A Taste for Squid: Red-billed Tropicbird (*Phaethon aethereus*) Diet in the South Atlantic

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Abstract.—Examining prey choice is important for understanding the drivers of survival, breeding success and population change of tropical seabirds. We analysed spontaneous regurgitations from Red-billed Tropicbirds (*Phaethon aethereus*), at St Helena, South Atlantic between 2013 to 2018. Squid from four families, eight fish families, and one octopus species were identified. Dietary composition and diversity revealed Red-billed Tropicbirds fed predominantly on epipelagic juvenile squid, especially neon flying squid (*Ommastrephes cylindraceus*). Commercially exploited fish and species commonly used as bait and/or discarded, had a low frequency of occurrence (8.8%) and by number (3.5%) in regurgitations, indicating tropicbirds may have a low risk of mortality by bycatch. The consumption and size of squid varied among years; 2017 had significantly smaller squid consumed in greater proportions than in the other years observed. Chicks had larger meals (by ~41 g ± 39 g SD) and higher diversity of prey compared to adults, suggesting adults favoured selecting higher quality, heavier prey as a provisioning strategy for their chicks. The high frequency of occurrence of juvenile squid, and reliance on neon flying squid, suggests future dietary measures may provide valuable information about juvenile squid abundance and development, before they are mature and become commercially important to fisheries. *Received 9 Jan 2024, accepted 14 Jun 2024.*

Key words.—*Phaethon aethereus*, diet, St Helena, foraging ecology, squid, fish, bycatch, fisheries management

Seabirds are often used as bio-indicators of marine ecosystems (Rajpar *et al.* 2018), for example, to evaluate primary productivity (Velarde *et al.* 2013) and ecosystem health (Aliakbari *et al.* 2011). Studying seabird foraging ecology through diet composition is essential for the comparative interpretation and understanding of the relationships between seabirds as apex predators, and the marine environment (Catry *et al.* 2009). Monitoring fluctuations in the composition and availability of prey has been used to evaluate changes in marine resources and food webs (Cherel and Weimerskirch 1995; Einoder 2009), providing information, for example, on the effects of changes in limate (Montevecchi and Myers 1997; Ito *et al.* 2009), fish stocks (Miller and Sydeman 2004) and contaminants affecting ocean health (Cifuentes *et al.* 2003; Carravieri *et al.* 2016; Seco *et al.* 2021). In tropical oceans, where productivity is generally low and prey is generally patchily distributed, seabirds typically use foraging strategies such as feeding in multispecies flocks associated with sub surface predators (Soanes *et al.* 2021). In contrast, seabirds in temperate or polar environments have access to food resources that show less seasonal variation and tend to forage in habitats of known enhanced productivity such as upwellings, frontal zones and shelf edges (Ashmole and Ashmole 1967; Weimerskirch 2007).

Tropicbirds (Order Phaethoniiformes) are long lived pelagic seabirds with long incubation and fledging periods, lasting up to 43 and 119 days, respectively (Boeken 2016). Tropicbirds forage by surface plunging; previous studies using conventional analysis of prey remains (Le Corre *et al.* 2003) suggest they predominantly feed on

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surface-dwelling flying fish (Exocetidae) and squid (Ommastrephidae). Red-billed Tropicbirds (*Phaethon aethereus*) have a pan-tropical oceanic distribution, occurring in the Atlantic, Pacific and Indian Oceans (Orta 1992; Lee and Walsh-McGehee 2000). Unlike White-tailed Tropicbirds (*Phaethon lepturus*) that commonly forage in multi-species seabird flocks (Ainley *et al.* 1986; Hyrenbach *et al.* 2013), Red-billed Tropicbirds and Red-tailed Tropicbirds (*Phaethon rubricauda*) forage solitarily for prey, primarily at dawn and dusk (Diop *et al.* 2018; Madden *et al.* 2022, Madden *et al.* 2023) in the upper 3–10m of the water column (Sommerfeld and Hennicke 2010; Castillo-Guerrero *et al.* 2011). Whilst annual differences in diet composition have been observed in Red-tailed Tropicbird (Le Corre *et al.* 2003), few have been described in Red-billed Tropicbirds (Castillo-Guerrero *et al.* 2011).

Likewise, few studies of tropicbirds have compared information of the diet of adults and chicks. Indeed, when adult and chick diet has been examined, data has either been pooled together e.g., Diamond 1975; Harrison *et al.* 1983 or used in a comparative analysis of diet composition between species from the same locality (Stonehouse 1962; Ashmole and Ashmole 1967; Catry *et al.* 2009), or to quantify provisioning effort and frequency (Schaffner, 1988), inter-annual variation (Le Corre *et al.* 2003) or environmental effects (Castillo-Guerrero *et al.* 2011). Understanding seabird prey requirements and dietary flexibility through different life stages is important for effective conservation and management (Cherel and Bocher 2022), especially in a changing climate where distribution and abundance of available prey may vary significantly over time (Reynolds *et al.* 2019).

Assessing the threat that fisheries pose to long lived seabird species through either deletion of/competition for certain fish species or bycatch (incidental mortality) is vital for species conservation, but also to inform evidence-based fisheries management. St Helena Island holds one of the southernmost breeding sites of Red-billed Tropicbirds in the South Atlantic, representing a regionally and internationally important part of the global population (Beard *et al.*

2023a). The artisanal tuna fishery operating throughout the year at St Helena consists of a small inshore fishing fleet, targeting primarily yellowfin tuna (*Thunnus albacares*) and Skipjack (*Katsuwonus pelamis*) using pole and line and/or rod and reel. Tuna are attracted to the vessel using small pelagic fishes as bait. Trolling is also used and involves lines with artificial lures being towed behind the vessel, near to the surface at a certain speed (around 7 knots) to attract large pelagic fish such as wahoo (*Acanthocybium solandri*). Assessing the potential risk of this fishery for Red-billed Tropicbirds is urgently required, particularly to ensure that sustainable fisheries practices are used within the "category VI sustainable use" (St Helena Government 2022) 200 nautical mile Marine Protected Area (MPA) around St Helena. Assessing the susceptibility of this species to threats from fisheries is also fundamental for its effective global conservation management.

The primary goal of his study was to describe the diet of Red-billed Tropicbirds at St Helena, combining conventional identification methods with molecular diagnostics, to firstly assess whether existing fisheries might pose a risk of bycatch or prey depletion based on tropicbird prey composition, and secondly to assess differences in prey composition in relation to life stage and annual variation. We hypothesised that 1) diet would consist primarily of fish and squid, as documented by observations of Red-billed Tropicbirds in the eastern Atlantic Ocean (Diop *et al.* 2018), Gulf of California (Castillo-Guerrero *et al.* 2011), and Pacific Ocean (Spear *et al.* 2007), 2) that the diet of breeding and non-breeding adults would differ in taxonomic composition due to differences in foraging strategies and the constraints imposed by central-place foraging whilst breeding, 3) that the diet of adults and chicks would differ in taxonomic composition, and 4) that there would be variation between years in dietary composition.

METHODS

Prey Sampling and Identification

This study was conducted at St Helena, South Atlantic Ocean (15° 57' 57" S, 5° 42' 39" W) during colony

visits carried out between 21 August and 21 December, to coincide with the peak breeding period (Beard *et al.,* 2023a) from 2013 to 2018. Red-billed Tropicbird food samples (henceforth referred to as "regurgitations") were obtained opportunistically from adults and chicks which regurgitated spontaneously when handled. Regurgitations were processed at the Environment and Natural Resources Division laboratory of St Helena Government, St Helena. Each regurgitation was washed with water, weighed, and then prey items were separated and items individually weighed. Red-billed Tropicbirds sometimes regurgitated a large number of cephalopod beaks (keratinous mandibles), which accumulate in the gizzard. These accumulated prey items were separated from fresh (recently consumed/intact) prey items and processes separately. Identification of prey was made based on Smith and Heemstra 2012 for fishes, Clarke 1986 and Nesis 1987 for squid, and our own reference collection. Where identification was uncertain, prey samples were preserved and stored in alcohol (70%) until they could be sent off island for external verification by experts and/or DNA analysis.

The total length (TL, to the nearest 0.1 mm) and fresh mass (M to the nearest 1 g) of all intact fish, lower rostral length (LRL) for squid beaks, and lower hood length (LHL) for octopus beaks were measured to the nearest 0.01 mm using Vernier callipers. Regression equations given by Clarke 1986 and Xavier and Cherel 2009 for cephalopods and Smale 1993 for octopods, were used to estimate dorsal mantle length (ML) and fresh mass (M) from LRL or LHL. Where no relationships were available for a species, ML and M were extrapolated from equations for closely related species or for species with a similar morphology.

Three indices were used to describe Red-billed Tropicbird diet: percentage frequency of occurrence (%FO), average percentage abundance (%A) and percentage prey specific number (%PN) (Amundsen, Gabler and Staldvik, 1996). Species commonly encountered by local fisheries (Collins 2016) were used to further categorise identified prey items into commercially fished species (CFS), species commonly used as bait (B) and species accidentally caught during fishing practices and discarded (D). Risk of bycatch was assessed from the cumulative percentage frequency of occurrence (%FO) and numerical abundance of each category.

Prey DNA Isolation and Amplification

A representative selection of tissue from prey items that could not be formally identified to species level from examination (15 squid) were then used to extract genomic DNA for genetic barcoding. A standard lysis/ Proteinase K digestion was conducted, followed by a ammonium acetate salt and subsequent ethanol precipitation (Aljanabi and Martinez, 1997). Primers (for two COI and one 16S rRNA mtDNA gene fragments) and the conditions for DNA amplification by polymerase chain reaction (PCR) are provided in Supplementary Material Appendix 1. Resulting PCR products were sent for Sanger sequencing in both directions by Eurofins (Wolverhampton, UK).

Taxonomic Assignments of Prey

Chromatograms from sequence data were checked for quality and edited using MEGA, v.7.0.21 (Kumar *et al.* 2016), then compared to those in the GenBank nucleotide database using the BLAST algorithm (Altschul *et al.* 1990) (http://blast.ncbi.nlm.nih.gov/ cgi). Assignments were made to the highest possible taxonomic level (e.g., order, family, genus, species) based on percentage of similarity. Matches of 98-100% to the best hit in BLAST were recorded, or adjusted if the top-matching species did not occur in the south Atlantic. Sequences belonging to taxa other than the possible prey species (e.g., humans etc) were repeated and/or discarded from further analysis.

Statistical Analyses

All statistical analyses were performed in R 3.5.1 (R Development Core Team 2021). All general linear models (GLMs) and chi-squared tests were implemented in the *stats* package (R Core Team and contributors worldwide 2021), and all generalised additive mixed models (GAMMs) in the *gamm4* package (Wood and Scheipl 2020) following Thomas *et al.* (2017). Significant effects ($P < 0.05$) in final models were plotted using the *ggplot2* R package (Wickham 2016).

For the calculation of %FO, %A and %PN we excluded 27 regurgitations (23%) including 49 fresh and 6 accumulated prey items as they did not contain at least one item we could identify to species level. We were unable to examine relationships between fresh prey mass and taxon due to the high level of digestion observed in fish fresh prey items. As none of the dietary characteristics investigated varied with Red-billed Tropicbird sex (GLM, all $P > 0.10$; $n = 48$ regurgitations from males and 24 for females), data from males and females were pooled for comparisons between years, ages and breeding status. We used unpaired t-tests to examine differences in the fresh wet mass of regurgitations between ages (adults vs. chicks) and breeding status (breeding vs. non-breeding). A one-way analysis of variance (ANOVA) was used to assess differences in the fresh wet mass of food samples between years (2014–2018), using a subset of data from adults only, to avoid possible influence of age, as chicks were only sampled across two years (2017 and 2018).

The numerical frequency of a given prey taxon (fish or squid) was defined as the percentage of fresh prey items of that taxon in a regurgitation. A weighted binomial general linear model (GLM; Thomas *et al.* 2017) was then used to assess differences in diet between ages, and using adult data only we examined differences between breeding status and among years (2013-2018). In each model, the variable of interest (year, age or breeding status) was used as an additive and interactive effect, with taxon as a categorical variable.

A GLM was used to investigate differences in the occurrence of only fish, or only squid or both fish and squid, as fresh prey in regurgitations among years. Similarly, Chi-squared tests were used to test for differences

in fresh prey composition (fish, squid, or both) between ages and breeding status.

We used GAMMs to investigate whether cephalopods consumed by Red-billed Tropicbirds varied in size among years (2015–2018), between breeding status and between ages using lower rostral length (LRL) instead of mantle length (ML) to avoid extrapolation errors, and a subset of cephalopod species with a %FO > 5. In all models we used a gamma distribution and log link function, with individual identity as a random effect to account for repeated measures from the same individuals. Julian day (day of the year) was treated as a smoothed fixed effect to account for seasonal variation in prey size. Year was included as a fixed factor in models of age and breeding status differences in diet, to account for annual variation in prey size between years. The significance criterion for all tests was set at < 0.05. When applicable, data are presented as means ± standard deviation (SD).

RESULTS

A total of 117 regurgitations were collected from chicks ($n = 23$), as well as breeding ($n = 32$) and non-breeding ($n = 62$) adult Red-billed Tropicbirds between 2013 and 2018. Regurgitations came from 81 individuals (Supplementary Material Table S1) and of those individuals, 21 yielded more than one regurgitation (on different occasions).

Diet Composition

The mean fresh wet mass, determined from 93 of 117 regurgitations was 55.32 ± 38.37 g SD (range 3-147 g). The regurgitations contained an average of 2.21 ± 1.64 (range 1-9 items) fresh prey items and 15.78 ± 28.85 (range 0-150 items) accumulated prey items ($n = 117$). A total of 259 fresh prey items were found in the regurgitations. Fresh prey consisted of squid (i.e., flesh and squid beaks with transparent parts) and fish (Fig. 1A). Significantly more squid were observed (numerical frequency) than fish as fresh prey items in regurgitations (GLM; 73.3% vs. 26.6%, z = 9.322, $P < 0.001$; $n = 234$, Fig. 1B). Squid also occurred as the only fresh prey in 50.4% of regurgitations (i.e., 59 out of 117 regurgitations), compared to 27.4% where both fish and squid occurred and 22.2% with only fish.

Eleven families, ten genera and nine species were identified from fresh prey (Table 1). Prey sequences were obtained for nine out of

F1

T1

the 15 analysed regurgitated tissue samples (Supplementary Material Appendix 1). neon flying squid (*Ommastrephes cylindraceus*) had the highest overall prey occurrence (51.1% FO), while flying fishes (Exocoetidae) were the most frequently occurring fish family (22.2% FO). Three items from two regurgitations could not be assigned to either fresh or accumulated prey items: one parasitic fish isopod (unidentified Malacostraca spp.) and two parasitic copepods (*Brachiella thynni*); the latter were found in a food sample from a chick approximately four days old.

Accumulated prey items occurred in 23% of regurgitations (in 27 out of 117 regurgitations with fresh prey items), of which cephalopod beaks accounted for 97% ($n = 411$) and fish otoliths for 3% ($n = 3$) of all items. 119 cephalopod beaks from fresh prey items were collected from 52 regurgitations, resulting in a total of 276 upper and 254 lower beaks. 238 of the lower beaks were measured. Ommastrephidae was the most frequently identified prey family in the diet of Red-billed Tropicbirds, and *O. cylindraceus* the most common squid species in terms of number of lower beaks encountered (Table 2). The estimated ML of cephalopods for which beaks could be estimated ranged from 3.1 mm to 167.8 mm (mean = 98.9 ± 26.3 mm, $n = 238$, Fig. 2). The species with the smallest estimated ML was blanket octopus (*Tremactopus violaceus*, 3.1 mm) and the largest was the neon flying squid (*Ommastrephes cylindraceus*, 167.8 mm). In terms of mass, the smallest cephalopod consumed was *O. cylindraceus* with an estimated wet mass of <1 g, whereas the largest cephalopod was diamondback squid (*Thysanoteuthis rhombus*) with an estimated wet mass of ~287 g.

T2

F2

Species of Fisheries Interest

One commercially fished species, chub mackerel *Scomber japonicus*, was found in 2.2% of fresh diet samples (by frequency of occurrence). Two taxa were commonly used as bait: Chub Mackerel and *Decapterus* sp., collectively representing 3.3%. Four species occasional discarded from commercial inshore fishing practices represented 8.8% (club mackerel, *Decapterus sp*, Atlantic trumpetfish
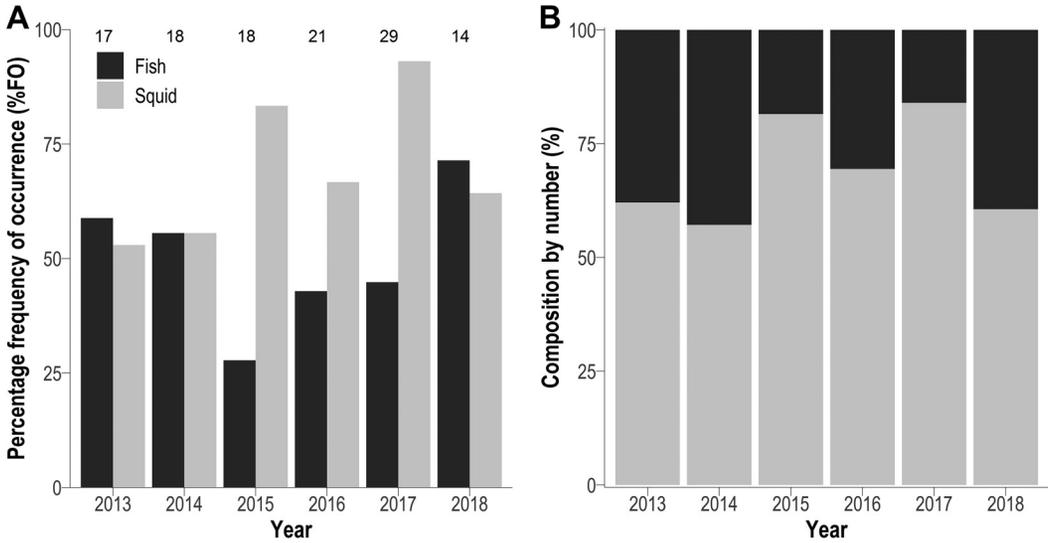
Figure 1. Composition of fresh regurgitations from Red-billed Tropicbirds at St Helena (adult and chicks) (A) frequency of occurrence (%FO) and (B) composition by number (%), per year monitored.

(*Aulostomus strigosus*) and keeltail needlefish (*Platybelone argalus trachura*) (Table 1).

Annual variation

There was no significant difference between years in the mean fresh wet mass of regurgitations (ANOVA, $F = 1.37$, df = 4, $P = 0.25$; $n = 71$), or evidence of differences between years in the consumption (numerical frequency) of fresh prey per regurgitation (GLM; $z = −1.58$, $P = 0.11$; $n = 188$). However, there were significant differences in the consumption of squid among years compared to fish (GLM; year: taxon: $z = 2.02$, $P = 0.04$; $n = 188$), notably 2017 had a considerably higher consumption of squid per regurgitation (mean proportion of squid: 73.1%) than in 2013 (mean: 46.1%). The number of regurgitations containing only fish as fresh prey differed significantly from regurgitations containing both fish and squid ($t = 2.44$, $P = 0.03$; $n = 18$), and the strength of these differences varied significantly between years (fish only: years: $t = −2.44$, $P = 0.03$; $n = 18$). After accounting for variation between prey species and across Julian Days, cephalopods in Red-billed Tropicbird diet were significantly smaller in size during 2017 and 2018 than in 2015 (2017: $−0.26 \pm 0.09$, $t = −2.70$, $P = 0.008$, 2018: $−0.46$

± 0.13, $t = −3.62$, $P < 0.001$; $n = 198$, reference year = 2015, model $R^2 = 0.20$, Fig. 3).

Breeding and non-breeding adult diet

Breeding adult regurgitations had a heavier wet mass than non-breeding adults (mean $\pm$ SD for breeding: 63.07 ± 41.12g, mean for non-breeding adults: 38.82 ± 27.66g; unpaired t-test, $t = 2.43$, $P = 0.02$, $n = 71$). There was little evidence of differences in the occurrence of only fish, squid or both as fresh prey ($X^2_2 = 3.07$, $P = 0.22$, $n = 94$), or differences in overall consumption (numerical frequency) of fresh prey items (GLM; $\beta = −0.41 \pm 0.24$, $z = −1.74$, $P = 0.08$, $n = 188$) between breeding and non-breeding adults. However, non-breeding adults consumed more squid (numerical frequency) as fresh prey items than breeding adults did (mean proportion of squid per non-breeding adult regurgitation: 66.9% vs. breeding adult: 57.3%, GLM; $z = 2.16$, $P = 0.03$, $n = 188$). After accounting for differences within seasons (Julian Day), between years and prey species, non-breeding adults consumed only marginally smaller squid than breeding adults ($\beta = −0.08 \pm 0.08$, $t = −0.89$, $P = 0.38$, $n = 84$, $R^2 = 0.24$, Supplementary Material Fig. S1a).

Table 1. Fresh prey items from breeding and non-breeding Red-billed Tropicbird regurgitations, and an indication of their occurance in fisheries practices. Number of prey items assessed (*n*), percentage frequency of occurance (%FO), percentage abundance (%A), percentage prey-specific number (%PN), inshore commercially fished species (CFS), inshore discards (D), bait species (B). * indicates newly recorded prey families or species in Red-billed Tropicbird diet.

Class	Order	family	Species	Totals	n	%FO	%A	%PN	CFS	D	B
Actinopterygii											
	Beloniformes			**69**			**26.6**				
				30							
		Belonidae	Platybelone argalus trachura*		5	4.4	1.9	55.6	—	✓	—
		Exocoetidae	Unidentified Exocoetidae		25	22.2	9.7	34.7	—	—	—
	Carangiformes			1							
		Carangidae	Decapterus sp.		1	1.1	0.4	100	—	✓	✓
	Perciformes			2							
		Scombridae	Scomber japonicus*		2	2.2	0.8	100	✓	✓	✓
	Syngnathiformes			1							
		Aulostomidae*	Aulostomus strigosus*		1	1.1	0.4	33.3	—	✓	—
	Tetraodontiformes			22							
		Molidae*	Ranzania laevis*		9	8.9	3.5	81.8	—	—	—
		Tetraodontidae	Lagocephalus lagocephalus		13	14.4	5.0	50	—	—	—
	Anguilliformes			2							
		Congridae*	Unidentified sp.		2	1.1	0.8	50	—	—	—
	Unidentified fish			11	11	6.7	4.2	33.3			
				190			**73.4**				
Cephalopoda				190							
	Oegopsida	Ommastrephidae	Ommastrephes cylindraceus*		69	51.1	26.6	52.7			
			Unidentified Ommastrephidae		2	2.2	0.8	33.3			
		Onychoteuthidae*	Onykia carriboea*		11	6.7	4.2	44.0			
			Walvisteuthis rancureli*		6	4.4	2.3	50.0			
		Thysanoteuthidae*	Thysanoteuthis rhombus*		1	1.1	0.4	100			
		Unidentified squid			101	35.6	39.0	47.6			
Total					**259**		**100**				

Table 2. Lower rostral length (LRL) for squid and lower hood length (LHL) for Octopodidae measurements (lower beaks), estimated dorsal mantle length (ML) and wet mass (g) of cephalopods identified in Red-billed Tropicbird diet (n = 238). % represents the proportion of each prey species in squid prey. * indicates newly recorded prey families or species in Red-billed Tropicbird diet.

Prey Species	n	%	LRL/LHL (mm)			ML (mm)			Estimated mass (g)		
			Mean ± SD	min	Max	Mean ± SD	min	Max	Mean ± SD	min	Max
Decapoda											
Octopoteuthidae*											
Octopoteuthis spp.	1	0.42	1.26	—	—	—	21.44	—	—	2.01	—
Ommastrephidae											
Ommastrephes cylindraceus*	170	71.43	1.94 ± 0.70	0.40	4.17	106.20 ± 19.22	63.74	167.83	19.23 ± 23.68	0.09	145.17
Hyaloteuthis pelagica*	8	2.84	1.62 ± 0.36	1.09	2.35	60.00 ± 8.86	47.15	77.78	6.39 ± 2.83	2.92	12.46
Onychoteuthidae*											
Walvisteuthis jeremiahi*	5	2.10	1.90 ± 0.32	1.54	2.25	87.12 ± 19.62	65.04	108.35	13.07 ± 6.12	6.50	20.26
Walvisteuthis rancureli*	18	7.56	2.13 ± 0.46	0.54	2.77	100.93 ± 27.89	4.04	140.07	19.04 ± 8.07	0.28	37.81
Onykia carriboea*	28	11.76	1.87 ± 0.42	0.70	2.65	84.97 ± 25.89	13.80	132.75	13.24 ± 7.84	0.61	33.10
Thysanoteuthidae*											
Thysanoteuthis rhombus*	2	0.84	2.23 ± 0.39	1.95	2.50	88.80 ± 11.63	80.58	97.02	210.46 ± 107.99	134.10	286.82
Octopoda											
Tremoctopodidae*											
Tremoctopus violaceus*	5	2.10	1.54 ± 0.47	1.00	2.18	16.79 ± 11.89	3.09	32.81	4.47 ± 4.42	0.76	10.98
Unknown	1	0.42	—	—	—	—	—	—	—	—	—
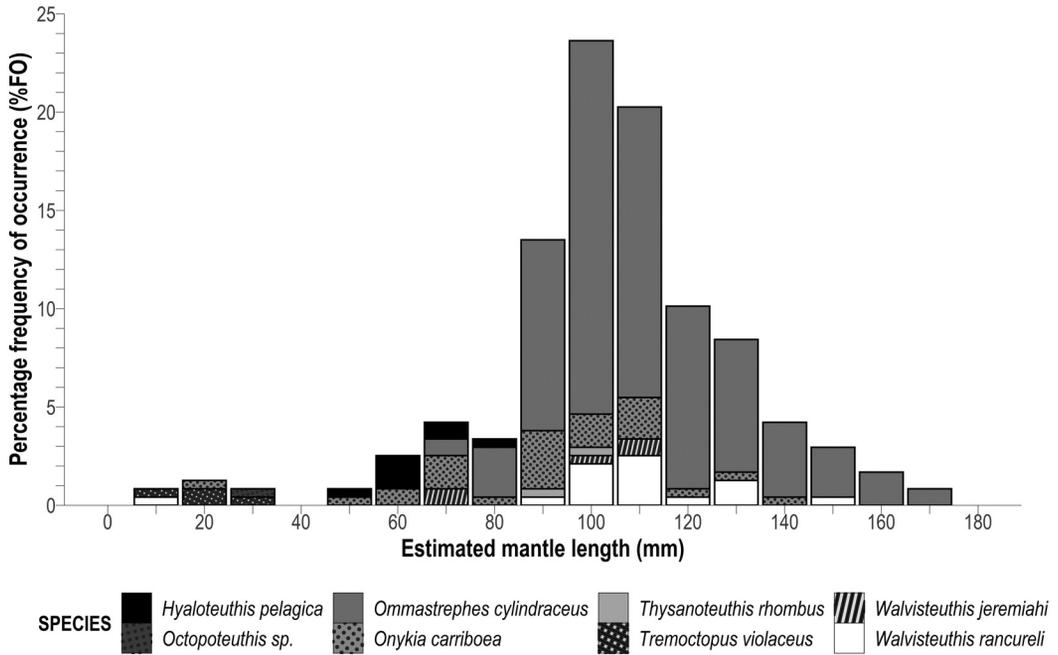
Figure 2. Percentage frequency of occurrence (%FO) of estimated mantle length (ML) of cephalopods consumed by Red-billed Tropicbird adults and chicks at St Helena in the South Atlantic (n = 237).

Adult and chick diet

Adult regurgitations were on average 41g lighter than that of chicks (mean for adults: 45.65 ± 38.19 g, mean for chicks: 86.54 ± 37.35 g; unpaired t-test, t = −4.65, df = 32.56, $P < 0.001$; n = 93). The diet of adults was considerably less diverse than that of chicks; adult regurgitations consisted predominantly of only squid as fresh prey items (54%), whereas chick regurgitations were more likely to contain both fish and squid (61%, χ^2 = 17.27, df = 2, $P < 0.001$; n = 117). We found no evidence of differences in overall consumption (numerical frequency) of fresh prey between chicks and adults (GLM; z = −1.71, P = 0.09; n = 234); however, chicks had a higher consumption of squid as fresh prey in regurgitations than adults (GLM, mean proportion of squid per regurgitation 74.9% vs. 63.6%, z = 2.18, P = 0.03; n = 234). After accounting for variation within a breeding season (Julian Day), variation between years, and prey species, chicks consumed only marginally, and not significantly smaller squid than adults (β − 0.06 ± 0.08, R^2 = 0.19, t= −0.72, P= 0.47; n = 198, Supplementary Material Fig. S1b).

DISCUSSION

This is the first detailed study of Tropicbird diet in the South Atlantic, enabling us to assess the susceptibility of Red-billed Tropicbirds to fisheries-related threats. We found that the diet consisted primarily of squid, containing only small proportions (< 10%) of species that are commercially exploited in local fisheries. This indicates that Red-billed Tropicbirds on St Helena are currently unlikely to be strongly affected by the current existing local fisheries. However, squid fisheries operating in international waters, which tropicbirds frequently use for foraging (Diop *et al.* 2018) often target the same squid species and may pose a bycatch risk or reduce the squid available for foraging tropicbirds.

Diet Composition

Diet consisted predominantly of squid, accounting for 73.4% of fresh prey items and fish (26.6%), in contrast to Red-billed Tropicbirds in the North Atlantic which fed exclusively on fish (Diop *et al.* 2018; Madden *et al.* 2022). Our study found both a high
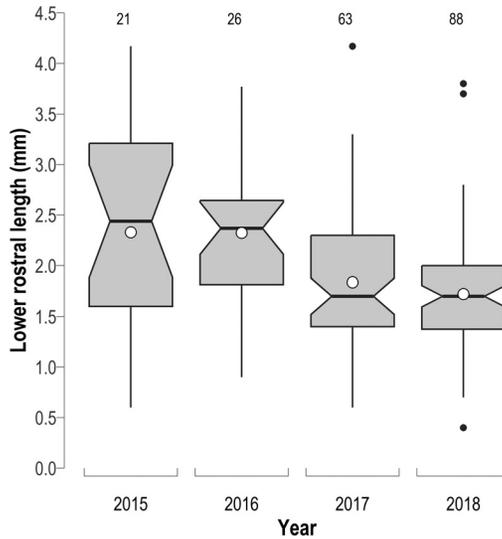
Figure 3. Relationship between lower rostral length (LRL) (mm) of consumed squid *Ommastrephes cylindraceus* and *Onykia carriboea* between 2015–2018 by Red-billed Tropicbird adults and chicks. Tropicbirds in 2015 consumed squid of significantly larger beak size than 2017 and 2018. The boxes show the 25th and 75th percentile, notches the median, and whiskers the maximum value excluding outliers (black dots). *n*: number of measured lower beaks is indicated at the top. White dots represent the mean value for each year.

occurrence (%FO) and numerical abundance of squid in the diet at St Helena, which is rare not only for Red-billed Tropicbirds, but for tropicbirds in general. In one of the two years during which Red-billed Tropicbird diet was observed using regurgitations in Farallon de San Ignacio, Mexico, cephalopods had a higher occurrence than fish (Castillo-Guerrero *et al.* 2011), but in all other known Red-billed Tropicbird diet studies which examined regurgitations, fish had a higher abundance and/or occurrence than squid (Diop *et al.* 2018; Llamas 2019; Madden *et al.* 2022; Madden *et al.* 2023).

Red-tailed Tropicbirds in the eastern Pacific and at Europa Island, Indian Ocean showed a lower occurrence of squid but a higher abundance (% number) than fish in regurgitations (Le Corre *et al.* 2003; Spear *et al.* 2007). Yet, on Christmas Island (Indian Ocean), Red-tailed Tropicbirds showed a higher occurrence of squid than fish (Ashmole and Ashmole 1967) in contrast to Aldabra Atoll (Indian Ocean), where the opposite was observed (smaller abundance and occurrence of squid than fish) (Diamond 1975). Similar findings apply to diet studies of White-tailed Tropicbirds: all but one study, at Aldabra Atoll, Indian Ocean (Diamond 1975), showed either higher occurrence and/or abundance of fish over squid (Schaffner 1988; Ramos and Pacheco 2003; Catry *et al.* 2009). These differences in diet between locations from examining regurgitations are likely to be influenced by prey availability, the geographical position of the colony in relation to the proximity to one or more oceanic fronts, species foraging preferences on continental shelves or oceanic waters or a combination, as seen in albatross species (Xavier *et al.* 2003).

We recorded for the first time four families of cephalopods in the diet of Red-billed Tropicbirds, including eight genera and seven species not previously documented in the diet. Three genera of fish including two families and four species were recorded in the diet for the first time. All species of squid recorded in the present study were new in the diet of Red-billed Tropicbirds in the South Atlantic. Although the tropical clubhook squid (*Onykia carriboea*) was recorded in the diet of Fairy Terns (*Gygis alba*) as early as 1965 at St Helena (Bolstad, 2008), it has not been recorded to date in the diet of any other seabird species at St Helena.

Despite evidence of fish not playing a major role in the diet, and the high level of digestion limiting identification of many fish to species level (~56%), our study demonstrates that Red-billed Tropicbirds prey on both coastal/neritic (e.g., needlefishes Belonidae) and oceanic fish (e.g. molas Molidae). Given the small number of other prey types that were included occasionally in the diet of some individuals, and all LRL were on average < 4 mm, indicating that ingested squid were epipelagic juveniles (Zuyev *et al.* 2002), we consider it likely that the population of Red-billed Tropicbirds at St Helena specialises in foraging on juvenile neon flying squid (*O. cylindraceus*).

Slender sunfish (*Ranzania laevis*) that were present in regurgitations are known to feed predominantly on squid of the family Ommastrephidae (Nyegaard *et al.* 2017), and it is therefore possible some accumulated cephalopod prey found in the Red-billed Tropicbird diet could have accrued through a secondary predator such as slender sunfish. Likewise, the copepods and isopod taxa could have been directly ingested by prey, attached to prey, or directly consumed by Red-billed Tropicbirds. Given the size of the parasitic isopods (< 1cm) it seems unlikely that the latter occurred. Ashmole and Ashmole (1967) also noted parasitic copepods and isopods in the diet of Red-tailed Tropicbirds in the Pacific Ocean, but considered them to be accidental prey, given the small number observed. It is nevertheless feasible that adult tropicbirds purposely forage for copepods to feed to small, young chicks.

Excluding the copepods and isopod that could not be defined as intentional or accidental prey, we found no additional evidence of crustaceans in Red-billed Tropicbird diet, although Penrose (1879) postulated that crabs formed a staple of tropicbird diets on nearby Ascension Island, and crabs have been recorded in small quantities in Red-billed Tropicbird regurgitations at the Gulf of California (Castillo-Guerrero *et al.* 2011). Similarly, at Cabo Verde small quantities of crab as well as DNA from a crustacean and mollusc were found in the regurgitations of Red-billed Tropicbirds (Llamas 2019; Carreiro *et al.* 2023) and small quantities of shrimp have also been found in Red-tailed Tropicbird regurgitations in Hawaii (Harrison *et al.* 1983). This likely reflects regional availability instead of prey preference, given the low energetic value of such small prey items.

The current understanding of prey behaviour, and vertical distribution of oceanic squid is very limited (Boyle and Rodhouse 2005). Squid are known to rise to the surface during the night to feed then descend to the ocean depths, 200-1000m in daytime (Zuyev *et al.* 2002). Given that the maximum recorded diving depth of Red-billed Tropicbirds is relatively shallow (25.6 m; Sommerfeld and Hennicke 2010) one might expect foraging to occur more frequently at night when squid are closer to the surface during their vertical migration; however, the timing of foraging behaviour has yet to be examined at this locality. Red-billed Tropicbird populations in the Caribbean and Senegal tend to be inactive and rest at night, with a foraging peak in the early hours after sunrise (Diop *et al.* 2018; Madden *et al.* 2022; Madden *et al.* 2023), but this may be explained by their diet being exclusively composed of fish. Similarly, Red-billed Tropicbirds at Cabo Verde and Mexican colonies were found to dive to much shallower depths (< 3m; Castillo-Guerrero *et al.* 2011; Saldanha *et al.* 2023), which may also explain their preference for fish over squid. Prey can also be made available at the upper layers of the ocean through the action of other foraging animals, e.g., tuna and dolphins, which herd prey to the surface. Tropicbirds are thought to often forage in association with sub-surface predators (Ashmole and Ashmole 1967; Ballance and Pitman 1999; Spear *et al.* 2007) such as tuna, which have shown a high consumption of epi- and mesopelagic prey (Perrin *et al.* 1973). Examining the spatial, temporal and interspecific occurrence of foraging behaviour of the St Helena Red-billed Tropicbird population may offer more insights into their foraging ecology. The use of additional taxonomic identification techniques such DNA metabarcoding of regurgitations or faecal samples as well as Stable Isotope Analysis could also

provide further insight into predator prey tropic interactions (Carreiro *et al.* 2023; Piña-Ortiz *et al.* 2024).

Susceptibility to Bycatch

The fish *S. japonicus* was the only locally commercially fished species of high global commercial value (Sumaila *et al.* 2007) found in the Red-billed Tropicbird diet samples. *S. japonicus,* along with other species commonly used as bait are predominantly caught before dawn within 30 nautical miles of the coastline (Laptikhovsky *et al.* 2020) when Red-billed Tropicbirds may forage (Diop *et al.* 2018; Madden *et al.* 2022; Madden *et al.* 2023). However, given that their foraging range from St Helena extends considerably outside the Marine Protected Area (MPA; Diop *et al.* 2018), the risk that local commercial fisheries pose to Red-billed Tropicbirds at St Helena is probably low.

Another component of tropicbird diet around St Helena is roughear scad (Carangidae; *Decapterus tabl*), which is found in small numbers in tropicbird regurgitations (A. Beard pers. obs.). In contrast, Carangidae (*Decapterus* sp.) account for over 70% of bait used in commercial tuna fisheries around St Helena (Laptikhovsky *et al.* 2020). The low incidence of commonly used bait fish (Carangidae and Scombridae) and species commonly discarded (Table 1) implies that Red-billed Tropicbirds are not dependent on fisheries for food, unlike other seabird species, for example Northern Gannets *Morus bassanus* (Patrick *et al.* 2015). These results agree with findings by Diop *et al.* 2018, who concluded that Red-billed Tropicbird breeding at Senegal may partly feed on discards but are not reliant on them. Overall, neither the risk of bycatch nor the risk of prey competition or depletion appear to currently affect tropicbirds at St Helena.

Annual Variation

The observed differences in diet among years in our study suggest that this may have been driven by annual differences in the availability of prey species. Squid were the predominant prey favoured by Red-billed Tropicbirds; despite considerable variability in availability as the number and size of squid caught per regurgitation varied among years, there were no observed differences in the occurrence of only squid in regurgitations or occurrence between years compared to regurgitations containing only fish or both fish and squid. In a patchy oceanic environment where there is annual variation in food availability it suggests that in the South Atlantic, squid are the more predictable and/or preferred prey species than other species such as pelagic fish. Indeed inter-annual variability in oceanographic conditions caused by El Niño Southern Oscillation (ESNO) have been shown to affect Red-billed Tropicbird foraging behaviour and diet (Castillo-Guerrero *et al.* 2011). Whilst fluctuations in the Angola Benguela upwelling system and its influence of prey availability in the south Atlantic were not examined in this study, 2015 coincided with a moderate cooling event along the Angolan-Namibian coastline (Imbol Koungue *et al.* 2019) and a considerable increase in the occurrence and number of squid found in Red-billed Tropicbird regurgitations (Figure 1ab). There was also among year variability in frequencies of regurgitations containing both fish and squid prey to those just containing fish, suggesting fish were caught opportunistically. The degree of digestion of fish prey meant that reliable estimation of length and mass was not possible. However, fish could be larger in size and mass compared to squid, which would reduce a tropicbird's limited payload capacity to carry multiple prey items such as both fish and squid. During the 2015 Angola Benguela cooling event, adult Red-billed Tropicbirds at St Helenian showed significantly lower body condition than in other years 2013-2017 (Beard *et al.* 2023b), suggesting that environmental fluctuations may have impacted both prey and predators in this little-studied upwelling system. It was not possible during this study to examine within year dietary differences due to sample sizes, further investigation of within and

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between year dietary preferences of Red-billed Tropicbirds in relation to environmental and oceanographic parameters would be advantageous. Our findings highlight the importance of multiyear studies to identify drivers of variation in diet composition.

Breeding vs. Non-breeding Adult Diet

There were few differences observed in the diet between breeding and non-breeding adults. Although regurgitations were generally lighter in mass and contained a higher proportion of squid as fresh prey in non-breeding adults compared to breeding adults, there was little evidence of differences in the occurrence or frequency of fresh prey, or the size of squid consumed. This is surprising given that breeding adults are often constrained to forage within an accessible distance to the breeding colony, whereas non-breeders can forage for longer over larger distances, and therefore likely exploit different areas at sea.

Adult vs. Chick Diet

This is the first study using a traditional approach to compare Red-billed Tropicbird adult and chick diets, however Piña-Ortiz *et al.* (2024) compared adult and chick diets using stable isotopes. Diets of adults were considerably less diverse than those of chicks. Adult regurgitations consisted predominantly of only squid, whereas chicks were more likely to contain both fish and squid. This is consistent with findings by Piña-Ortiz *et al.* (2024) where higher stable isotopic ratios were observed for chicks than adults, suggesting that adults select higher quality prey for their chicks compared to what they consume themselves. This is a common strategy in many bird species during chick rearing; for example, among Crested Terns (*Sterna bergii*) (McLeay *et al.* 2009). As a chick grows there is a high energy demand, and energy-rich prey species, which have a high protein or lipid content, are often preferentially collected for delivery to young, compared to prey ingested by parents (Ojowski *et al.* 2001). Squid are considered to have a lower calorific content due to lower contents of lipids

than fish (Croxall and Prince 1982; Zuyev *et al.* 2002).

As expected, Red-billed Tropicbird chicks consumed larger quantities (proportion per regurgitation) of squid than adults, and slightly (although not significantly) smaller sized squid as fresh prey than adults. This may be because chicks are often constrained by their physical capacity; smaller squid would be easier to ingest, and as chicks grow in size, so does their ability to ingest larger volumes in one feed (Schaffner 1988). Our results suggest that Red-billed Tropicbirds at St Helena select higher quality, heavier prey for their chicks over quantity as a provisioning strategy. However, all differences must be interpreted cautiously as it is possible that during the return flight from foraging areas, adult prey had been digested for a longer period than meals delivered to chicks. Similarly, it is unclear if regurgitations from adults rearing chicks were intended for themselves or for the chick however, the limited dietary differences observed between breeding vs. non-breeding adults suggests minimal influence on the differences in adult vs. chick diet. Our study did not allow a comparison of the differences in prey species between adults and chicks or between different breeding stages (non-breeding, incubation and chick rearing) due to the small sample size. It would be advantageous to examine this further in the future.

Management Implications

Both trolling and pole and line are highly selective fishing methods (Santiago *et al.* 2016), with pole and line fishery considered to have a low associated bycatch relative to other tuna fishing methods e.g., purse seine (Miller *et al.* 2017). Given the low incidence of commercial, bait and discarded fish species identified in the diet of Red-billed Tropicbirds, totalling 3.5% of fresh prey (Table 1), the fact that current fisheries management prohibits the use of less selective fishing methods (e.g., purse seine, bottom trawls, gill and tangle nets and longlining) and that illegal, unreported and unregulated fishing within the MPA is thought to be rare (St Helena Government

2022), we consider the threat posed by current commercial fishing practices pose on Red-billed Tropicbirds within the St Helena MPA, to be low.

The Ommastrephidae family was identified as the predominant cephalopod family in Red-billed Tropicbird diet. This family is the most important commercial group of cephalopods globally, representing almost 50% of the total fished cephalopod biomass (FAO, 2019). Despite squid not currently being a target of local commercial fisheries (St Helena Government 2022), Red-billed Tropicbirds are known to forage extensively outside of the 200 nautical mile MPA (Diop et al. 2018), where commercial cephalopod fisheries occur. Future changes in fisheries practices therefore, could have important implications for sustainable management of resources and potential change in susceptibility of tropicbird populations which show a high consumption of cephalopods e.g., Red-billed Tropicbirds at St Helena (this study) and White-tailed Tropicbirds in Puerto Rico (Schaffner 1988) to bycatch mortality.

The large component of juvenile squid found in the diet of Red-billed Tropicbirds from St Helena suggests that further studies could not only use Red-billed Tropicbirds as indicators of the marine environment to inform about future changes in local juvenile squid abundance and fisheries practices, but demonstrate that the known sensitivity of tropicbirds to changes in climatic conditions (Le Corre et al. 2003; Castillo-Guerrero et al. 2011) can be important in developing our understanding of climate change in tropical marine systems, through monitoring its impacts on prey distribution and abundance (Diamond and Devlin 2003).

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