

SPECIAL ISSUE ARTICLE

Genetic analysis of the diet of red-footed boobies (*Sula sula*) provisioning chicks at Ulupa'u Crater, O'ahu

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Abstract

1. The diet of red-footed boobies (*Sula sula*) provisioning chicks was quantified using 106 regurgitations collected from 81 adults over two study years with contrasting oceanographic conditions: 2014 and 2015.
2. A total of 1,049 prey items were sorted into three broad categories (fish, squid, and other, consisting of highly-digested 'mush' and parasitic isopods) and assigned a categorical freshness value of 1 (perfect condition), 2 (superficial digestion), or 3 (highly digested and incomplete).
3. A total of 492 undigested prey items (freshness 1 and 2) were measured and sampled for genetic identification with 401 (82%) successfully identified. Rarefaction analyses suggested robust diet sampling, with 32 documented species: 29 fishes and three squids.
4. While the prey-specific index of relative importance highlighted that fish dominated the diet in 2014 (32.3% fish, 15.1% squid) and in 2015 (29.9% fish, 22.3% squid), diet composition varied significantly between the two study years.
5. The purpleback flying squid (*Stenoteuthis oulaniensis*) accounted for 98.7% (232 of 236) of the identified squid specimens. Mantle lengths were significantly longer in 2015 (7.2 ± 1.1 SD cm) than in 2014 (6.3 ± 1.7 SD cm).
6. These results indicate a higher relative abundance and size of the epipelagic squids available to foraging red-footed boobies in 2015, during warm-water conditions associated with the positive phase of the El Niño Southern Oscillation.
7. Due to their diverse diet, red-footed boobies are useful bioindicators of epipelagic nekton assemblages during changing oceanographic conditions. Moreover, their reliance on the purpleback flying squid makes these seabirds ideal samplers of the juveniles of this poorly studied species.
8. Understanding how interannual and longer-term oceanographic variability affects central-place foraging seabirds and the recruitment and abundance of their squid prey is critical for precautionary ecosystem-based fisheries management of oceanic islands and the surrounding marine environment.

KEYWORDS

diet, El Niño, El Niño Southern Oscillation, flying squid, genetic analysis, Hawaii, purpleback squid, red-footed booby, *Stenoteuthis oulaniensis*, *Sula sula*

1 | INTRODUCTION

The structure of marine food webs and the distribution of upper-trophic predators shift in response to large-scale changes in ocean circulation and productivity over interannual and longer time scales (Lehodey, Bertignac, Hampton, Lewis, & Picaut, 1997; Mantua, Hare, Zhang, Wallace, & Francis, 1997; Wolter & Timlin, 2011). In particular, seabirds respond to oceanographic forcing from the El Niño Southern Oscillation and longer-term decadal variability (Hyrenbach & Veit, 2003; Montevecchi & Myers, 1997; Schreiber & Schreiber, 1984). For example, during the 1982–1983 El Niño, red-footed booby (*Sula sula*, RFBO) diet at French Frigate Shoals (north western Hawaiian Islands, NWHI) switched from flying fish to flying squid, indicating that epipelagic squids became more available to these central-place foraging seabirds during warm-water conditions (Seki & Harrison, 1989).

Tropical seabirds rely on a variety of behavioural and physical mechanisms to locate and capture widely-dispersed prey, and many species forage with subsurface predators (SSPs) that drive epipelagic prey to the surface or into the air (Ballance & Pitman, 1999; Harrison, Hida, & Seki, 1983; Spear, Ainley, & Walker, 2007). Species in the 'tuna bird' guild, including frigatebirds, terns, noddies, shearwaters, and boobies, often forage in association with cetaceans and large predatory fishes (Au & Pitman, 1986; Harrison et al., 1983; Hebshi, Duffy, & Hyrenbach, 2008). Therefore, the distribution and foraging behaviour of these seabirds provide a broader ecological context for interpreting the effects of oceanographic variability on the food webs that support other economically-important upper-trophic marine predators, such as tunas (Au & Pitman, 1986; Lyday, Ballance, Field, & Hyrenbach, 2015; Seki & Harrison, 1989).

In this study, RFBO diet was used as an indicator of year-to-year variability in epipelagic prey assemblages, by virtue of four key traits: (i) their ability to sample localized prey distributions during the breeding season, when their foraging ranges are restricted around their colonies (maximum range = $201.6 \text{ km} \pm 87.1 \text{ SD}$ for multi-day trips from O'ahu (Adams, Felis, & Czapanskiy, 2020); (ii) their generalist foraging habits and opportunistic diet (Harrison et al., 1983; Seki & Harrison, 1989); (iii) their demonstrated sensitivity to changes in ocean productivity (Schreiber & Schreiber, 1984; Weimerskirch, Le Corre, Jaquemet, & Marsac, 2005); and (iv) the logistical ease involved in the study of their diet and movements, due to their non-migratory habits and colonial breeding (Ashmole & Ashmole, 1967a; Schreiber, Schreiber, & Schenk, 1996).

More specifically, RFBO diet was used to detect changes in available prey within the surface waters surrounding their breeding colony on the island of O'ahu, Hawaii. In particular, the goal of this study was to quantify the RFBO diet during two years of contrasting oceanographic conditions (2014, 2015) and to address three hypotheses. First, to account for potential biases due to differential digestion, total mass and sample freshness were compared based on collection time. We expected that samples after tag retrievals (post-foraging) would be heavier (greater mass) and fresher than samples before tag deployments (pre-foraging). Second, the influence of RFBO sexual size

dimorphism in diet composition was tested. Because females are, on average, larger than males ($954 \text{ g} \pm 41 \text{ SD}$ for ♀; $839 \text{ g} \pm 44 \text{ SD}$ for ♂, $n = 24$; Young et al., 2010), we expected sex-specific differences in the prey loads (overall mass) and prey composition (fish and squid). Third, inter-annual differences were tested to determine whether RFBO change their diet during years with contrasting oceanographic conditions. Due to diverse epipelagic prey assemblages and the observed variability in RFBO diets (Ashmole & Ashmole, 1967b; Harrison & Seki, 1987; Seki & Harrison, 1989), the importance of both individual species and functional groups were considered. Based on previous findings from the NWHI (Seki & Harrison, 1989), we expected squid to become a more important component of the RFBO diet in 2015 (a warm-water year), compared with 2014 (a year of 'average' water temperature).

2 | METHODS

2.1 | Study area

The study colony (21.458394°N ; 157.723361°W), is located on the eastern crest of Ulupa'u Crater, within the Marine Corps Base Hawai'i (MCBH), on the island of O'ahu, Hawai'i. RFBO build their nests in the available kiawe trees (*Prosopis pallida*) and haole koa shrubs (*Leucaena leucocephala*). Colony size estimates range from a minimum of 337 in 2008, to a maximum of 2,380 in 1969 (Russell & Vanderwerf, 2010). Currently, the RFBO breeding population is estimated at a minimum of 700 individuals (T. Russell, pers. comm., July 2015).

2.2 | Collection and preservation of diet samples

Regurgitations were collected opportunistically from tagged and non-tagged adults, during field work to deploy and retrieve GPS tags for a tracking study (Adams et al., 2020). Overall, 81 birds provided 106 regurgitations (diet samples) collected over two years (51 in 2014 and 55 in 2015), comprising 9,698.4 g of sorted prey (Table 1). Each individual regurgitation was immediately placed into a labelled gallon-sized Ziploc bag, stored on ice in a cooler, and placed in a freezer for long-term storage. No preservatives were used during sample collection and all samples remained frozen until they were sorted in the laboratory at the Oceanic Institute.

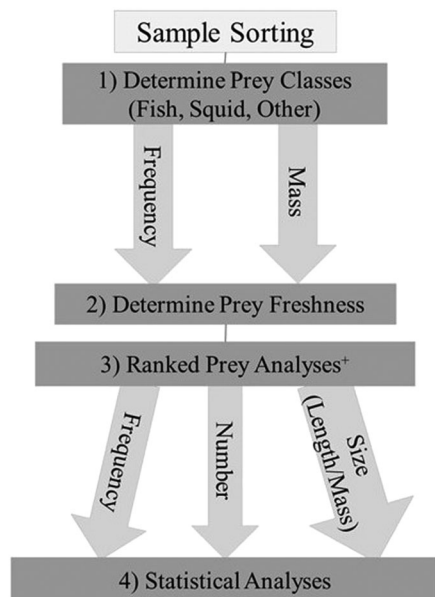
2.3 | Diet analysis

All diet sample processing and sorting was completed by the same person (S.E.D.) following a standardized five-step protocol (Figure 1): (i) two passes for sorting, the first to remove foreign items and the second to quantify prey classes; (ii) determination of prey freshness; (iii) quantification of the frequency, number and size of 'fresh' prey (freshness scale of 1 or 2); (iv) genetic analysis to identify species and calculate richness and diversity; and (v) statistical analysis of the

TABLE 1 Sample sizes, showing the yearly number of regurgitations and the mass of prey sorted

Year	Regurgitation samples	Regurgitation mass (g)	Deployment samples (dates)	Recapture samples (dates)
2014	51	4521.9	15 (1 June) 9 (4 June)	9 (4 June) 18 (5–7 June)
2015	55	5176.5	13 (17 June) 12 (29 June)	14 (22–23 June) 16 (6–7 July)
Total	106	9698.4	49	57

Note: The number of regurgitation samples obtained during tag deployments and recaptures of tagged birds is shown per study year.



*Rank of 3 may not have enough tissue or important hard parts present for identification; only mass recorded

FIGURE 1 Flow chart illustrating the sequential steps of diet analysis

relative occurrence and relative abundance of the different prey classes and species.

2.3.1 | Step 1: Quantify prey classes

Sorting involved removing foreign (non-prey) items collected accidentally with the sample (e.g. sand, gravel, leaves, sticks), then separating each sample into three distinct prey classes: 'Fish', 'Squid', and 'Other' (isopods and 'mush' – indistinguishable matter). The presence or absence, and the mass of each prey class were used to quantify their incidence and relative abundance across all samples.

2.3.2 | Step 2: Determine prey completeness and freshness

Because digestion may lead to the underestimation of the mass of the stomach contents, diet studies often quantify the freshness of individual prey items (Ashmole & Ashmole, 1967a; Diamond, 1983). Following these previous studies, prey items were categorized as either complete (C) or incomplete (I), and ranked based on their degree of digestion, using a three-level freshness scale (Table 2, Figure S1).

TABLE 2 Criteria used to determine the freshness of prey items (modified from Ashmole & Ashmole, 1967b)

Freshness Scale	Criteria
1	'Perfectly intact': good condition, fully intact with colourful skin/scales. Fish with head attached, squid mantle with pink–purple skin and buccal mass and finlet often attached
2	'Slightly digested': fair condition, partially intact with colourful (may begin to be discoloured) skin/scales. Usually fish head or tail present, tissue missing, still able to collect information
3	'Incomplete': not fresh, fish tail with little to no muscle and showing vertebrae, just head present or whole skeleton. Part of mantle tissue, not firm, extremely discoloured (grey/white/pale pink)

Note: See Figure S1.

The mush was assigned a freshness rank of 3 because it consists of unidentifiable tissue, which could represent a mixture of the three other categories (fish, squid, crustacean). Thus, mush only contributed to the total mass and total freshness of a sample and did not influence the occurrence and the relative mass of fish and squid. Prey items were categorized as one of four completeness and freshness codes: C1, C2, I2, I3 (Table 3).

To investigate the potential influence of digestion on the apparent diet composition, a weighted average of freshness (WAF) metric was used to estimate the freshness of each sample, based on the freshness of the individual prey items it contained. The WAF of each sample was calculated by averaging the freshness scores of each individual prey item, the fish parts, the squid parts, and the mush, weighted by their masses. WAF was calculated as follows:

TABLE 3 Classification of fish ('F') and squid ('S') prey items by completeness (C: Complete, I: Incomplete) and freshness (1, 2, 3), by study year

YEAR	CLASS	C1	C2	I2	I3	Total
2014	F	10	7	79	137	233
	S	54	38	37	57	186
2015	F	3	18	65	275	361
	S	84	42	56	88	270
Total	F + S	151	105	237	557	1,050

$$WAF = (\sum(M_{Pi} * F_{Pi}) + (M_{Fp} * F_{Fp}) + (M_{Sp} * F_{Sp}) + (M_m * F_m)) / TM \quad (1)$$

where M represents mass, F : freshness rank, Pi : prey item, Fp : fish parts, Sp : squid parts, m : mush, and TM : total mass. The unitless WAF index ranges between 1 and 3, with 1 representing a fresh sample and 3 representing a highly digested sample.

2.3.3 | Step 3: Ranked prey analyses

All prey samples were sorted and categorized using four metrics (Ashmole & Ashmole, 1967a, 1967b; Diamond, 1983; Schreiber & Hensley, 1976):

1. Percentage frequency of occurrence (%FO): the presence/absence of each prey class within and across samples as a function of the total number of prey items across classes.
2. Percentage number (%N): the count of complete and incomplete individuals within each freshness rank category, relative to the total number of prey individuals.
3. Percentage mass (%M): the wet mass (g) of complete and incomplete individuals within each freshness rank category, relative to the total wet mass of all prey items.
4. Size (length or mass): complete prey items were weighed to the closest 0.1 g using a benchtop balance and the mantle length (squid) and the standard length (fish) were measured to the closest 0.1 cm with callipers.

The percentage prey-specific index of relative importance (%PSIRI) was used to quantify the importance of each prey class (at the lowest possible taxonomic level) during each study year, as follows:

$$\%PSIRI = (\%FO \times (\%PN + \%PM)) / 2 \quad (2)$$

where %FO is percentage frequency of occurrence, %PN is the percentage number of prey individuals for the samples where the given species was present, and %PM is the percentage prey mass for those samples where the given species was present. The %PSIRI provides a bounded index, ranging from 0% (the prey class never occurred in a sample) to 100% (only the prey class occurred in all the samples) (Brown, Bizzarro, Cailliet, & Ebert, 2012).

2.3.4 | Step 4: Genetic identification

All genetic analyses relied on previously developed methods for Sanger sequencing (Longmire, Maltbie, & Baker, 1997; Renshaw, Olds, Jerde, McVeigh, & Lodge, 2015). Briefly, a comprehensive list of potential prey species was assembled using published lists for the Main Hawaiian Islands (MHI) and the NWHI (Table S1). Cut genomic sequences (mitochondrion and cytochrome oxidase subunit I, COI) for these species were compiled from National Center for Biotechnology

Information (Genbank, 2016) and used to identify ingested prey items, following detailed protocols (Table S2). Briefly, tissue samples were incubated at 65°C for 2 hours in 1.5-ml microcentrifuge tubes with 500 µl Longmire's lysis buffer (Longmire et al., 1997) and 400 µg of Proteinase-K; DNA was extracted from prey items with a chloroform-isoamyl alcohol protocol (Renshaw et al., 2015). A fragment of the mitochondrially-encoded COI was polymerase chain reaction amplified following the protocols for the mCOLintF/jgHCO2198 primer pair (Leray et al., 2013) and sequenced on an ABI 3130xl Capillary Sequencer. Sequence chromatograms were quality checked by eye and primer sequences removed. Taxonomy of resulting sequences was identified using nucleotide BLAST (2016).

The species richness of the prey was quantified using four metrics: the bias corrected Chao1 and Chao2 indicators, estimated the lower bound of species richness, accounting for species contributing one and two prey items (Chao, 1987), and the Jack 1 and Jack 2 richness estimators, resampled to reduce bias after removing unique species (found within only one sample; Jack 1) and duplicates (species found in exactly two samples; Jack 2) (Burnham & Overton, 1979). These indices and rarefaction curves were calculated using the EstimateS biodiversity statistical software (Colwell, 2013).

2.3.5 | Step 5: Statistical analyses

All statistical tests were performed using the SYSTAT13 software (SYSTAT, 2016) and significance was assessed using $\alpha = 0.05$. Data normality was quantified using the Kolmogorov-Smirnov test, and the homogeneity of variances was assessed with the Levene's test (Zar, 1984).

2.4 | Hypothesis testing

The statistical analysis of the diet data followed a hierarchical approach using three different datasets with different sample sizes to address three separate hypotheses involving sampling biases, colony-wide interannual patterns, and sex-based differences.

2.4.1 | Sampling biases

To account for the potential biases associated with regurgitations collected before or after a tracked foraging trip, a subset of 23 individuals that yielded two regurgitations (during tag deployment and recapture) were used to compare the total mass and freshness of paired samples from the same individuals with paired t-tests.

2.4.2 | Interannual differences

The entire sample set (106 regurgitation samples from 81 adults: 51 from 2014 and 55 from 2015) was used to quantify year-to-year

changes in the incidence and importance of prey functional groups and the presence and size of 'indicator' prey species. This entailed comparing the WAF between years (2014, 2015) using a one-way analysis of variance (ANOVA), and exploring the potential influence of freshness on sample mass and composition, by comparing total mass and mush mass during the two study years, while controlling for WAF using analysis of co-variance (ANCOVA).

2.4.3 | Sex differences

A subset of 82 diet samples from 59 birds of known sex was used to test for 'sex-based' dietary differences: 34 diet samples from 23 birds (14 Male, 9 Female) in 2014, and 48 diet samples from 36 birds (19 Male, 17 Female) in 2015. Birds were sexed using a blood sample taken during tag retrieval (Longmire et al., 1993). The sexual dimorphic index (SDI) was used to quantify morphometric differences between males and females (Angel et al., 2015; Ashmole & Ashmole, 1967b). SDI was expressed using the following ratio:

$$SDI = \left| -\left(\frac{\text{mean male}}{\text{mean female}} \right) + 1 \right| \times 100 \quad (3)$$

where a value of 0 implies that the sexes are equal, and positive or negative values signify that the females or males are larger, respectively. Three morphometric measurements were used to sex RFBO without blood samples: body mass (g), culmen length (mm), and wing chord (mm). Additionally, to explore differences in the diet composition of males and females, the SDI was applied to the relative abundance (% total mass) of the different prey classes.

2.5 | Exploratory analyses

In addition to testing these three hypotheses, multivariate methods were used to explore the diet composition using the statistical software PC-ORD 6.10 (McCune & Mefford, 2011). All analyses compared prey mass, using a data matrix with samples (regurgitations) as rows and prey (species or functional groups) as columns. To facilitate a successful ordination, those 'rare' prey species that were present in only one sample, and all 'empty' samples that did not contain identified prey items, were discarded. The pair-wise distances between the resulting samples were computed using the relative Sorensen distance measure, to ensure each sample contributed the same weight to the analysis (McCune & Grace, 2002).

Two tests were performed using the same sample-species matrix: a free ordination and a group comparison. First, a non-metric multi-dimensional scaling (NMDS) ordination was performed using 500 iterations of the real data and the significance was assessed by comparing the observed stress to an expectation from 999 runs of randomized data. The *P* value for each axis was calculated using the minimum stress, as follows: (number of random values equal to or larger than the observed +1) / (number of randomized runs +1). Note that the

estimated *P* value can range from 1 (1,000/1,000) to 0.001 (1/1,000) (Peres-Neto, Jackson, & Somers, 2005).

A maximum of six axes was selected for the ordination, and the stability criterion was set to 0.000001. Next, a multi-response permutation procedure (MRPP) test was performed to compare all the prey species at once, across four different 'sex by year' categorical groupings: males 2014, females 2014, males 2015, and females 2015. Finally, following the significant MRPP, indicator species analysis (ISA) was used to identify significant indicator prey associated with the four 'sex by year' groups.

To explore the potential influence of individual prey taxa, these analyses were performed twice: (1) using individual species (68 diet samples and 20 prey species), and (2) using functional groups (62 diet samples and four functional groups) defined on the basis of published species accounts (Fishbase, 2016). Similar to other descriptive groupings (i.e. schooling versus non-schooling prey; Litzow & Piatt, 2003) the functional groups were based on species-specific habitat associations and habits: reef-associated fishes, pelagic-oceanic fishes, flying fishes, and squids. Flying fishes included the two families (Exocoetidae and Hemiramphidae) that leap out of the surface ocean to escape sub-surface predators. All other pelagic fishes that do not fly were included in the pelagic-oceanic group. Reef-associated fishes were defined as those species whose adults inhabit reef habitats. Squids included flying squids of the family Ommastrephidae (Table 7).

2.6 | Oceanographic conditions

Four metrics were used to characterize interannual variability in environmental conditions during the two study years. The Pacific decadal oscillation (PDO; NCD, 2016) and the Multivariate El Niño southern oscillation index (MEI; ESRL, 2016) quantified basin-wide conditions. Wind speed (WSP) and sea surface temperature (SST) measurements compiled by Ocean Watch (2016) quantified regional conditions north of O'ahu.

The MEI quantifies ocean-atmosphere conditions along the Tropical Pacific (~10°N to 10°S) by integrating six cross-correlated variables: sea-level pressure, zonal and meridional sea surface WSP, SST, air temperature, and total cloudiness fraction within the sky. 'Average' conditions range from slightly positive (+0.5) to slightly negative (−0.5) deviations from the long-term mean. El Niño is characterized by positive anomalies (≥+0.5) and La Niña is characterized by negative anomalies (≤−0.5) (Wolter & Timlin, 2011). The PDO is the first principal component of detrended SST across the Pacific Ocean, north of 20°N (Mantua et al., 1997). Monthly MEI and PDO index values, before and during the breeding season (January–August) of both study years (2014, 2015) were compared using paired t-tests.

Regional oceanographic conditions were quantified using WSP (m s^{-1}) and SST (°C) taken from a $5^\circ \times 5^\circ$ grid centred on National Data Buoy Center Buoy 51,000 (23.5380°N 153.8081°W). Average daily observations during the breeding season (May–July) of both study years (2014, 2015) were compared using two-way ANOVA.

3 | RESULTS

3.1 | Oceanographic conditions

The basin-wide (MEI, PDO) and the regional (SST, WSP) metrics varied significantly between 2014 and 2015, indicating that sampling occurred in two years with contrasting oceanographic conditions. The monthly (December–January through June–July) MEI values were greater in 2015 than in 2014 (paired t -test, $t = -10.733$, $df = 6$, $P < 0.0001$; Figure 2A). The monthly PDO index was also significantly greater in 2015 than in 2014 (paired t -test, $t = -2.698$, $df = 6$, $P = 0.036$; Figure 2B).

Daily WSP during the RFBO breeding season (May–August) was greater in 2014 than in 2015 (ANOVA, $F_{1,235} = 12.723$, $P < 0.0001$). There was also a significant month $\times$ year interaction ($F_{3,235} = 8.492$, $P < 0.0001$), with different seasonal trends in WSP across the two study years, increasing in 2014 and decreasing in 2015 (Figure 3A).

Although daily SST was not significantly different in 2014 and 2015 (ANOVA, $F_{1,235} = 0.268$, $P = 0.605$), there were different seasonal trends during the two study years, as evidenced by the significant month $\times$ year interaction ($F_{3,235} = 86.021$, $P < 0.0001$). Namely, while May SST was lower in 2015 than in 2014, SST increased during subsequent months in 2015 (Figure 3B). Nevertheless, daily SST was, on average, not significantly different between the two years (2014 mean: $25.4^{\circ}\text{C} \pm 0.5$ SD and 2015 mean: $25.4^{\circ}\text{C} \pm 1.0$ SD).

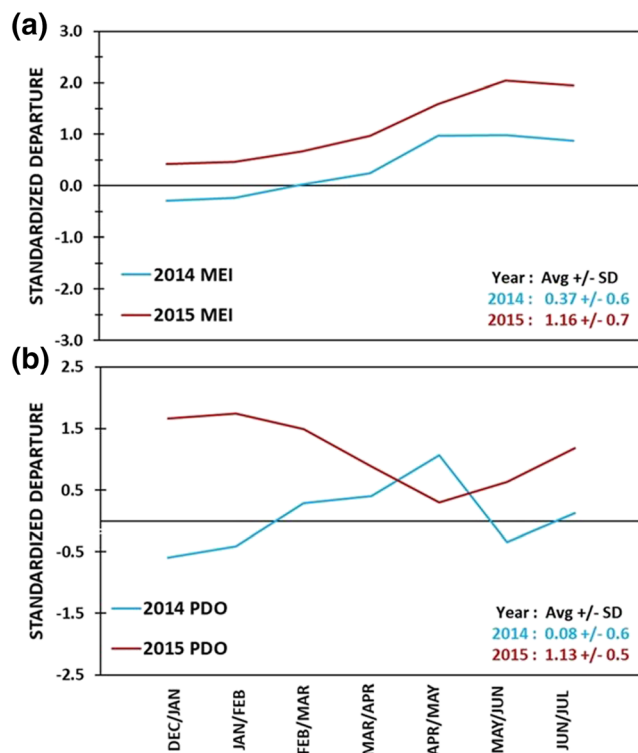


FIGURE 2 Average ($\pm$ SD) monthly multivariate El Niño Southern Oscillation index (MEI; a) and monthly Pacific decadal oscillation (PDO; b) values before and during the sampling period (January–July) of the two study years (2014 and 2015)

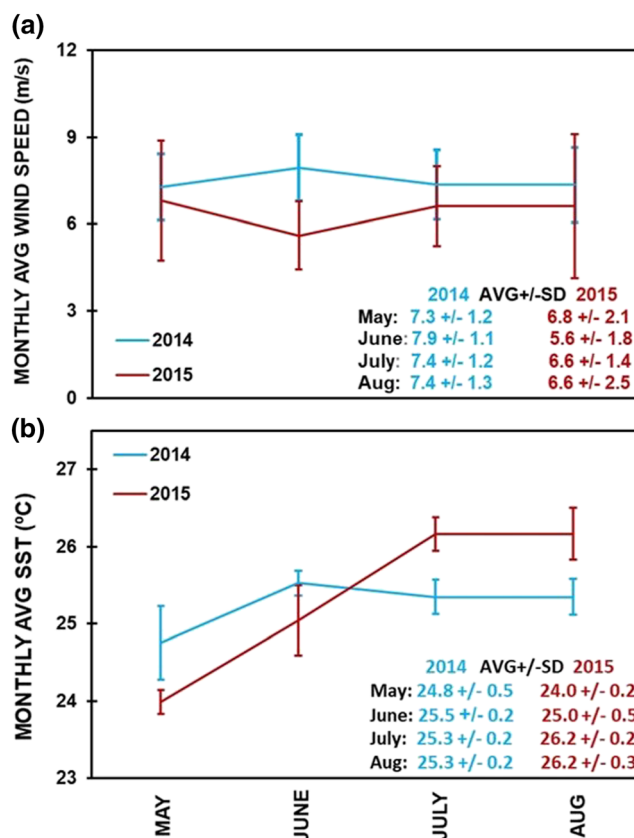


FIGURE 3 Average ($\pm$ SD) monthly wind speed (m s^{-1} ; a) and sea surface temperature (SST, $^{\circ}\text{C}$; b) during the red-footed booby breeding season of the two study years (2014 and 2015, 1 May–31 August)

3.2 | Testing sampling biases in diet composition

The first step in the dietary analysis, involved investigating potential biases between regurgitation samples obtained from tagged birds before or after tracked foraging trips, by comparing a subset of 23 pairs of samples obtained from the same birds during deployment and recapture. This analysis involved two females and nine males in 2014 and one female and 11 males in 2015, with four of these birds (two males and two females) sampled repeatedly during both study years.

The total mass of the deployment and recapture samples from the same individuals was not different (paired t -test, $t = -1.004$, $df = 22$, $P = 0.327$), and the paired differences were normally distributed (one-sample Kolmogorov–Smirnov test, $n = 23$, $\text{Max Diff} = 0.092$, $P = 0.979$). By contrast, the WAF was significantly lower for the recaptures than for the deployments (paired t -test, $t = 2.124$, $df = 22$, $P = 0.045$), and the paired differences were normally distributed (one-sample Kolmogorov–Smirnov test, $n = 23$, $\text{Max Diff} = 0.090$, $P = 0.984$). These results indicated that the food samples obtained from birds returning from a tracked foraging trip were fresher, as evidenced by the lower WAF. Nevertheless, since the total mass of the deployment and recapture samples did not differ, every sample was used for further analyses, regardless of the time of collection (i.e. before or after a tracked foraging trip).

3.3 | Interannual differences in diet composition

3.3.1 | Total sample mass

The colony-wide analysis used all 106 regurgitations collected from 81 birds. The total mass of the regurgitations was not significantly different between years (ANOVA, $F_{1,103} = 0.412$, $P = 0.522$), and the residuals were normally distributed (one-sample Kolmogorov-Smirnov, $n = 106$, $Max\ Diff = 0.065$, $P = 0.976$). The average total mass of the regurgitations was $88.1\text{ g} \pm 59.8\text{ SD}$ (median = 66.2, range = 6.8–229.6 g) in 2014 and $95.6\text{ g} \pm 61.0\text{ SD}$ (median = 89.4, range = 8.8–225.2 g) in 2015 (Figure 4). Nevertheless, the total mass of a sample was significantly related to its WAF score (ANCOVA, $F_{1,103} = 31.993$, $P < 0.0001$).

The WAF scores, which range between 1 and 3, were not significantly different between years (ANOVA, $F_{1,103} = 0.051$, $P = 0.823$) and averaged $2.2 \pm 0.5\text{ SD}$ (median = 2.1, range = 3–1) in 2014 and $2.3 \pm 0.5\text{ SD}$ (median = 2.1, range = 3–1) in 2015.

3.3.2 | Prey classes

Four different metrics underscored the dominance of fish in the diet during both study years, with squid playing a secondary role: (1) absolute prey masses, (2) absolute prey numbers, (3) average number of prey items per sample, and (4) %PSIRI. In 2014, fish accounted for 67.9%M and 54.2%N and squid accounted for 29.8%M and 45.8%N. In 2015, fish accounted for 57.4%M and 53.8%N, and squid accounted for 42.1%M and 46.2%N. A minor proportion of the sample mass (2.3% in 2014 and 0.5% in 2015) was classified as mush, which did not involve any discernible prey items.

The dominance of fish was also evident when the average masses were compared across samples (Figure 5). In 2014, the average fish mass per sample was $60.2\text{ g} \pm 55.4\text{ SD}$, more than twice the average squid mass per sample ($26.4\text{ g} \pm 30.1\text{ SD}$). In contrast, during 2015,

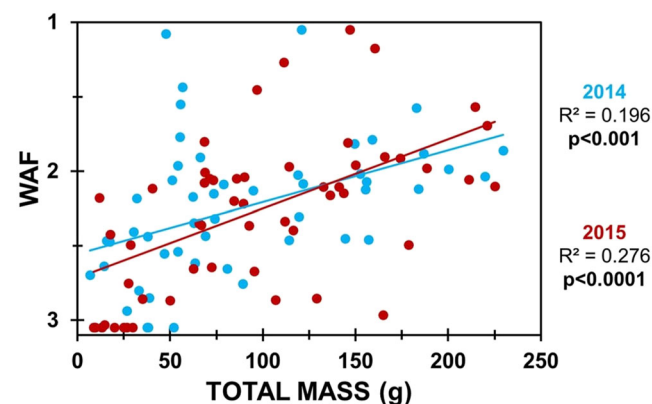


FIGURE 4 Significant relationship between weighted average freshness (WAF, 1: fresh–3: highly digested) and total mass (g) of the regurgitation samples collected from red-footed boobies in 2014 and 2015

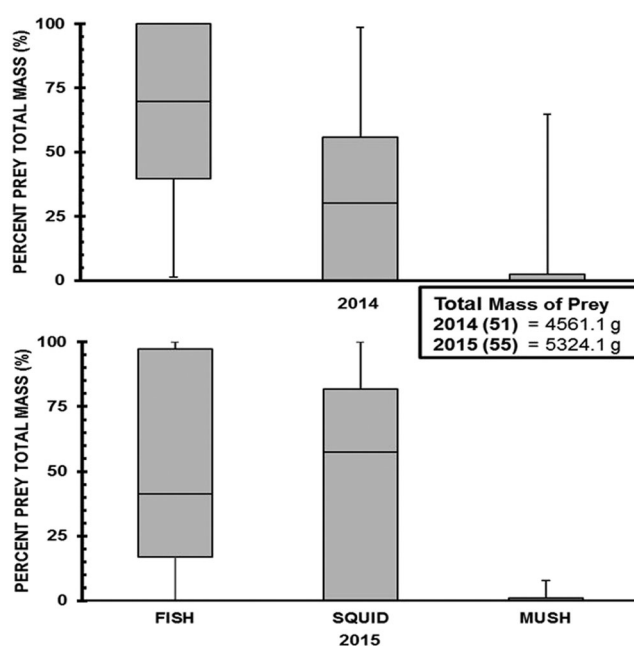


FIGURE 5 Boxplots (5%, 25%, median, 75%, 95%) of percent of total mass by prey class (fish, squid, mush) consumed by red-footed boobies in 2014 and 2015

the average mass per sample of fish ($54.0\text{ g} \pm 57.8\text{ SD}$) and squid ($39.6\text{ g} \pm 50.9\text{ SD}$) were similar (Figure 5). Overall, the average mass of fish (ANOVA, $F_{1,103} = 0.313$, $P = 0.577$) and the average mass of squid (ANOVA, $F_{1,103} = 2.59$, $P = 0.111$) in a regurgitation sample did not differ between years. However, the average mass of mush was greater in 2014 than in 2015 (ANOVA, $F_{1,103} = 7.676$, $P = 0.007$).

In 2014, a total of 232 fish and 187 squid were sampled, with an average of $8.1 \pm 5.7\text{ SD}$ prey per sample (median = 7.0, range = 1–24). In 2015, a total of 315 fish and 270 squid were sampled, with an average of $10.6 \pm 8.4\text{ SD}$ prey items per sample (median = 9.0, range = 1–34). The majority (53.1%) of the prey items were categorized as I3 (incomplete, with the lowest freshness of 3), with 22.6% as I2, 14.4% as C1 and 9.9% as C2. Overall, 24.3% (255 of 1,049) of the sorted prey items were complete and their lengths were measured (Table 3). Based on the rank scale assigned to each prey item (C1, C2, I2, I3), squid freshness did not differ between years ($\chi^2 = 6.9489$, $df = 3$, $P = 0.666$) but fish freshness did ($\chi^2 = 29.323$, $df = 3$, $P < 0.0001$), with fresher prey items in 2014. Nevertheless, the overall sample WAF values did not differ between years (ANOVA, $F_{1,103} = 0.051$, $P = 0.823$).

Altogether, the %PSIRI indicated that fish was the most important prey class in 2014 (32.3%) and 2015 (29.9%), followed by squid (15.1% in 2014 and 22.3% in 2015). Mush had the lowest %PSIRI in both study years (0.5% in 2014 and 0.4% in 2015; Table 4).

3.3.3 | Prey size

In 2014, 109 complete items (17 fish and 92 squid) were measured, with fish standard lengths ranging from 5.0–19.2 cm and squid mantle

TABLE 4 Percent prey specific index of relative importance (%PSIRI) of prey classes (fish, squid, mush) by study year

		%FO	%PN	%PM	%PSIRI
2014	Fish	1.0	4.4	60.2	32.3
	Squid	0.7	5.1	36.4	15.1
	Mush	0.4	0.4	2.1	0.5
2015	Fish	1.0	5.9	56.1	29.9
	Squid	0.7	6.6	53.2	22.3
	Mush	0.5	0.5	0.9	0.4

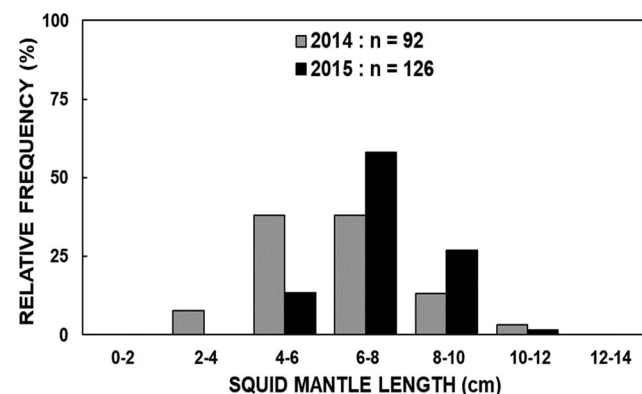
Note: The %PSIRI values integrate frequency of occurrence (%FO), percent average prey number (%PN), and percent average prey mass (%PM).

lengths ranging from 3.0–10.3 cm. In 2015, 146 complete items (19 fish and 126 squid) were measured, with fish standard lengths ranging from 4.1–20.5 cm and squid mantle lengths ranging from 4.5–10.0 cm. The largest fish were a 19.2 cm (120.7 g) narrowhead flying fish, *Cypselurus angusticeps*, in 2014 and a 20.5 cm (125.6 g) glider flying fish, *Cheilopogon atrisignis*, in 2015. The largest squids were two purpleback flying squids, *Sthenoteuthis oualaniensis* of 10.3 cm (35.1 g) in 2014 and 10.0 cm (24.0 g) in 2015.

After excluding three individuals of other species, the mantle lengths of the purpleback flying squids sampled during the two study years were significantly longer in 2015 (ANOVA, $F_{1,216} = 23.977$, $P < 0.0001$; Figure 6). In 2014, the size distribution involved smaller-sized juveniles (6.3 cm $\pm$ 1.7 SD, median = 6.2 cm, range = 2.0–10.3 cm, $n = 92$). There was a slight shift into larger-sized juveniles in 2015 (7.25 cm $\pm$ 1.1 SD, median = 7.3, range = 4.5–10.2 cm, $n = 126$; Figure 6). Fish size frequencies were not tested due to small sample sizes (<20 prey items per sample year).

3.3.4 | Identified prey species

Out of 1,050 sorted prey items, 492 were used for genetic identification. Prey with a freshness of 3 were not used, to ensure positive

**FIGURE 6** Histogram of squid mantle lengths consumed by red-footed booby over the two study years (2014 and 2015)

identifications due to the limitations of Sanger sequencing with potentially mixed species templates from highly digested prey. Eighty-two percent (401) of these prey items produced successful COI sequences and were genetically identified (Table 5). Thirty-two species (29 fish and 3 squid), belonging to nine fish families and two squid families, were identified. Three families (Acanthuridae, Molidae, Onychoteuthidae) occurred solely in 2014. All other fish and squid families were sampled in both study years.

The %PSIRI indicated that flying fishes (Exocoetidae) and flying squid (Ommastrephidae) were the most important prey in 2014 (15.6 and 10.8%, respectively) and in 2015 (15.0 and 10.6%, respectively; Table 5). The fish species with the highest importance were the tropical two-winged flying fish (*Exocoetus volitans*, 9.4%) in 2014, and the glider flying fish (*Cheilopogon atrisignis*, 8.3%) in 2015. The purpleback flying squid comprised 98.7% (233 of 236) of the identified squids and had the greatest importance (10.7% in 2014 and 10.5% in 2015; Table 5). The other three identified squids were two neon flying squids *Ommastrephes bartamii* (one from each year), and one *Onykia* sp. individual in 2014.

Rarefaction analyses of the pooled species data, revealed robust estimation of species richness (Figure 7). According to the empirical observations and the four estimators (Chao 1, Chao 2, Jack 1, Jack 2), the species richness reached an asymptote. While the estimator asymptotes surpassed the observed species richness asymptote by up to 10 species, all four estimators remained within the 95% confidence intervals of the estimated species richness.

3.4 | The effect of sexual dimorphism on diet composition

3.4.1 | Sexual size dimorphism

This analysis involved 56 birds of known sex with a complete set of morphometric data (body mass, culmen length, wing chord): 26 in 2014 (9 females and 17 males) and 30 in 2015 (14 females and 16 males; Table 6). All SDI values were positive, indicating that, on average, females were larger than males. Body mass was the most strongly sexually dimorphic trait, with SDI values of 13.1% (2014) and 13.5% (2015), followed by culmen length (2014: 3.9%, 2015: 3.8%), and wing chord (2014: 0.7%, 2015: 3.0%; Table 6). Thus, these morphometric data (body mass, culmen length, wing chord) were used to assign four birds of unknown sex using z-scores, where $z = (\text{bird value} - \text{sample mean for males or females}) / (\text{sample SD for males or females})$.

3.4.2 | Total sample mass and prey class comparisons

These 56 birds of known sex yielded a total of 82 regurgitations; 34 from 2014 (11 from females (some birds had more than one regurgitation), 23 from males) and 48 from 2015 (18 from females,

TABLE 5 Species list of 401 genetically identified prey items from 106 RFBO regurgitations from 2014 (A) and 2015 (B), showing the number, frequency of occurrence (FO), prey-specific percent mass (%PN), percent number (%N), prey-specific percent number (%PM) percent mass (%M), and percent prey specific index of relative importance (%PSIRI)

A. 2014								
Family	Scientific name	Total #	%FO	%PN	%N	%PM	%M	%PSIRI
Acanthuridae		1	0.0	1.0	0.0	7.7	0.0	0.09
	<i>Zebrasoma flavescens</i>	1	0.0	1.0	0.0	7.7	0.0	0.09
Carangidae		3	0.1	1.0	0.0	10.3	0.0	0.33
	<i>Decapterus macarellus</i>	2	0.0	1.0	0.0	12.4	0.0	0.26
	<i>Seriola dumerili</i>	1	0.0	1.0	0.0	6.1	0.0	0.07
Exocoetidae		48	0.4	2.7	0.2	85.8	0.3	15.62
	<i>Cheilopogon atrisignis</i>	2	0.0	2.0	0.0	152.4	0.0	1.51
	<i>Cheilopogon nigricans</i>	2	0.0	1.0	0.0	34.5	0.0	0.70
	<i>Cypselurus angusticeps</i>	1	0.0	1.0	0.0	120.7	0.0	1.19
	<i>Exocoetus monocirrhous</i>	2	0.0	1.0	0.0	34.7	0.0	0.70
	<i>Exocoetus volitans</i>	32	0.3	2.3	0.1	66.0	0.2	9.37
	<i>Exocoetidae</i> sp.	7	0.1	1.4	0.0	19.3	0.0	1.02
	<i>Hirundichthys coromandelensis</i>	1	0.0	1.0	0.0	10.3	0.0	0.11
	<i>Prognichthys occidentalis</i>	1	0.0	1.0	0.0	102.8	0.0	1.02
Gempylidae		7	0.0	3.5	0.0	47.3	0.0	1.00
	<i>Gempylus serpens</i>	7	0.0	3.5	0.0	47.3	0.0	1.00
Hemiramphidae		4	0.1	1.3	0.0	38.4	0.0	1.17
	<i>Euleptorhamphus viridis</i>	3	0.1	1.0	0.0	35.5	0.0	1.07
	<i>Oxyporhamphus micropterus</i>	1	0.0	1.0	0.0	8.8	0.0	0.10
Molidae		1	0.0	1.0	0.0	56.4	0.0	0.56
	<i>Ranzania laevis</i>	1	0.0	1.0	0.0	56.4	0.0	0.56
Mullidae		23	0.2	2.6	0.1	4.5	0.0	0.63
	<i>Mulloidichthys flavolineatus</i>	1	0.0	1.0	0.0	3.1	0.0	0.04
	<i>Mulloidichthys martinicus</i>	1	0.0	1.0	0.0	6.2	0.0	0.07
	<i>Mulloidichthys vanicolensis</i>	1	0.0	1.0	0.0	9.9	0.0	0.11
	<i>Parupeneus chrysonemus</i>	12	0.1	2.4	0.1	2.8	0.0	0.25
	<i>Parupeneus multifasciatus</i>	2	0.0	1.0	0.0	1.1	0.0	0.04
	<i>Parupeneus pleurostigma</i>	6	0.1	1.2	0.0	1.1	0.0	0.11
Nomeidae		1	0.0	1.0	0.0	3.4	0.0	0.04
	<i>Psenes cyanophrys</i>	1	0.0	1.0	0.0	3.4	0.0	0.04
Scombridae		5	0.1	1.0	0.0	40.3	0.0	2.03
	<i>Auxis thazard</i>	1	0.0	1.0	0.0	43.3	0.0	0.43
	<i>Katsuwonus pelamis</i>	4	0.1	1.0	0.0	39.6	0.0	1.59
Ommastrephidae		124	0.6	3.9	0.6	30.4	0.2	10.76
	<i>Ommastrephes bartramii</i>	1	0.0	1.0	0.0	3.2	0.0	0.04
	<i>Sthenoteuthis oualaniensis</i>	123	0.6	3.8	0.6	30.3	0.2	10.72
Onychoteuthidae		1	0.0	1.0	0.0	0.6	0.0	0.02
	<i>Onykia</i> sp.	1	0.0	1.0	0.0	0.6	0.0	0.02
B. 2015								
Family	Scientific name	Total #	%FO	%PN	%N	%PM	%M	%PSIRI
Carangidae		8	0.1	1.3	0.0	10.0	0.0	0.62
	<i>Decapterus macarellus</i>	6	0.1	1.2	0.0	9.8	0.0	0.50
	<i>Selar crumenophthalmus</i>	2	0.0	1.0	0.0	5.6	0.0	0.12

(Continues)

TABLE 5 (Continued)

B. 2015								
Family	Scientific name	Total #	%FO	%PN	%N	%PM	%M	%PSIRI
Exocoetidae		36	0.4	1.8	0.2	80.8	0.3	15.01
	<i>Cheilopogon atrisignis</i>	11	0.2	1.2	0.1	99.8	0.2	8.27
	<i>Cypselurus hiraii</i>	1	0.0	1.0	0.0	133.3	0.0	1.22
	<i>Cypselurus angusticeps</i>	5	0.1	1.7	0.0	70.8	0.0	1.98
	<i>Exocoetus monocirrhus</i>	2	0.0	1.0	0.0	1.8	0.0	0.05
	<i>Exocoetus volitans</i>	5	0.1	1.3	0.0	14.1	0.0	0.56
	<i>Exocoetidae</i> sp.	8	0.1	2.7	0.0	72.4	0.0	2.05
	<i>Hirundichthys coromandelensis</i>	1	0.0	1.0	0.0	23.4	0.0	0.22
	<i>Hirundichthys oxycephalus</i>	1	0.0	1.0	0.0	59.1	0.0	0.55
	<i>Parexoctetus brachypterus</i>	1	0.0	1.0	0.0	3.8	0.0	0.04
	<i>Prognathodes</i> sp.	1	0.0	1.0	0.0	7.8	0.0	0.08
Gempylidae		1	0.0	1.0	0.0	0.4	0.0	0.01
	<i>Gempylus serpens</i>	1	0.0	1.0	0.0	0.4	0.0	0.01
Hemiramphidae		7	0.1	1.2	0.0	24.7	0.0	1.41
	<i>Euleptorhamphus viridis</i>	6	0.1	1.2	0.0	28.8	0.0	1.36
	<i>Oxyporhamphus micropterus</i>	1	0.0	1.0	0.0	4.3	0.0	0.05
Mullidae		11	0.1	3.7	0.1	6.9	0.0	0.29
	<i>Mulloidichthys flavolineatus</i>	2	0.0	2.0	0.0	5.7	0.0	0.07
	<i>Mulloidichthys martinicus</i>	1	0.0	1.0	0.0	1.1	0.0	0.02
	<i>Mulloidichthys vanicolensis</i>	4	0.1	1.3	0.0	3.1	0.0	0.12
	<i>Parupeneus multifasciatus</i>	2	0.0	1.0	0.0	1.4	0.0	0.04
	<i>Parupeneus pleurostigma</i>	2	0.0	1.0	0.0	1.0	0.0	0.04
Nomeidae		7	0.0	7.0	0.0	28.3	0.0	0.32
	<i>Psenes cyanophrys</i>	7	0.0	7.0	0.0	28.3	0.0	0.32
Scombridae		2	0.0	1.0	0.0	2.4	0.0	0.06
	<i>Katsuwonus pelamis</i>	1	0.0	1.0	0.0	3.0	0.0	0.04
	<i>Thunnus albacares</i>	1	0.0	1.0	0.0	1.7	0.0	0.02
Ommastrephidae		111	0.5	3.8	0.6	36.2	0.2	10.55
	<i>Ommastrephes bartramii</i>	1	0.0	1.0	0.0	4.0	0.0	0.05
	<i>Sthenoteuthis oualaniensis</i>	110	0.5	3.8	0.6	36.0	0.2	10.50

Note: Bold %PSIRI values indicate highly important prey families and species.

30 from males). On average, female regurgitations had greater mass than male regurgitations in 2014 (females = 106.5 ± 68.1 SD; males = 90.8 ± 59.9 SD) and in 2015 (females = 105.3 ± 70.7 SD; males = 88.7 ± 64.2 SD; Table 6). However, the mass of the regurgitations was not significantly different between years or between sexes, and there was no significant interaction of these two factors (two-way ANOVA, Year: $F_{1,78} = 0.012$, $P = 0.915$; Sex: $F_{1,78} = 1.107$, $P = 0.296$; Year $\times$ Sex: $F_{1,78} = 0.001$, $P = 0.976$). Moreover, the fish mass in the regurgitations was significantly different by sex, with females delivering more fish than males (two-way ANOVA, Year: $F_{1,78} = 0.038$, $P = 0.847$; Sex: $F_{1,78} = 5.299$, $P = 0.024$; Year $\times$ Sex: $F_{1,78} = 2.339$, $P = 0.130$); however, the squid mass in the regurgitations was not (two-way ANOVA, Year: $F_{1,78} = 0.138$, $P = 0.711$; Sex: $F_{1,78} = 2.230$, $P = 0.139$; Year $\times$ Sex: $F_{1,78} = 3.725$, $P = 0.057$). While these patterns were consistent between the two study years, the marginally

significant Year $\times$ Sex interaction suggests that females delivered more fish and males delivered more squid in 2015.

The SDI values illustrated the observed patterns of sexual dimorphism, when we analysed the total mass and the composition (fish versus squid) of the regurgitation samples. In 2014, females consistently delivered heavier food loads (14.7%) than males, with more fish mass (15.0%), and more squid mass (13.1%). Whereas female diet samples were also heavier in 2015 (15.8%), females delivered more fish (59.2%) and males delivered more squid (–191.4%; Table 6).

3.5 | Exploratory analysis: Individual prey species

NMDS revealed a 1-dimensional solution with a final stress of 12.09, indicating that the ordination results were 'fair' and should be

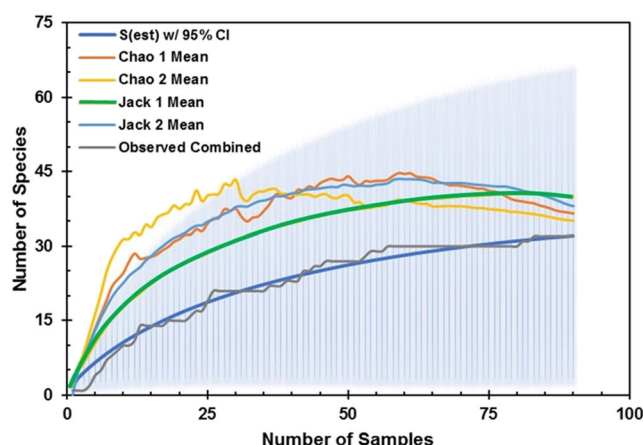


FIGURE 7 Rarefaction curves derived from 90 identified prey items collected over the two study years (2014 and 2015), showing the combined observed number of species (32), the 95% estimated confidence interval (CI), and four estimates: Chao1, Chao2, Jack1, and Jack2

interpreted with caution (McCune & Grace, 2002). The correlations of the prey species with the single resulting axis captured a fish–squid gradient, with samples being dominated (by mass) by either glider flying fish ($\tau = +0.432$) or purpleback flying squid ($\tau = -0.378$).

The MRPP chance-corrected within-group agreement, showed that the heterogeneity within groups was not different than that expected by chance ($A = 0.015$), and the groups were not significantly different ($P = 0.141$). Because the MRPP was not significant, the pair-wise tests were not considered, and no ISA was performed.

3.6 | Exploratory analysis: Prey functional groups

To facilitate the diet characterization, given the many rare species in the regurgitation samples, the species data were pooled into four functional groups with similar habitat-associations and habits: reef-associated fish, pelagic-oceanic fish, flying fish, and squid (Table 7).

The NMDS ordination of the functional groups generated a 2-dimensional solution, with a final stress of 4.46, indicating an ‘excellent’ ordination (Clarke, 1993; McCune & Grace, 2002). Consistent with the individual species NMDS, the two-dimensional ordination revealed that squids were positively correlated with both axes (axis 1, $\tau = +0.849$; axis 2, $\tau = +0.612$) and that flying fish were negatively correlated with both axes (axis 1, $\tau = -0.790$; axis 2, $\tau = -0.556$; Figure 8). The other two functional groups were not significantly correlated with the ordination axes: pelagic-oceanic fish (axis 1, $\tau = -0.087$; axis 2: $\tau = -0.338$) and reef-associated fish (axis 1, $\tau = -0.114$; axis 2, $\tau = +0.251$).

The MRPP chance-corrected within-group agreement, showed that heterogeneity within groups was greater than what was expected by chance ($A = 0.107$) and the groups were significantly different ($P = 0.002$). Pairwise comparisons, revealed three significant pair-wise group differences: 2014 males versus 2015 females ($A = 0.076$, $P = 0.02$), 2014 males versus 2015 males ($A = 0.047$, $P = 0.03$), and 2015 females versus 2015 males ($A = 0.258$, $P = 0.0003$). The other three pair-wise group comparisons were not significant: females from both years ($A = 0.054$, $P = 0.139$), males and females from 2014 ($A = -0.017$, $P = 0.705$) and 2014 females and 2015 males ($A = 0.022$, $P = 0.176$). Following the significant MRPP, ISA based on 9,999 iterations of the randomized data ($P = 0.0155$) confirmed that in 2015, females ate more flying fish ($P < 0.0036$) and males ate more squid ($P = 0.0397$; Figure 8). The other two functional groups (epipelagic fish, reef-associated fish) were not significant indicators.

4 | DISCUSSION

Hawaiian RFBOs have been considered generalist foragers because they consume a large variety of fish and squid prey, and associate with a wide range of sub-surface predators (dolphins, dolphinfish, tunas) (Ashmole & Ashmole, 1967a; Harrison et al., 1983; Hebshi et al., 2008; Seki & Harrison, 1989). Moreover, because RFBOs provisioning chicks range up to ~300 km from their colonies and dive up to

TABLE 6 Comparison of average ($\pm$ SD) female and male morphometrics and prey classes in regurgitation samples, using the sexual dimorphic index (SDI) (Angel et al., 2015), whereby positive (negative) values indicate that females have larger (smaller) values than males

Morphometrics	2014 ($n = 26$)			2015 ($n = 30$)		
	F ($n = 9$)	M ($n = 17$)	SDI (%)	F ($n = 14$)	M ($n = 16$)	SDI (%)
Sex (# Bird)						
Mass (g)	1131.7 $\pm$ 83.7	983.8 $\pm$ 70.2	13.1	1084.6 $\pm$ 63.2	938.4 $\pm$ 63.1	13.5
Culmen (mm)	85.0 $\pm$ 2.2	81.7 $\pm$ 2.9	3.9	84.5 $\pm$ 3.9	81.3 $\pm$ 2.0	3.8
Wing chord (mm)	402.1 $\pm$ 8.6	399.2 $\pm$ 7.3	0.7	405.1 $\pm$ 13.3	392.9 $\pm$ 8.3	3.0
Diet	2014 ($n = 34$)			2015 ($n = 48$)		
	F ($n = 11$)	M ($n = 23$)	SDI (%)	F ($n = 18$)	M ($n = 30$)	SDI (%)
Sex (# Regurgitations)						
Fish (g)	69.1 $\pm$ 53.7	58.7 $\pm$ 56.0	15.0	87.1 $\pm$ 71.3	35.6 $\pm$ 48.5	59.2
Squid (g)	33.8 $\pm$ 39.2	29.4 $\pm$ 30.0	13.1	18.1 $\pm$ 32.4	52.7 $\pm$ 55.6	-191.4
Total mass (g)	106.5 $\pm$ 68.1	90.8 $\pm$ 59.9	14.7	105.3 $\pm$ 70.7	88.7 $\pm$ 64.2	15.8

Note: Samples sizes are shown in parentheses.

TABLE 7 Classification of the 32 identified prey species into four functional groups (reef-associated, pelagic-oceanic, flying fishes, squids) defined based on taxonomic and habitat characteristics from Fishbase (www.fishbase.org)

Species	Reef-associated	Pelagic-oceanic	Flying fishes	Squids
<i>Zebрасoma flavescens</i>	*			
<i>Decapterus macarellus</i>		*		
<i>Selar crumenophthalmus</i>	*			
<i>Seriola dumerili</i>	*			
<i>Cheilopogon atrisignis</i>			*	
<i>Cheilopogon nigricans</i>			*	
<i>Cypselurus angusticeps</i>			*	
<i>Cypselurus hiraii</i>			*	
<i>Exocoetus monocirrhous</i>			*	
<i>Exocoetus volitans</i>			*	
<i>Exocoetidae</i> sp.			*	
<i>Hirundichthys coromandelensis</i>			*	
<i>Hirundichthys oxycephalus</i>			*	
<i>Paraxocoetus brachypterus</i>			*	
<i>Prognichthys occidentalis</i>			*	
<i>Gempylus serpens</i>		*		
<i>Euleptorhamphus viridis</i>		*		
<i>Oxyporhamphus micropterus</i>		*		
<i>Ranzania laevis</i>		*		
<i>Mulloidichthys flavolineatus</i>		*		
<i>Mulloidichthys martinicus</i>		*		
<i>Mulloidichthys vanicolensis</i>		*		
<i>Parupeneus chrysonemus</i>		*		
<i>Parupeneus multifasciatus</i>		*		
<i>Parupeneus pleurostigma</i>		*		
<i>Psenes cyanophrys</i>		*		
<i>Auxis thazard</i>		*		
<i>Katsuwonus pelamis</i>		*		
<i>Thunnus albacares</i>		*		
<i>Ommastrephes bartramii</i>				*
<i>Sthenoteuthis oualaniensis</i>				*
<i>Onykia</i> sp.				*

7.6 m (1.1 m on average), they can sample widely distributed prey in near-surface waters (Adams et al., 2020).

The goal of this study was to characterize the diet of chick-provisioning RFBOs using standardized morphological methods augmented with genetic identification. Moreover, to place these results in a broader ecological context, we investigated the diet during two years with contrasting oceanographic conditions (2014, 2015), characterized by changes in basin-wide (MEI and PDO) and regional (SST and WSP) environmental conditions.

The RFBO diet was very diverse, including 32 different species (29 fish and three squid; Table 7), indicating they are generalist foragers and consume a wide variety of prey, including reef-associated,

epipelagic, and mesopelagic fishes. However, flying fishes (Exocoetidae) and flying squids (Ommastrephidae) were the most important prey, and together accounted for 80% (by number) and 50% (by mass) of the regurgitation samples in both study years. In particular, a single species, the purpleback flying squid, accounted for 99% of all the identified squids (233/236), and was the most numerous identified prey during both study years; accounting for 21% (by mass) and 58% (by number) of the prey in the regurgitation samples. Our results that flying fish and flying squid are the most important RFBO prey are consistent with previous studies from other locations in the central Pacific (French Frigate Shoals, Palmyra, Christmas Island) and the Indian Ocean (Europa Island) (Ashmole &

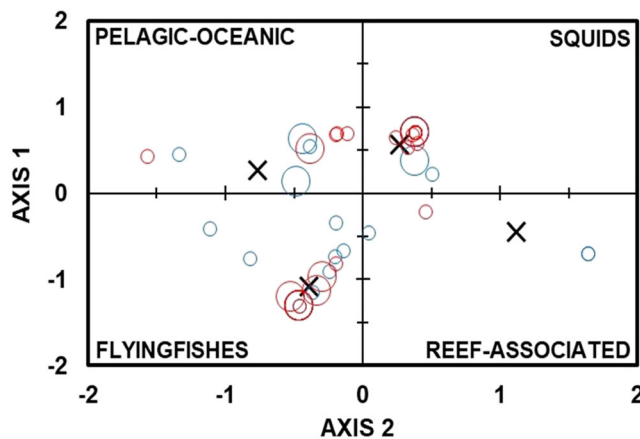


FIGURE 8 Non-metric multi-dimensional scaling and multi-response permutation procedure relationships showing the two-dimensional relationship amongst samples, where each quadrant represents one of four prey functional groups (flying fishes, squids, pelagic-oceanic fishes, reef-associated fishes). The 'Xs' are the coordinates of each prey functional group and the circles are the individual regurgitations by male (small) and female (big) for the two years, 2014 (blue) and 2015 (red)

Ashmole, 1967b; Harrison et al., 1983; Schreiber et al., 1996; Seki & Harrison, 1989; Weimerskirch, Le Corre, Ropert-Coudert, Kato, & Marsac, 2006; Young et al., 2010).

The RFBO diet changed in 2015, during a period of warmer SST and lower WSP associated with an El Niño event, with a concurrent increase in squid and a decrease in fish. Furthermore, the purpleback flying squids consumed were, on average, about 1 cm longer in 2015 than in 2014. However, these larger individuals were still juveniles, which remain in surface waters and are thus available to foraging RFBOs (Harman et al., 1989; Young & Hirota, 1998). Given the observed changes in SST during the two study years, the warmer water conditions in 2015 could have influenced the juvenile squid growth and survivorship, leading to greater abundance and larger size.

In the Indian Ocean, purpleback flying squids have been observed spawning sooner and growing faster during warmer-water conditions (Xinjun, Bilin, Siquan, Weiguo, & Xiaohu, 2006). This same environmental response was invoked off the windward (eastern) shore of O'ahu, where the majority of the large-sized fertile adult squids occurred in late spring and early summer (Suzuki, Yamamoto, Ishii, & Matsumoto, 1986; Young & Hirota, 1998). In the MHI, squids mature when they have a mantle length between 160 and 205 mm (females) and between 130 and 175 mm (males) (Young & Hirota, 1998). Thus, because all the individuals taken by the RFBO were smaller than 103 mm mantle length, these juveniles probably originated from a spawning event during the preceding spring (March–May).

Interestingly, because this species can spawn multiple times, adults may reproduce throughout the year (Harman et al., 1989). Around the MHI, ommastrephid paralarvae have been caught in large numbers along the windward side of the islands (Bower et al., 1999), and our results together with Adams et al. (2020) indicated the RFBO

foraging habitat overlaps the spawning grounds of this species. However, annual spring–summer squid surveys during multiple years with contrasting oceanographic conditions would be needed to better characterize the phenology and environmental drivers of *Sthenoteuthis oualaniensis* spawning and development.

Because little is known about flying fish distributions and responses to oceanographic variability in the central Pacific Ocean, it is unclear whether their abundance or spawning was affected by the warming documented in 2015. However, a study in the Caribbean found that, because growth rates were faster when SST was warmer, juveniles hatched in warmer water grew faster than juveniles in colder water (Oxenford, Hunte, Deane, & Campana, 1994).

Similar to other colonies in the central and south Pacific (Harrison et al., 1983; Lewis et al., 2005; Schreiber & Hensley, 1976; Weimerskirch et al., 2005; Young et al., 2010), Hawaiian RFBOs were sexually dimorphic, with larger females (Table 6). When we compared the diets of males and females, the larger-bodied females delivered significantly heavier food loads than males. However, the relative diet composition was not different between males and females in 2014, during a period of 'average' environmental conditions. However, females and males ate disproportionately more fish and squid, respectively, in 2015. While this disparity during the El Niño year potentially indicated sexual segregation of foraging niches, additional analyses of the tracking data collected from these individuals will provide further insights into the spatial and behavioural segregation of males and females.

This study documented temporal changes in RFBO diet during 2 years with contrasting oceanographic conditions and underscored the potential use of seabirds to study broader shifts in the marine ecosystem structure over time, by sampling their epi-pelagic nektonic prey. Unfortunately, the only historical RFBO diet data from this site involve notes published in 1967 (Ashmole & Ashmole, 1967a). Nevertheless, because this site is easily accessible, it is an ideal setting to study changing RFBO foraging and diet using consistent methods and metrics during periods with changing oceanographic conditions (e.g. Barrett et al., 2007). To this end, this study used novel genetic methods to develop a comprehensive understanding of the RFBO diet composition, by describing prey-specific importance, and species richness. Despite underscoring the generalist nature of the RFBO diet, involving 29 fish species, the high consumption of purpleback flying squid juveniles highlights the ecological importance of this poorly understood prey species, which is consumed by a wide range of Hawaiian seabirds and is an important fishery resource in tropical waters (Harrison et al., 1983; Harrison & Seki, 1987; Xinjun et al., 2006; Young & Hirota, 1998).

Studying the diet and foraging behaviour of seabirds can also provide insights into how ocean productivity and prey availability are likely to affect other upper-trophic marine predators (Harrison & Seki, 1987; Hebshi et al., 2008). In particular, RFBO forage with a large variety of SSPs throughout the eastern tropical Pacific and the central North Pacific (Au & Pitman, 1986; Harrison et al., 1983; Hebshi et al., 2008). Dietary studies at breeding colonies in the NWHI have documented that RFBO consume prey up to 20 cm in length,

which were attributed to feeding associations with large SSPs, including skipjack tuna (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*), dolphinfish (*Coryphaena hippurus*), and spotted dolphins (*Stenella attenuata*) (Harrison et al., 1983). While RFBOs most commonly forage with skipjack tuna around O'ahu, (80.0%), they associate with a wide variety of SSPs: cetaceans (12.5%), dolphinfish (5.0%), and yellowfin tuna (2.5%) (Hebshi et al., 2008). Skipjack tuna are most numerous around O'ahu during the RFBO chick-rearing season (spring–summer), further underscoring the ecological importance of their foraging association (Senina, Sibert, & Lehodey, 2008).

Because tuna and dolphinfish are commercially important fish in Hawai'i (Loke, Geslani, Takenaka, & Leung, 2012) developing fishery-independent metrics of the abundance and composition of their nektonic prey would allow fisheries managers to better understand and predict how changes within the marine environment can influence the availability and abundance of these predatory fishes around the MHI (e.g. Lehodey et al., 1997; Lyday et al., 2015). In particular, we contend that the RFBO diet is ideal for monitoring the composition of epipelagic fish and squid assemblages, and the abundance and development of their premier prey, juvenile purpleback flying squid. To this end, ongoing monitoring of RFBO diet over the next decade would provide valuable insight to better understand how ocean warming will affect the regional food web supporting pelagic predators around Hawai'i. Based on our results, we hypothesize that, as SSTs continue to increase, the purpleback flying squid will become an increasingly more important prey for epipelagic upper-trophic predators in Hawai'i.

In the 1980s, experimental night-time jigging around the MHI revealed large (>200 mm mantle length) and sexually mature purpleback flying squid spawning on the windward side of the archipelago, strongly suggesting that this species spawns repeatedly throughout the year, whenever they encounter suitable environmental conditions (Harman et al., 1989; Suzuki et al., 1986). However, it is unknown if the frequency and magnitude of these spawning events is changing through time, and whether the abundance of purpleback flying squid has changed since the 1980s. Annual winter–spring surveys for spawning and larval squid would be needed to characterize the phenology and environmental drivers of purpleback flying squid spawning and development (e.g. Bower et al., 1999). Additionally, spring–summer data from marine predators could provide information about the relative abundance and development of the juveniles not targeted by fisheries (e.g. Cherel & Weimerskirch, 1995).

Ecosystem modelling and fisheries catches have documented increasing squid populations in response to the fishing down of marine food-webs and the warming of tropical oceans (Baum & Worm, 2009; Doubleday et al., 2016). More favourable oceanographic conditions in the central Pacific could lead to more abundant purpleback flying squid in the future, and potentially to renewed interest in developing a jigging fishery for this species around the MHI. However, before this species can be commercially exploited, an ecosystem-based assessment, incorporating an understanding of the environmental drivers and the population dynamics will be needed, to

ensure the precautionary management of this fishery (Lyday et al., 2015; Rodhouse et al., 2014). To this end, RFBO could serve as biological samplers of purpleback flying squid. More specifically, RFBO regurgitations could provide information about juvenile squid relative abundance and development, before they mature and are accessible to the fishery.

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SUPPORTING INFORMATION

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