Niche partitioning among and within sympatric tropical seabirds revealed by stable isotope analysis

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STABLE ISOTOPES SUGGEST NICHE PARTITIONING AMONG SYMPATRIC TROPICAL SEABIRDS

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Running Head: Stable isotopes show resource partitioning in tropical seabirds

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ABSTRACT

Despite the low productivity and ephemeral and patchy nature of resources in tropical waters, stable isotopic data from this study suggests that substantial resource partitioning occurs among tropical seabird communities. In this study we compared δ\(^{13}\)C and δ\(^{15}\)N levels in feathers across eight sympatric tropical seabird species; for a subset of these species we also compared isotopic levels in blood, and examined variation across years and sexes. We found that while there is low total variation in both δ\(^{13}\)C and δ\(^{15}\)N across the eight seabird species examined, all species occupied a distinct isotopic niche when both breeding and non-breeding periods were evaluated. There was only slight variation in the pattern of resource partitioning between breeding and non-breeding periods. Notably, there was a strong correlation between body mass, wing span, and wing loading ratios on foraging area, evaluated by δ\(^{13}\)C levels, which is also coincident with estimates of field metabolic rate. Isotopic separation by age and year, within species was also observed; however separation by sex appeared to be relatively uncommon even in sexually dimorphic species. As a group, seabirds were isotopically distinct both from their prey and from other marine predators. Overall, the results are generally consistent with what is known about the at sea distribution and diet of these seabirds, and with patterns of stable isotope partitioning among these species in other locations. Still, several results, including low δ\(^{13}\)C of black noddies (*Anous minutus*), high δ\(^{15}\)N of white terns (*Gygis alba*), and strong correlation of δ\(^{13}\)C to body size and metabolic rate merit further examination. More research on isotopic cartography of tropical oceans, species specific fractionation rates, and stable isotopes of prey are needed to evaluate the usefulness of stable isotopes in identifying resource partitioning in tropical marine environments.
INTRODUCTION

Open oceans in tropical environments generally have low productivity, patchy and unpredictable distribution of prey, and low structural complexity (Ballance et al 1997, Longhurst and Pauly 1987, Weimerskirch 2007). High level marine predators thus face many foraging challenges in locating prey and are generally physiologically constrained for energetically efficient travel and foraging behavior (Weimerskirch et al 2004, Bertrand et al 2002). For tropical seabirds this has generally limited them to foraging within the first several meters of the sea surface, and they are often reliant on subsurface predators to drive food to the surface, further increasing patchy nature of food resources (Ballance et al 1997). Yet, despite these strong constraints, diverse predator, and particularly seabird communities, occur; this leads to questions about the degree, nature, and mechanisms of resource partitioning in these extremely homogenous and resource poor environments.

Resource partitioning in tropical oceanic environments has been well documented for temperate and polar seabird species (Ainley et al 1992, 1994), but remains an area of much inquiry for tropical seabird species particularly in open ocean environments (Harrison and Seki et al 1987, Catry et al 2009). Tropical seabird diets are more diverse than their temperate and polar counterparts, and there can be a high degree of overlap in diets and foraging areas (Ainley and Boekelheide 1990, Ballance et al 1997, Catry et al 2009). Thus, these observations have led to questions about the degree to which tropical seabird species are able to partition resources.

There have been multiple studies of diets of seabird communities in tropical environments, but these have generally been constrained to the breeding season (Ashmole...
to comprehensively explore the diet of non-breeding tropical seabird communities found
higher degrees of resource portioning than found in a similar study of polar seabirds
(Spear et al 2007). They found partitioning by species, sex, age, foraging strategy, and
body size. However, at-sea surveys of seabird diets have some inherent limitations for
answering questions about resource partitioning. Since seabirds are typically sampled
lethally, each animal contributes only a single data point in space and time, and it is thus
not possible to examine changes in individual foraging behavior across space and time.
Also, since reproductive status cannot be confirmed, it is not possible to link foraging
behavior to reproductive status, although it is clear that reproductive status influences
foraging ability. Spatially extensive survey efforts may also cross community boundaries
for both seabirds and prey species, resulting in comparisons of foraging ecology of
species that have limited co-occurrence. Collections across longer time periods may
compare foraging across heterogeneous temporal periods.

Data on resource partitioning of tropical seabirds is also available from direct
comparisons of foraging behavior across species via various methods of electronic
2010). However, cost, size, and logistical constraints associated with these methods have
been generally limited to comparisons involving two or three species, with emphasis on
larger species during breeding periods (Ropert-Coudert and Wilson 2005).

The usefulness of stable isotope analysis (SIA) as a tool for understanding
resource partitioning is providing a low impact way to examine resource partitioning,
both spatially and by trophic level. Stable isotopes of nitrogen and carbon in seabird
tissue reflect those values seen in their prey and give insight into origin and type of prey consumed. Stable carbon isotopes of seabirds and seabird prey show evidence of an increasing enrichment in inshore as compared to offshore feeding animals in both tropical and temperate areas (Hobson et al. 2004, Cherel et al. 2008). Stable nitrogen isotopes increase in a predictable matter with each trophic exchange, and thus indicate trophic position of a consumer (Vanderklift and Ponsard 2003). Crucial to seabird studies, since different tissues integrate these stable isotopes over different periods of time, a single bird can provide integrated information on diet over a period of time ranging from days to even years, depending on the tissue or tissues analyzed (Dalerum and Angerbjörn 2005).

Multiple studies have now used these SIA to understand resource partitioning among seabird communities (REFS). SIA have now yielded important insights into subtle changes in foraging ecology based on reproductive stage (e.g. Awkerman et al. 2007), age (e.g. Forero et al. 2002), colony location (e.g. Jaquemet et al. 2008), sex (e.g. Bearhop et al. 2006), migratory patterns (e.g. Cherel et al. 2000), as well as other factors (e.g. Cherel et al. 2005, Phillips et al. 2009). These studies have been consistent with data observed via direct tracking both in temperate and tropical systems (Phillips et al. 2009, Weimerskirch et al. 2009b, Young et al. 2010). Since SIA allows for fairly robust, low impact method to resolve spatial and trophic separation among species, it is a promising tool to examine resource partitioning among seabirds in tropical environments, particularly for those species (e.g. terns, noddes, and small petrels) that are too small for current tracking methods. There have been a few studies that used SIA to examine resource partitioning on a community scale in the tropics (Cherel et al. 2008, Catry et al. 2008, Kojadinovic 2008). Those studies, all conducted in Southern Indian Ocean, reached very different
conclusions, both in the degree of resource partitioning and the usefulness of isotopes as a tool for identifying foraging patterns in the tropics. Our study site, Palmyra Atoll (equatorial Pacific), is quite different from the other studies in that Palmyra is far (1000’s km) from any continental shelf or coastal habitat, and has lower heterogeneity in oceanic conditions (productivity, SST, bathymetry) and thus may more typify open ocean conditions experienced by many tropical seabirds (Weimerskirch et al 2005, Catry et al 2008).

The aims of the present study are 1) determine if there is significant isotopic partitioning in tropical seabirds living in an open ocean environment; 2) compare SIA results to data from conventional stomach content analyses, at-sea surveys, and known natural history to evaluate the usefulness of SIA as a tool for examining resource partitioning in among sympatric tropical seabirds; 3) compare patterns of isotopic partitioning in blood and feathers to evaluate differences in partitioning in breeding (from blood) and non-breeding (from feathers) periods; and 4) compare isotopic levels among different age classes and sexes of the same species and over multiple consecutive years. Specifically, we examined stable isotopes of carbon and nitrogen from nine species of sympatric seabirds at Palmyra Atoll. This included multiple congeneric species, and species with similar foraging strategies, where fine-scale niche partitioning might be particularly important for mitigating competitive interactions.

**METHODS**

Our research was conducted at Palmyra Atoll National Wildlife Refuge (5.867° N, 162.067° W). Palmyra Atoll is a low-lying tropical atoll located in the Line Island chain
of the central Pacific Ocean. It is situated at the boundary of the eastern cool tongue and western warm pools of the Pacific, on the boundary of the intertropical convergence zone (Longhurst and Pauly 1987). Palmyra is composed of a ring of calcium carbonate derived islets encircling three saltwater lagoons. The land of Palmyra is predominantly forested; *P. grandis* and *T. argentea* forest provide extensive nesting habitat for tree nesting birds and occasional herbaceous and bare patches (including two maintained areas) serve as nesting areas for ground nesting birds (Young et al 2010). The surrounding waters are uniformly low in productivity (mean of 0.14 mg chlorophyll a /m³), warm (mean sea surface temperature of 21.3˚C), and deep (except in immediate vicinity of the Line Island chain, surrounding waters are > 1000 m). The seabird community at Palmyra consists of 10 breeding species from 1) Order Charadriiformes - sooty terns *Sterna fuscata* (125,000-220,000 pair), white terns *Gygis alba* (~200 pair), brown noddies *Anous stolidus* (~500 pair), black noddies *A. minutus* (~1000 pair), and 2) Order Pelecaniformes - greater frigatebird *Fregata minor* (~250 pair), red-footed boobies *Sula sula* (~2500 pairs), brown booby *S. leucogaster* (~400 pairs), masked booby *S. dactylatra* (~35 pairs), and red-tailed tropicbirds *Phaethon rubricauda* (~150 pairs), and white-tailed tropicbirds *P. lepturus* (~10 pairs) (Fefer 1987, Young et al *unpublished data*). At Palmyra these birds breed asynchronously throughout the year.

**Sample collection**

We collected feather samples from 9 of the 10 species that breed at the atoll (all except white-tailed tropicbirds). Samples were collected from breeding adults (either incubating or chick rearing), with the exception of samples from white terns, where reproductive...
status was unknown. All samples were collected in July 2009, except for sooty terns, which did not breed in summer 2009. Samples from sooty terns were thus collected in July 2008. For red-footed boobies, additional samples were collected from breeding adults in two previous years (July 2007 and July 2008) for interannual comparisons. For five species we also analyzed feathers collected from chicks in 2009. Feather tissue from brown noddies and wedge-tailed shearwaters (not a breeding species at Palmyra) were not included in statistical analyses due to small sample sizes. Birds were sexed using voice, plumage, and/or molecular sexing methods (Young et al 2010). We used unabraded underwing contour feathers (Jaeger et al 2009). Body masses of remaining species, plus wingspan and wingloading for all species were estimated from Hertel and Ballance (1999) and Spear and Ainley (1999). Field metabolic rate (kJ/day) was estimated from allometric equations for Pelicaniformes, Charadriformes, and Procellariformes (Shaffer in review).

For the three boobies, the frigatebird, and the sooty tern, blood was also collected from a brachial vessel from a subset of the individuals that were sampled for feathers. Muscle tissue (breast) was collected from red-footed boobies, masked boobies, black noddies, and sooty terns (chicks only) found dead in the breeding colony.

Diet samples were collected when spontaneously regurgitated. The best-preserved specimens of the common Exocetidae (flying fish) and Ommastrephidae (flying squids) from these diet samples were used for the isotopic analyses. Muscle tissue of Clupeiformes (herring and anchovy), small baitfish common in the diets of many of these species (Catry et al 2009) was directly collected from below a seabird feeding aggregation immediately off the atoll. Muscle tissue from pelagic fish predators (wahoo,
Acanthocybium solandri; yellowfin tuna, *Thunnus albacores*) was collected from animals captured from within 3km of the reef immediately surrounding the atoll. *Sthenoteuthis* spp. (jumbo flying squid) was captured approximately 700 km from the atoll.

**Sample preparation and isotopic analyses**

Feathers were washed in DI water, dried at 60°C for storage, and subsequently cut into fine pieces for analysis. Blood, diet, and muscle tissue samples were all preserved frozen at -80°C. They were then freeze dried and ground to a fine powder. We did not extract lipid from any tissues as C:N ratios were always less than 4.0, and usually less than 3.5, suggesting lipid levels were low across all samples (Post et al 2007).

Stable isotopic ratios of C and N were analyzed at the Stanford Stable Isotope Biogeochemistry Laboratory (SIBL) using a Thermo Finnegan Delta-Plus XP IRMS. Replicate laboratory standards of graphite (USGS 24), ammonium sulfate (IAEA N1), and acetelanalide internal to each run show analytical error of less than 0.2‰ for both C and N.

**Isotopic interpretations**

The interpretation of carbon isotope values presented in this paper, are based on the assumption that the established inshore/offshore gradient of carbon-13 in seabird diets (Cherel and Hobson 2007, Graham et al 2009) is likely the primary driver for changes in stable carbon isotopes observed in this study. Benthic to pelagic gradients in carbon isotopes may partially cause this pattern; however the steep drop off in waters immediately surrounding Palmyra, and the fact that all seabirds in this study consume...
prey found on or near the ocean surface, make it unlikely to be the primary driver of differences in carbon isotopes. Changes in carbon isotopes due to trophic level differentiation are generally small in seabirds, and given the limited range of $\delta^{15}N$ measured and the lack of correlation between $\delta^{15}N$ and $\delta^{13}C$ observed in this study, it is not likely to be an important explanatory factor here. Variation in $\delta^{15}N$ within a tissue type is likely primarily due to sequential enrichment in consumer tissues, such that $\delta^{15}N$ is interpreted as a measurement of trophic position. However, since there are established $\delta^{15}N$ gradients on large scales across the Pacific Ocean (Graham et al. 2009) we also consider the possibility that $\delta^{15}N$ changes could be caused by these spatial gradients.

We further assume that there is no size or age specific fractionation of either carbon ($\Delta C$) or nitrogen ($\Delta N$) isotopes within seabirds (Cherel et al. 2005). We do not directly compare $\delta^{15}N$ or $\delta^{13}C$ across tissue types, given different isotopic signatures and fractionation rates of these tissues (Cherel et al. 2005). The period of isotopic integration in blood is assumed to be days to weeks, such that blood taken from a breeding bird is assumed to give diet information on the breeding period (Hobson and Clark 1992a, 1992b). Since feathers are usually molted after reproduction, and are inert thereafter, feather samples were assumed to represent the composition of the diet during the nonbreeding period (Bearhop et al. 2002). Muscle tissue likely integrates over intermediate time periods (4-6 weeks) (Hobson and Clark 1992a, 1992b).

**Statistical analyses**

To examine differences in resource partitioning among species, we used multivariate analysis of variance (MANOVA), with subsequent univariate ANOVA tests of $\delta^{13}C$ and
δ¹⁵N difference, with post-hoc Tukey HSD analyses. Statistical analyses were performed in JMP 7 (SAS Institute, Cary, NC, USA). When necessary to meet assumptions of normality, data was transformed using Box-Cox transformation. All figures/tables depict untransformed data. All mean values are shown with ± 1 SD, also untransformed.

**RESULTS**

*Species comparisons*

The nine species of adult seabirds showed significant overall isotopic segregation in feather samples (MANOVA, Wilks’ lambda, F₁₆,₂₈₈ = 12.49, p < 0.0001; Fig 1A). Univariate tests of feathers also show significant difference both by δ¹³C (ANOVA, F₇,₁₄₅ = 30.41, p < 0.0001) and by δ¹⁵N (ANOVA, F₇,₁₄₅ = 5.20, p < 0.0001). Results from post hoc Tukey pairwise comparisons show significant differences in δ¹³C among most species, although there is some overlap (Table 1). For δ¹⁵N, the three booby species were all significantly different from the white tern and the great frigatebird; there are no other significant differences (Table 1).

The five species sampled for blood also showed significant overall isotope segregation (MANOVA, Wilks’ lambda, F₈,₉₀ = 36.63, p < 0.0001). Univariate tests showed significant differences both by δ¹³C (ANOVA, F₄,₄₆ = 129.10, p < 0.0001) and by δ¹⁵N (ANOVA, F₄,₄₆ = 30.41, p < 0.0001). In post hoc analyses, all seabird species partitioned separately for δ¹³C, except for greater frigatebirds, which were indistinguishable from either masked or red-footed boobies. For δ¹⁵N, greater frigatebirds and brown boobies were distinct from the other three species (Fig 1B).
All seabirds differed from one another in at least one of the two stable isotopes measured in blood or feathers. The total variation in values among species was between 1.2‰ (in feathers and 1.8‰ (in blood) in δ¹³C and between 2.5‰ (in feathers) and 2.3‰ (in blood) in δ¹⁵N. The pattern of trophic partitioning among species changed somewhat between blood and feathers, with great frigatebirds having a relatively lower δ¹³C, in feathers compared to blood, and brown boobies had a relatively higher δ¹³C in feathers compared to blood.

Comparison of δ¹³C by mean body mass per species yielded a significant positive relationship (R² = 0.67, p < 0.01), with more enriched δ¹³C for larger birds (Fig 2A). The relationship improved significantly when δ¹³C was compared to wing loading (R² = 0.82, p < 0.01), where birds with lower wing loading were more enriched with δ¹³C (Fig 2B). There was also a strong positive relationship between estimated field metabolic rate and δ¹³C (R² = 0.74, p < 0.01). There were no significant relationships between any of the above variables and δ¹⁵N values.

Although there were few muscle tissue samples per species, there were significant differences in isotope levels between species in this tissue. Black noddies had lower δ¹⁵N in muscles than sooty terns (F₃,12 = 4.64, P = 0.02). For δ¹³C black noddies were significantly more depleted than either red-footed or masked boobies.

**Effects of year, age, and sex within species**

There was a slight but significant interannual difference in δ¹³C for breeding adult red-footed boobies (MANOVA, Wilks’ Lambda, F₄,126 = 4.10, p < 0.01), where boobies in 2007 had slightly higher δ¹³C values than birds in either 2008 or 2009 (ANOVA, F₂,50 =
3.61, p = 0.03; Fig 3). No such trend was detected in δ¹⁵N. There were slight differences in feather isotope levels across age classes for red-footed boobies within 2009 (MANOVA, Wilks' lambda, \(F_{4,44} = 3.21, p = 0.02\)), where chicks had slightly higher δ¹⁵N values \((F_{2,23} = 5.49, p = 0.01)\) than either adult or juvenile birds. No such trend was apparent in δ¹³C.

Of the five species for which adult and chick feathers were compared, four showed significantly higher δ¹⁵N values in chicks than in adults (black noddy, \(t = 2.47, p = 0.02, df = 24\); red-footed booby \(t = 2.92, p = 0.01, df = 18\); red-tailed tropicbird, \(t = 5.63, p < 0.0001, df = 39\)) and one, great frigatebirds showed marginally significant increases \((t = 1.82, p = 0.08, df = 24; \text{Fig 4})\). Only one species, red-tailed tropicbirds showed significant differences in δ¹³C by age, with adult birds having lower δ¹³C than chicks \((t = 5.63, p < 0.0001, df = 39)\).

Comparisons by sex (among adult birds from the same year) were conducted for red-footed, masked, and brown boobies, as well as greater frigatebirds. The only species that showed significant differences by sex were brown boobies where males had lower δ¹³C \((t = 4.23, p < 0.001, df = 14)\) and lower δ¹⁵N \((t = 2.40, p = 0.03, df = 14)\) than females.

**Comparisons to prey and other marine predators**

Comparisons among seabirds, other marine predators, and prey only included the five species for which blood data was available. As a group, seabirds were segregated in trophic space both from their prey and from other large pelagic predators (MANOVA, Wilks' Lambda, \(F_{6,298} = 23.26, p < 0.0001; \text{Fig 5}\)). Seabirds and predatory fish (wahoo,
yellowfin tuna) had higher $\delta^{15}N$ than prey (flying fish, squid, and anchovies) or than large predatory invertebrates (jumbo flying squid) ($F_{6,149} = 15.87, p < 0.0001$). The patterns of $\delta^{13}C$ was different, with the large predatory fish having higher $\delta^{13}C$ than seabirds, seabird prey, or predatory invertebrates ($F_{6,149} = 64.93, p < 0.0001$).

Post-hoc analysis on a species by species comparison, showed less clear patterns of partitioning among seabirds and their prey. With regard to prey, diet items from seabird stomachs (flying fish, squid) had large SD ($\pm XX$), particularly in $\delta^{15}N$, and were significantly elevated in $\delta^{15}N$ over anchovy. Squid were indistinguishable in $\delta^{15}N$ from any of the seabird species, although $\delta^{13}C$ distinguished them from all seabird species except red-footed boobies and greater frigatebirds. Flying-fish were indistinguishable in either parameter from red-footed and masked boobies.

Seabirds had relatively little overlap with other pelagic predators. Only the brown boobies and yellowfin tuna, and red-footed boobies and jumbo flying squid were indistinguishable from each other in both $\delta^{13}C$ and $\delta^{15}N$.

**DISCUSSION**

**Resource partitioning by species**

The eight sympatric seabird species studied each occupy a distinct ecological niche across the breeding and non-breeding periods. The degree of partitioning observed in non-breeding period was greater than that detailed by at-sea surveys and diet analyses (Surman and Wooler 2003, Spear et al 2007).

The patterns observed in $\delta^{13}C$ in non-breeding period were generally consistent with data from tracking and at-sea surveys, where sooty terns and greater frigatebirds
being highly pelagic, white terns and red-footed boobies less pelagic, followed red-tailed
tropicbirds, brown and masked boobies, the least pelagic (Ballance et al 1997, Jaquemet et al 2005). The one surprising result based on carbon isotope levels was the highly
pelagic signal of black noddies (i.e. values were more negative than that of any other
species). This species is generally considered to be an opportunistic nearshore feeder that
is often seen foraging near jacks and in lagoons (Ashmole 1968, Seki and Harrison 1989).
Yet values of carbon isotope levels in lagoons at Palmyra are particularly elevated, and
black noddie isotope levels do not resemble those of reef jacks at Palmyra (McCauley et al unpublished data). Given the small total range of $\delta^{13}C$ observed, controlled
measurements of species-specific fractionation rates would be helpful to confirm that
species-specific fractionation rates do not drive these patterns (Becker et al 2007).
Likewise, while $\delta^{13}C$ maps available for the equatorial Pacific Ocean do not suggest high
variation in $\delta^{13}C$ around this region, that is based on limited sampling near Palmyra
(Graham et al 2007); better isotopic sampling of oceans in this region would help interpret these results.
Species level changes in $\delta^{13}C$ were highly correlated to body mass, wing loading
and metabolic rates; small species with high metabolic rates and species with low wing
loading exhibit a more pelagic signature than larger species and species with high wing
loading. Generally, birds with low body mass and high wing loading should have low
costs of flight, perhaps enabling a more pelagic lifestyle (Pennycuick 1989). However,
since these factors covary with metabolic rate, it is also possible that metabolic rate
drives this pattern.
Nitrogen isotopes from non-breeding periods showed the greater frigatebird to have elevated $\delta^{15}N$ levels over many species including all the boobies (consistent with Cherel et al. 2008). Although direct analysis of diets of non-breeding greater frigatebirds is quite similar to red-footed boobies, these birds also often consume pulli of sooty terns and noddies, potentially explaining this variation (Schreiber and Hensley 1986, Megyesi and Griffin 1996, Spear et al 2007). Kleptoparasatism which seems to be of particular importance in non-breeding birds could potentially explain these elevated $\delta^{15}N$ values, as these partially digested food items may have elevated $\delta^{15}N$ levels (Gilardi 1994). As in the Seychelles (Catry et al 2008), the white tern also showed high $\delta^{15}N$ levels. While Catry et al suggested that this might be reason to discount results of $\delta^{15}N$, the consistency across studies perhaps merits further consideration for biologically valid explanations. It is possible that these high levels may be due to the large portion of its diet (>40%) composed of small, predatory Scombridae (*Euthynnus* sp; Spear et al 2007). This predatory species might well be higher in $\delta^{15}N$ than flying fish and squid dominating diet of other species; direct measurements of $\delta^{15}N$ of these prey would be necessary to resolve this. The three booby species, which feed primarily on flying fish and squid (Schreiber and Hensley 1986, Spear et al 2007) show particularly low $\delta^{15}N$ values. Controlled studies of species specific fractionation patterns would help understand if physiological or ecological factors drive $\delta^{15}N$ patterns (Becker et al 2007)

Examination of blood samples, representing the breeding interval, also showed distinct niches for each of the species examined. There were small changes in foraging areas in non-breeding as opposed to breeding periods, with greater frigatebirds and brown boobies looking comparatively less pelagic than during non-breeding interval. For the
two species for which tracking data is available at Palmyra (red-footed and masked
booby), the \( \delta^{13}C \) results are high consistent with tracking results, even though the
differences in foraging distances was not extremely large (Young et al in press).

The relative positions of \( \delta^{13}C \) and \( \delta^{15}N \) in both breeding and non-breeding periods
observed at Palmyra were highly consistent with those observed in Europa Island and in
the Seychelles (Catry et al 2008, Cherel et al 2008) suggesting that niche partitioning is
consistent in very different parts of the species’ ranges. The absolute values of \( \delta^{13}C \) were
also consistent in both studies in nonbreeding interval, but slightly depleted at Palmyra
during breeding interval, perhaps due to more oceanic location of Palmyra itself which
might lead to lower \( \delta^{13}C \) values (Graham et al 2009). In contrast, absolute values of \( \delta^{15}N \)
observed at Palmyra were greatly elevated (by about 2‰) over that observed at Europa in
both breeding and non-breeding periods. This suggests a higher baseline of nitrogen at
Palmyra than at Europa; similar results were seen in comparison of Seychelles to
Mozambique Channel (Jaquemet et al 2008). This is consistent with latitudinal variations
of \( \delta^{15}N \) in isotopic cartography. This suggests that while it is possible to compare
relative \( \delta^{15}N \) positions across studies, to compare absolute values of \( \delta^{15}N \) strong good
knowledge of baseline \( \delta^{15}N \) is needed (Graham et al 2009).

There was a relatively few samples of muscle tissue, but patterns were fairly
consistent with that seen in feathers and blood. Only the \( \delta^{15}N \) of black nodies were
significantly different (lower in \( \delta^{15}N \)).

**Partitioning within species**
Comparison of adult feathers to chick feathers shows elevated levels of $\delta^{15}N$ in chicks across all five species examined. This varies from the pattern seen for greater frigatebirds in Europa Island, but is consistent with results seen for sooty terns at the same site (Cherel 2008). This could reflect a shift to higher trophic level prey items during the breeding period, selective feeding of food items to young, age specific fractionation, or some effect of regurgitation of food. Other studies have shown differential provisioning of chicks with higher quality food, or different trophic level food sources (Hodum and Hobson 2000, Cherel 2008). While seabird studies have not documented changes in $\Delta N$ by age, this has been seen in other taxa and could be a viable explanation (Roth and Hobson 2000). Even without different $\Delta N$, by feeding on partially digested food, they may be incorporating $\delta^{15}N$ from their parents’ bodies, thus explaining higher $\delta^{15}N$ values. Direct comparison of adult and chick blood and diets (not taken here) would help resolve this. The lack of any shift in $\delta^{13}C$ from adults to chicks was unexpected given that breeding places constraints on seabird foraging distances, but is consistent with lack of change seen in Seychelles (Catry et al 2008).

We saw relatively little resource partitioning by sex. Of the four species, all exhibiting reverse sexual dimorphism, for which sex differences in foraging were examined, we saw differences only for one (brown booby). The larger females of brown boobies showed higher $\delta^{15}N$ and $\delta^{13}C$, indicating higher trophic level and less pelagic food sources. This is consistent with other evidence of niche partitioning by sex in brown boobies (Gilardi 1992, Weimerskirch et al 2009b). Likewise, the lack of resource partitioning by sex in red-footed boobies, and masked boobies is also consistent other studies (Weimerskirch et al 2009a, Young et al in press), although sexual differences
have been observed in the red-footed booby in other locations (Weimerskirch et al 2006b).

In the one species for which we compared data across multiple years, we saw small but significant differences in $\delta^{13}C$ for one of the three years. This may point to small variability in food sources across time even in tropical resources. While it is not always possible to gather simultaneously, this suggests cautions in interpreting data from seabirds gathered different years in isotopic analyses.

**Comparisons with prey and predators**

We saw clear distinctions among seabirds, their prey, and other marine predators. The relatively little overlap between pelagic predators and seabirds on a species by species basis was unexpected, given that many of these seabirds feed so heavily in flocks over schools of predators. The primary differences in seabirds and other predatory fish was $\delta^{13}C$ and may reflect integration of benthic food sources into fish diets. However, it could also represent other differences in diet, as other studies of seabird and predator diets have shown substantial variation, even though the forage together (Catry et al 2009). The difference in $\delta^{15}N$ between *Sthenoteuthis spp.* and seabirds may reflect lower trophic diet of *Sthenoteuthis spp.* (Shchetinnikov 1992).

Seabirds were also not isotopically distinct from individual prey types in $\delta^{15}N$, which was unexpected given known fractionation rates for seabirds between 3 and 5 ‰ (REFS). This may be due to use of muscle/mantle from diet samples rather than whole animals; whole fish have been shown to have lower $\delta^{15}N$ levels (REFS). This may also be due to partial digestion of diet samples analyzed. Although diet samples selected
appeared to be in excellent condition, and interior muscle samples were, minimizing potential contamination with seabird digestive enzymes, SD was very high in these samples and overall $\delta^{15}N$ levels were high. Muscle from Clupeiformes from beneath bird foraging flocks did not show this elevated $\delta^{15}N$ or the high SD. We suggest care should be used in determining diet items based on items from prey items gathered via stomach contents. Analysis of whole additional diet items gathered directly, and sorted to lower taxonomic levels would be a good resource for future use of isotopes in diet analyses of tropical seabirds. This would also help resolve lingering questions about the merit of $\delta^{15}N$ in assessing food sources of tropical seabirds (Catry et al. 2008)

**Stable isotopes as a tool for evaluating resource partitioning in tropical seabirds**

There has been some discussion about the merit of using stable carbon and nitrogen in examining niche partitioning among tropical seabirds in open ocean sites. In this study, we find high consistency in species specific patterns of $\delta^{15}N$ and $\delta^{13}C$ across tissue types in this study, and between this study and other studies despite widely different habitats (Catry et al. 2008, Cherel et al. 2008). For the two species for which tracking data is available at this site, stable isotope levels are consistent (both within and across species) with tracking data and appear to be able to detect small scale changes in foraging (Young et al. *in press*). Alignment of tracking, at-sea surveys, and isotope data is also seen from various work in the Mozambique Channel (Jaquemet et al. 2005, Cherel et al. 2008). Also, for the great majority of analyses, patterns of partitioning are in keeping with the data available from at-sea surveys and stomach content analyses. All of
these factors appear to provide evidence for reliability of isotopes for identifying foraging patterns of tropical species. However the anomalously low $\delta^{13}\text{C}$ of black noddies and the strong correlation of $\delta^{13}\text{C}$ to field metabolic rate, does raise possibility that species specific fractionation rates might be an alternative explanation for significant variation observed in isotopes across species (repeated across both space and time). Other potential explanations (i.e. nutritional stress causing differential fractionation, mixing of benthic $\delta^{13}\text{C}$ signals, local variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) would be unlikely to be consistent across spatially and temporally distinct studies. However analyses of prey item isotope levels, controlled laboratory experiments of species specific fractionation rates, and better isotopic maps for the region would help confirm the interpretations presented here.

Conclusions

In general this study supports the idea that there is high niche partitioning among tropical seabirds even in ocean environments. Resource partitioning is not complete unless considered across both breeding and non-breeding periods. Partitioning appears to occur within species, as well as among species – with different isotopic signatures observed by sex, age, and year. Sexual partitioning of resources did not appear to be common even among species that had relatively high sexual dimorphism, consistent with tracking data from this site (Young et al in press). Total trophic range of seabird diet was small, and both seabirds and their diet showed substantially higher levels of $\delta^{15}\text{N}$ in this study than in studies from other sites despite apparently similar diets, pointing to potential variability in space in this value. There was a strong correlation between body
size, wing loading, and metabolic rate on δ¹³C; there are multiple possible explanations for this pattern.

While variability in both isotopes was smaller in this study than in comparable polar or temperate studies, the results suggest that niche partitioning is at least as prevalent in this system. This is despite more limited foraging techniques of tropical seabirds studied here, and despite patchier resources in the tropical ocean. The apparent ability to detect small differences in foraging changes via isotopes in tropical environments, continues to further suggestions of others that this minimally invasive tool can offer powerful insight to niche partitioning in tropical seabirds.

ACKNOWLEDGEMENTS

We thank the National Science Foundation, the National Geographic Society, the Stanford Vice Provost Office for Undergraduate Education summer field studies grants, the Stanford Gabilan Graduate Fellowship, and the Woods Environmental Institute for financial support. For logistical and material support we thank US Fish and Wildlife Service (Palmyra Atoll National Wildlife Refuge), The Nature Conservancy, and the Palmyra Atoll Research Consortium (PARC). For genetic analysis of sex, we thank Frank Hailer and Elizabeth Ann Schreiber at the Smithsonian Institute. For assistance in the field and laboratory we thank D. Mucciarone, K. Pollock, J. Svendson, S. Barclay, L. Anderegg, C. Depkin, A. Briggs, M. deGraff, P. de Salles, T. Jen, E. Hoffman, C. Burniske, N. Wenner, C. Hanson, L. Palumbi, and T. Robbins.
WORKS CITED


terns at a sub-tropical island in the eastern Indian Ocean. Journal of Zoology

Vanderklift, M. A., and S. Ponsard. 2003. Sources of variation in consumer-diet delta N-


Weimerskirch, H., M. Le Corre, H. Gadenne, D. Pinaud, A. Kato, Y. Ropert-Coudert, and
C. A. Bost. 2009a. Relationship between reversed sexual dimorphism, breeding
investment and foraging ecology in a pelagic seabird, the masked booby.

tropical seabird, the red-footed booby, in a dynamic marine environment. Marine
Ecology Progress Series 288:251-261.

strategy of a top predator in tropical waters: great frigatebirds in the Mozambique
Channel. Marine Ecology Progress Series 275:297-308.

Weimerskirch, H., M. Le Corre, Y. Ropert-Coudert, A. Kato, and F. Marsac. 2006b. Sex-
specific foraging behaviour in a seabird with reversed sexual dimorphism: the

Weimerskirch, H., S. A. Shaffer, Y. Tremblay, D. P. Costa, H. Gadenne, A. Kato, Y.
differences in foraging behaviour and foraging zones in blue-footed and brown boobies in the Gulf of California. Marine Ecology Progress Series 391:267-278.


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Table 1: Stable isotopic ratios of carbon and nitrogen and mass ratio of C:N in feathers, blood, and muscle tissue of seabirds, and muscles of other pelagic predators and prey. Letters following mean values denote significant differences among species in post-hoc analyses, values (within tissue type and isotopic ratio) not connected with the same letter are significantly different. Species marked with † are not considered in statistical comparisons due to small sample size. Values are mean ± SD.
Figure 1: Stable carbon and nitrogen isotope values (mean ± SD) from eight seabird species from Palmyra Atoll. Panel A shows values from feathers; solid black symbols denote those species for which isotopic values of blood are also presented panel B. BB = brown booby, BN = black noddy, MB = masked booby, GF = great frigatebird, RFB = red-footed booby, RTT = red-tailed tropicbird, ST = sooty tern, WT = white tern.
Figure 2: Stable isotope values of carbon compared to (a) body mass (b) wing loading and (c) estimated field metabolic rate. Species codes are the same as in Figure 1, plus BRN = Brown noddy; WSH = Wedge-tailed shearwater. Statistics here include all species depicted.
Figure 3: A comparison of stable carbon and nitrogen isotope values for feathers from red-footed boobies across multiple years and age classes. The first letter indicates age class (A = adult, I = immature, C = chick) and the number indicates the year samples were collected (2007, 2008, or 2009).
**Figure 4:** Stable carbon and nitrogen isotope values of feathers compared across age classes for multiple seabird species. Adults (A) are indicated by unfilled shapes and chicks (C) are indicated by filled shapes. Different species are indicated by codes (as same as in Figure 1) and shape.
Figure 5: Stable carbon and nitrogen isotope values of seabirds (filled circles), their prey (open squares), and other pelagic predators around Palmyra Atoll (grey triangles). AS = *Acanthocybium solandri* (wahoo); TA = *Thunnus albacores* (yellowfin tuna); E = Exocetidae (flying fish, various); C = Clupeiformes (anchovy and herring); S = *Sthenoteuthis* spp. (jumbo flying squid, various). Seabird codes are the same as in Fig 1.