lipid-extracted muscle and collagen samples in the same fashion as feathers, including normalization to keratin standards (Doucett et al. 2007; Finlay et al. 2010; Cole et al. 2011; Wiley et al. 2012).

Statistical analyses

We assessed sources of variation in δD associated with four data sets using general linear modeling (GLM). The first data set included Hawaiian petrel and Newell's shearwater feathers grouped by age and island of origin (termed island-age group with age equal to hatch-year bird or adult). In the case of Newell's shearwaters, species was used in place of an island designation. For this first data set, we investigated the influence of island-age group, El Niño southern oscillation (ENSO) and year (independent variables) on feather δD values (dependent variable). ENSO was modeled using the multivariate ENSO index (MEI) from the National Oceanic and Atmospheric Administration Earth System Research Laboratory. MEI data were averaged over the approximate period of feather growth as follows: (1) Hawaiian petrel adults from Maui, October to January; (2) Hawaiian petrel adults from all other islands and adult Newell's shearwaters, November to February; (3) Maui hatch-year Hawaiian petrels, August to November, and (4) all other hatch-year Hawaiian petrels and hatch-year Newell's shearwaters, September to December. These time periods derive from Hawaiian petrel and Newell's shearwater breeding phenology and primary molt sequence for procellariiforms (Simons 1985; Warham 1996; Edwards and Rohwer 2005; Pyle 2008; Deringer and Holmes 2009).

The second data set included δD values of feather, muscle, and bone collagen from adult Hawaiian petrels from Lanai, Maui, Hawaii and Kauai and hatch-year petrels from the islands of Maui and Kauai. In the GLM, these data were grouped by tissue and age to evaluate the effects of tissue and age on δD.

The third data set included δD data for muscle from the following groups: Maui hatch-year petrels, Kauai hatch-year petrels, adult Hawaiian petrels and adult Newell's shearwaters, and fish and squid derived from adult Maui Hawaiian petrel regurgitations. The GLM was used to assess if significant differences in δD existed among these groups.

The fourth set of data consisted of δD data for feathers from Black-footed albatross, Laysan albatross and Buller's shearwater, which differed in the relative contribution of teleost fish in their diets. The GLM evaluated the effect of species on δD. To circumvent the assumption of homoscedasticity, we used a GLM that did not assume equal variance among groups, and calculated effective degrees of freedom with a Satterthwaite approximation.

All statistical analyses were performed in SAS (version 9.03.01), where the normality of residuals was evaluated using normal quantile–quantile plots. No outliers were identified, based on the Bonferroni outlier test. In figures and text, isotope values are reported as averages ± standard errors.

Results

Among 80 Hawaiian petrel feathers, the range of δD values was −81 to 61 ‰. For 26 Newell's shearwater feathers, the range in δD was −67 to 16 ‰. The average δD values of adult Hawaiian petrel feathers were similar, regardless of colony (Fig. 1). Neither MEI nor year had a significant effect on feather δD values (Electronic supplementary material 1). However, isotope values of feather differed significantly among island age groups ($n = 106$, $F_{8, 22.1} = 189.62$, $P < 0.001$). Pair-wise comparisons showed that the δD values of Hawaiian petrel adults and hatch-years from the island of Hawaii were not different ($P = 0.0799$, Electronic supplementary material 1). In contrast, adult Hawaiian petrels from Maui and adult Hawaiian petrels and adult Newell's shearwaters from Kauai had significantly higher δD values relative to corresponding hatch-year birds

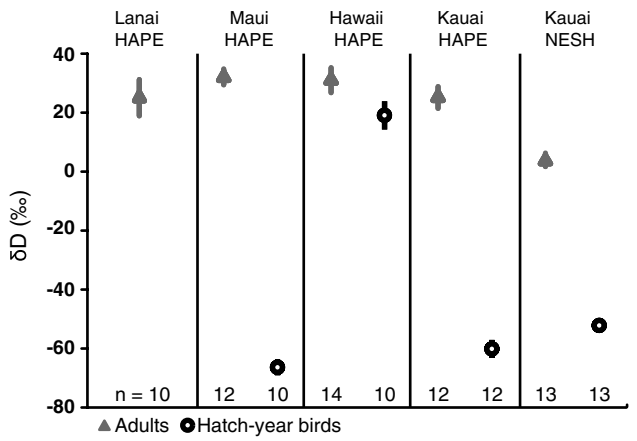

Fig. 1 Feather hydrogen isotope values (average ± standard error) of hatch-year (*open circle*) and adult (*closed triangle*) Hawaiian petrels from Lanai, Maui and Kauai and Newell's shearwaters from Kauai. The island of origin is shown at the *top* of each panel and sample size (*n*) at the *bottom* of each panel

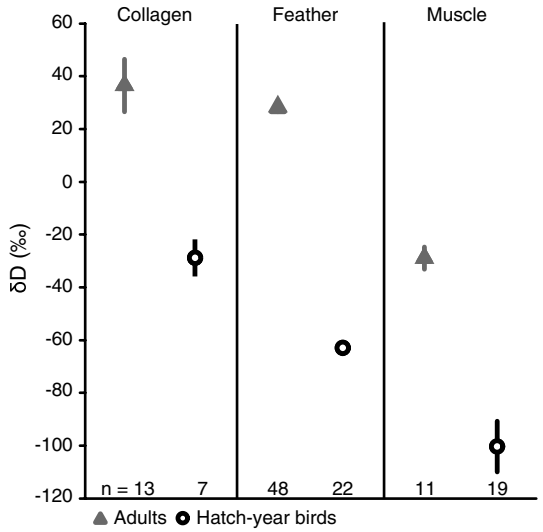

Fig. 2 Hydrogen isotope values (average ± standard error) of collagen, feather and muscle from Hawaiian petrels grouped by age. *Closed triangles* represent adults and *open circles* are hatch-year birds. Sample size (*n*) appears at the *bottom* of each panel. Collagen is from Lanai, Maui and Kauai adults and Maui and Kauai hatch-year birds. Feather is from Lanai, Maui, Hawaii and Kauai adults and Maui and Kauai hatch-year birds and muscle is from Lanai, Maui and Kauai adults and Maui and Kauai hatch-year birds. These data are used to assess whether all three tissue types exhibit a difference in δD values between adults and hatch-year birds. Feathers of hatch-year birds from Hawaii do not differ from adults, and therefore are excluded

($P < 0.001$ for all comparisons of adult and hatch-year birds from Maui and Kauai, Electronic supplementary material 1) (Fig. 1). Like other adults, adult Hawaiian petrels from Lanai had significantly higher δD values relative to hatch-year birds from Maui and Kauai ($P < 0.001$ for both comparisons, Electronic supplementary material 1) (Fig. 1).

The range of δD values of Hawaiian petrel muscle and collagen was between −112 and −6 ‰ and −53 and 111 ‰, respectively. Large differences occurred as a function of age class and tissue type ($n = 120$, $F_{5,\ 15.8} = 482.95$, $P < 0.001$) (Fig. 2). For each tissue type, adult and hatch-year δD values differed significantly ($P \leq 0.001$, pair-wise comparisons, Electronic supplementary material 2). In the case of adults, the δD values of muscle differed from those of collagen and feather, but collagen and feather δD values were similar (muscle vs. collagen, $P = 0.0003$; muscle vs. feather, $P < 0.001$; collagen vs. feather, $P = 1.000$). The δD values of collagen, feather and muscle of hatch-year birds all differed significantly from one another (collagen vs. feather, $P = 0.0039$; collagen vs. muscle; muscle vs. feather, $P < 0.001$).

Muscle δD values differed significantly between groups of consumers and prey ($n = 76$, $F_{5,\ 18.3} = 138.91$, $P < 0.001$) (Fig. 3). Pair-wise comparisons showed that hatch-year birds from Maui and Kauai did not differ significantly from either fish or squid provisioned to Maui hatch-year birds (prey found in the regurgitations of breeding Maui adults) ($P = 1.000$ for both comparisons, Electronic supplementary material 3). In contrast, the δD values of adult Hawaiian petrels and Newell's shearwaters were significantly higher than those of fish and squid provisioned

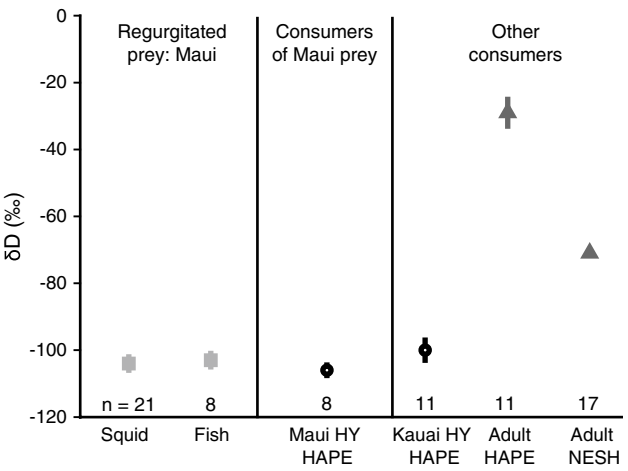

Fig. 3 Muscle hydrogen isotope values (average ± standard error) from prey regurgitated by adult Hawaiian petrels from Maui (regurgitated prey: Maui), Maui hatch-year birds (consumers of Maui prey), and Kauai hatch-year birds, adult Hawaiian petrel and Newell's shearwater (other consumers). Prey are *grey squares*, hatch-year birds are *open circles* and adults are *closed triangles*. Samples size (*n*) is at the *bottom* of each panel. Regurgitated prey from Maui adults are the putative prey of Maui hatch-year birds. Data from adults are the average of all islands

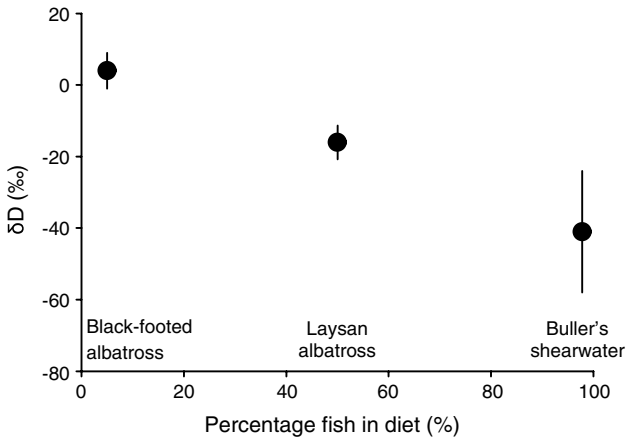

Fig. 4 δD values (average ± standard error) and samples sizes (n) of flight feathers from Black-footed albatross, Laysan albatross and Buller's shearwater vs. percent teleost fish in diet. Sample sizes for diet are 100–200 individuals, per species; data are from Gould et al. (1997) and Gould, Ostrom and Walker, unpubl. data

to Maui hatch-years ($P < 0.001$ for all comparisons, Electronic supplementary material 3).

In our data set consisting of feathers from Black-footed albatross, Laysan albatross and Buller's shearwater, the effect of species accounted for a significant proportion of the variance in δD ($n = 22$, $F_{2,\ 7.91} = 6.37$, $P = 0.023$) (Fig. 4).

Discussion

Our data show intra-specific variation in the δD values of feathers for the Hawaiian petrel and Newell's shearwater, with large differences between adult and hatch-year birds from Lanai, Maui and Kauai. While predictable continent-wide gradients in the δD of precipitation impart regional differences in the δD of bird populations of North America and Europe, large variation in δD is not expected for oceanic pelagic seabirds such as the Hawaiian petrel and Newell's shearwater (Hobson and Wassenaar 1997; Hobson et al. 1999, 2000). Similar to other seabirds such as penguins, procellariiform seabirds are not strongly influenced by precipitation or other sources of fresh water. Adult procellariiforms obtain relatively small amounts of water directly from the ocean (<2 0 % of total water influx) (Green and Brothers 1989), and instead derive the great majority of their hydrogen from dietary hydrogen (water and organic compounds in their marine prey). Penguins (*Sphenisciformes*) similarly acquire most of their water from prey (Green and Gales 1989). Hatch-year Hawaiian petrels spend <2 % of the nestling period outside of their burrows exercising their wings at the end of the fledging period, and are not known to have access to or to drink fresh water during this time (Simons 1985). Thus, hatch-year birds likely derive most, if not all, of their hydrogen from diet. Because the marine organisms that serve as a source of hydrogen to our primary study species ultimately rely on an isotopically homogeneous water source of 0 ‰, the wide range (e.g., 142 ‰ for feather) and systematic structure in δD values is remarkable (Craig 1961; Lecuyer et al. 1997).

We show that the presence of unexpected and systematic structure in the δD of seabirds (specifically, the disparity in δD values between Hawaiian petrel adults from all islands versus Maui and Kauai hatch-year birds) is independent of tissue type. In addition to feather, collagen and muscle show a range in δD values of 100 ‰ or more among individuals, with a large difference (at least 65 ‰) between the average δD values of adults and hatch-year birds. Our data also show that feather and collagen are significantly more enriched in deuterium than muscle; the average δD of adult feathers is 56 ‰ higher than that of adult muscle, and adult collagen is 65 ‰ higher than muscle. Similar trends of elevated δD values for feather relative to muscle are observed for captive Japanese quail and captive house sparrows (Wolf et al. 2011, 2012). While nutrient routing, protein turnover time and differences in the amino acid composition of tissues are known to influence carbon and nitrogen isotope variation among tissues, additional work is needed to determine the underlying processes that control δD variation among tissue types (Martinez del Rio et al. 2009).

The observation that the disparity in δD values between adult and most hatch-year Hawaiian petrels occurs in each tissue type suggests that the isotopic offset between age groups is not confined to a particular time period. Adult muscle tissue likely reflects the intake of dietary hydrogen during the past few months prior to death (Tieszen et al. 1983; Hobson and Clark 1992). As most Hawaiian petrel adults visited breeding colonies for at least 2 months before they died, their muscle δD values represent dietary hydrogen derived from prey taken during the breeding season. In contrast, adults molt and replace their flight feathers during the non-breeding season (Jehl 1982; Pyle 2008). Unlike feather and muscle, collagen integrates diet over the course of years (Rucklidge et al. 1992). Thus, year-round, adults appear to have high δD values relative to the δD values recorded by the feathers, muscle, and collagen of hatch-year birds during the breeding season.

We investigated the role of isotopic discrimination between the muscle tissue of consumer and prey muscle tissue (trophic fractionation, see Estep and Dabrowski 1980; Birchall et al. 2005; Peters et al. 2012) in controlling δD of Hawaiian petrels. There is no evidence of a trophic effect when δD values of muscle from Maui hatch-year Hawaiian petrels are compared to prey provisioned by parents. While δD values of Kauai hatch-year Hawaiian petrels

are isotopically similar to those of Maui hatch-year birds, adult Hawaiian petrels and Newell's shearwaters have δD values that are much higher than the prey provisioned to Maui hatch-years. This isotopic disparity implies that either (1) the diets of adult Hawaiian petrels and Newell's shearwaters have a much higher average δD value than do the fish and squid analyzed for our study, or (2) there is a physiological or biochemical mechanism responsible for the high δD values of adults. While our understanding of hydrogen isotope systematics in consumers of the oceanic pelagic realm is limited, the first possibility seems unlikely. Due to preferential incorporation of ^{1}H during photosynthesis, δD values of aquatic and coastal phytoplankton are typically more than 100 ‰ lower than the water from which they derive their hydrogen (Estep and Hoering 1980; Sternberg 1988). Owing to the low δD value of their water source, ca. 0 ‰, we expect oceanic phytoplankton to have δD values of ca. −100 ‰ or less. Because trophic fractionation associated with muscle appears to be negligible in our system, and because the δD of consumer tissues derives largely from food rather than water, we anticipate values of −100 ‰ or less for consumers's muscle (Wolf et al. 2011). Our data for fish, squid, and hatch-year Hawaiian petrels (average δD values of ca. −100 ‰) are consistent with this expectation. However, a deuterium-enriched dietary source would be required to account for the high δD values of adult Hawaiian petrel muscle (average −29 ‰), feather (average 27 ‰) and collagen (average 36 ‰). This seems unlikely because we do not know of a mechanism that would elevate δD values of prey from oceanic pelagic waters.

We considered the possibility that evaporative water loss, particularly associated with differences in breeding habitat and activity, could influence δD values of seabirds (McKechnie et al. 2004). Elevated cutaneous water loss and water loss during panting are expected in dry breeding habitats characteristic of Hawaii and Maui and in the case of increased physical activity (i.e., flight) (Simons 1985; Ainley et al. 1997; Hu et al. 2001). Water lost through evaporation is depleted in deuterium by ~50 ‰ relative to body water (McKechnie et al. 2004). However, a 50 ‰ change is too small to account for the >100 ‰ range observed in our data. Furthermore, we observe a large difference (86 ‰) between the average feather δD value of hatch-year petrels from Hawaii and Maui. Those two colonies are located above the cloud layer in the same dry subalpine moisture zone, where evapotranspiration is high even inside burrows (Whittow et al. 1984; Simons 1985; Ainley et al. 1997; Hu et al. 2001; Price et al. 2007). In contrast, petrel breeding colonies on Kauai are primarily in the wettest zones of the archipelago, within the montane cloud belt where evapotranspiration is low (Ainley et al. 1997; Simons and Hodges 1998; Price et al. 2007). Despite the large differences in expected evaporative water loss between Maui and Kauai hatch-year petrels, their mean δD values are very similar (Fig. 1). Thus, several lines of evidence suggest that isotope discrimination associated with water loss in arid environments is not a dominant control for our δD data. Alternatively, the inherent difference in activity levels between hatch-year petrels and adults might be expected to result in δD differences associated with evaporative water loss. Hatch-year birds grow feathers before they are able to fly, while adults grow feathers during the non-breeding season while they are in near continuous flight at-sea. However, because we see no difference between adults and hatch-year birds from the island of Hawaii, evaporative water loss associated with differences in activity level cannot be the primary factor that controls δD in Hawaiian petrels.

In addition to evaporative water loss, hydrogen isotope fractionation could occur when water is lost through salt glands, a phenomenon related to salt load. The supraorbital nasal salt glands are responsible for secreting the majority of a seabird's excess salt in the form of highly concentrated saline droplets (Peaker and Linzell 1975; Goldstein 2001). Higher salt load may occur in individuals that primarily consume prey such as squid and crustaceans, which are isosmotic with seawater as opposed to those that feed on hyposmotic teleost fish (Wiley et al. 2012). Consumption of isosmotic prey by seabirds can result in a doubling of salt load per unit energy and is associated with increased excretion through nasal salt glands (Green and Gales 1989; Goldstein 2001). Among seabirds, dietary salt load is known to differ between adults and hatch-year birds of some species. This is because parents sometimes feed their chicks a greater abundance of fish than they themselves consume (Baird 1991; Hodum and Hobson 2000). Furthermore, because the salt glands of growing hatch-year birds are not always fully developed, hatch-year birds may not have the salt tolerance of their parents and may benefit from diets relatively low in salt content (Johnston and Bildstein 1990; Dosch 1997). Even if diets do not differ, the salt load of hatch-year birds may also be lower than that of their parents because the salt content of food may be altered while in the stomach of adults. For example, in the krill-feeding Adelie penguin, stomach-pumped krill and food regurgitated for chicks contained less sodium than fresh krill (Janes 1997). Prey regurgitated for Maui hatch-year Hawaiian petrels include a combination of fish and squid (Simons 1985; Walker et al. unpublished data); we posit that adult diets are more heavily reliant on isosmotic prey, such as squid.

The possibility that dietary salt load imposes variation in δD values of seabirds is further supported by results from three seabird species that differ in the relative proportion of teleost fish in their diet: Black-footed albatross, Laysan albatross and Buller's shearwater. Samples of these species

were obtained in 1991 from the drift net fishery operating in the transition zone of the North Pacific Ocean between 1978 and 1991. We sampled individuals that had spent considerable time in the transition zone and chose feathers that had a high probability of being grown in the months preceding death, while the birds were at sea and not frequenting land (Edwards and Rohwer 2005; Pyle 2008). Our certainty about the diets of these individuals derives from a previous stomach content study (Gould et al. 1997, 1998). The Gould et al. (1997, 1998) study showed that while feeding within the transition zone, Black-footed albatross killed by drift nets consumed primarily neon flying squid (*Ommastrephes bartramii*), while Buller's shearwaters from the same drift net fishery relied primarily on teleost fish (Gould et al. 1998). Relative to these two species, Laysan albatross consumed an intermediate amount of fish and the rest of their diet was derived from squid and other invertebrates. Our data for Black-footed albatross, Laysan albatross and Buller's shearwater are consistent with the salt gland hypothesis: δD differs significantly among the three species and declines as the percent teleost fish in diet increases (Fig. 4). Thus, hydrogen isotope values may offer a useful biochemical signal of intra-specific and inter-specific dietary segregation in oceanic seabirds, by providing a simple proxy for the relative proportion of fish (or invertebrates) in the diet.

Conclusions

Contrary to expectation, our data show a broad range in δD values for five pelagic seabird species that forage within an isotopically uniform ecosystem. Conventionally, variation in δD values of avian tissues is ascribed to differences in isotope values of precipitation, evaporative water loss, or trophic level (Hobson and Wassenaar 1997, 2008; McKechnie et al. 2004; Birchall et al. 2005). We propose that salt load may be an important control of δD values for species that derive the majority of their dietary hydrogen from oceanic pelagic prey or that seasonally rely on saline resources. In either case, increased consumption of isosmotic prey may lead to elevated δD values. In our study species, δD data suggest that relative to adults and hatch-year Hawaiian petrels from the island of Hawaii, hatch-year Hawaiian petrels from the islands of Kauai and Maui and hatch-year Newell's shearwaters consume more teleost fish than they do isosmotic invertebrates. δD values may also be a useful tool in addressing questions related to the diet or salt load in other species that possess functional nasal salt glands, including a variety of seabirds (e.g., penguins, albatrosses, gulls, gannets, puffins) and other birds (some ducks, geese, grebes, hawks, roadrunners and ostriches) (Shoemaker 1972). Owing to the unexpected δD structure we found in oceanic pelagic seabirds, there is a platform for continued investigation into these possibilities. Moreover, the observation that a potential dietary signal is present in the δD of bone collagen affords the opportunity to study historical samples to reconstruct temporal changes in foraging habits.

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