

UNDERWATER BEHAVIOR OF CHRISTMAS SHEARWATERS *PUFFINUS NATIVITATIS* FROM KURE ATOLL

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ABSTRACT

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The Christmas Shearwater *Puffinus nativitatis* is an aquatic, tropical shearwater. While individuals of this species are known to forage by pursuit plunging and diving, there are no published reports quantifying their sub-surface foraging behavior. We obtained diving information from eight chick-rearing Christmas Shearwaters on Kure Atoll during June–August 2017. Over the 33 days during which instruments were deployed, 1521 dives were recorded. Individual deployments lasted three to eight days and were independent of the maximum depths recorded. Individual maximum depths were 10.7–24.1 m, averaging 15.4 m. While the deepest and longest recorded dive reached 24.1 m and lasted 31 s, the majority of dives were < 3.0 m deep (64%) and lasted ≤ 3.0 s (53%). Overall, deeper dives were longer. Almost all diving occurred during daylight, with peak diving activity (13%) occurring between 17h00 and 18h00 (dusk). Genetic metabarcoding identified digested prey items from regurgitations, distinguishing 11 species of fish and squid. This study highlights the way in which this species of concern uses the epipelagic foraging habitat within the Papahānaumokuākea Marine National Monument.

Key words: Christmas Shearwater, *Puffinus nativitatis*, diving, diet, Papahānaumokuākea Marine National Monument, seabird foraging behavior

INTRODUCTION

The Christmas Shearwater *Puffinus nativitatis* (CHSH) is a medium-sized seabird of the order Procellariiformes (body mass averages 354 ± 42 g (standard deviation, SD), $n = 89$; Harrison *et al.* 1983). CHSH are found throughout the tropical Pacific, breeding colonially on remote islands (Seto 2001). The breeding biology and demography of this species have been well described (Seto 2001, VanderWerf *et al.* 2015) and researchers have generalized CHSH foraging distributions and behavior from at-sea surveys (Ashmole 1971, Gould 1971), but there is no detailed information concerning their sub-surface foraging behavior. Namely, no study has quantified when, how often, and how deep they dive. However, Spear *et al.* (2007) found that CHSH have a diet dominated by flying fish (Exocetidae) and flying squid (Ommastrephidae), mostly forage over schools of yellowfin tuna *Thunnus albacares*, and are closely associated with Wedge-tailed Shearwaters *Ardenna pacifica* (WTSH), Sooty Terns *Onychoprion fuscatus*, and Great Frigatebirds *Fregata minor*.

Shearwaters in the genera *Puffinus* and *Ardenna* possess morphological adaptations for underwater propulsion (Kuroda 1954), and they forage by pursuit plunging and diving (Ashmole 1971). For these shearwaters, maximum-depth gauges and archival time-depth recorders (TDRs) have revealed a wide range of maximum depths (10–69 m, $n = 7$), a measurement that scales allometrically with mass (Shoji *et al.* 2016). Moreover, these

maximum depths cluster near the best-fit allometric regression for deep-diving penguins and alcid (Burger 2001, Shoji *et al.* 2016), indicating that *Puffinus* and *Ardenna* shearwaters are well adapted for diving. Specifically, CHSH are grouped with Manx-type shearwaters in the *P. puffinus* superspecies, which forage by wing-propelled diving and which have lower aspect ratios and higher wing loading than other shearwater groups (Spear & Ainley 1997a, Hertel & Ballance 1999, Spear *et al.* 2007). CHSH have three “highly aquatic” morphological characteristics associated with underwater movement: (i) laterally compressed tarsi (Kuroda 1954), (ii) low aspect ratio (~10), and (iii) particularly high wing loading for shearwaters (48 N·m⁻²) (Spear & Ainley 1997a, 1997b; Hertel & Ballance 1999). These morphological traits (Kuroda 1954, Hertel & Ballance 1999) and direct foraging observations (Ashmole 1971, Gould 1971, Spear & Ainley 1997a, 1997b; Spear *et al.* 2007) indicate that diving is a key part of CHSH life history.

“Tuna birds” are seabirds that feed with tuna in competitive multi-species flocks, with prey size (as a function of body/bill size) being an important segregating factor (Spear *et al.* 2007). Strong underwater pursuit capabilities offer CHSH an advantage over the other tuna birds, which practice depth-restricted plunging and surface-foraging (Harrison *et al.* 1983, Spear *et al.* 2007). By diving deeper, CHSH are able to target different prey than the somewhat morphologically similar WTSH (Spear *et al.* 2007). Studies of CHSH diet (Harrison *et al.* 1983, Spear *et al.* 2007), isotopic signatures (Bond *et al.* 2010), and metal contamination (Gochfeld

et al. 1999) corroborate field observations that the CHSH diet is distinct from other tuna birds. However, an integrated analysis linking prey selection with underwater behavior might well allow a fuller interpretation of these patterns.

Few descriptions of CHSH foraging behavior or epipelagic habitats have been published, and their foraging ranges in the central North Pacific are unknown (Seto 2001). In Hawaiian waters, most of the CHSH population breeds within the Papahānaumokuākea Marine National Monument (PMNM; Harrison *et al.* 1983), but the degree to which they use these waters remains a mystery. In 2016, the PMNM boundary was expanded from a radius of 50 nautical miles (92.6 km) around each island to the 200 nautical mile exclusive economic zone. As CHSH are considered a “species of high concern” due to inferred declining populations throughout the Pacific (PMNM 2008), evidence that locally breeding birds forage within the PMNM will underscore the importance of this protected area for the species.

This study reports the first TDR deployment on this species and describes both the foraging behavior and the diet of chick-rearing CHSH from Kure Atoll. More specifically, we first quantified five dive parameters: depth, duration, post-dive interval, hourly dive frequency, and behavioral aerobic dive limit. Then, we compared their observed maximum diving depth to 23.4 m, the empirical prediction based on an allometric regression developed using published maximum dive depth and body mass data from other TDR-tagged *Puffinus* and *Ardenna* shearwaters. Due to their highly aquatic morphological adaptations, we hypothesized that CHSH would exceed this predicted limit. Yet, due to their association with surface prey patches driven by sub-surface predators, we anticipated that most dives would be short and shallow. We also used the diving data and flight speeds to estimate their minimum foraging ranges from the colony, with the expectation that this species indeed forages within PMNM. Finally, we identified the prey items retrieved from tagged birds using gene sequences and discussed the CHSH diet considering the documented diving behavior.

STUDY AREA AND METHODS

Field methods

This study was conducted on Kure Atoll (28°25'N, 178°20'W), which is the island furthest to the northwest within PMNM. TDRs (Lotek LAT 1500) were 8 mm in diameter by 32 mm in length, had a cross-sectional area of 50 mm², and weighed 3.4 g in air and 0.9 g in seawater. The TDR pressure resolution was 0.05% of 1 dbar, with an accuracy of ± 1%. TDRs were < 1% of a CHSH's cross-sectional area (~166 190 mm²). TDRs were programmed to log continuously once every 30 minutes and to log conditionally every second once a pressure threshold of 0.5 dbar was reached, to indicate true dive activity. As one decibar is nearly equivalent to one meter of depth of seawater (1 dbar = 0.99 m depth in seawater), all pressure measurements will henceforth be expressed in meters. An activity (wet/dry) sensor was used to confirm whether the conditional pressure readings occurred underwater and to indicate the birds' location (on/off the water) at each 30-minute reading.

Two TDR devices were available for this study, and they were re-deployed sequentially on different birds throughout the summer of 2017 (Tag 1, Tag 2). Tag deployment occurred from 29 June to 09 August (Table 1). Adult birds were selected opportunistically, depending on accessibility and the presence of a large downy chick (body mass > 200 g), that had already been left unattended during the day. Ultimately, eight chick-rearing CHSH were outfitted with a TDR, with one adult per breeding pair tagged.

Birds were captured by hand at their nest site after dark (21h30–23h30, Hawai'i Standard Time, HST). The TDR was attached to the underside of the tail with Tesa Tape. Birds were marked with a small dot of white-out on the top of their head to distinguish them from their mate for tag retrieval, and they were released back to their nest site. To ensure the birds went out to sea at least once while tagged, the nest site was checked every 15 minutes (21h30–00h30) starting three days (72 hours) after tag deployment. Searches continued

TABLE 1
Summary of TDR deployment effort on Kure Atoll, 2017

| Bird # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|--------------------------------------|-----------------------------------|--|---------------------------------------|--------------------------------------|-------------------------------------|-----------------------------------|-----------------------------------|
| Deployment date | 6/28 | 6/28 | 7/13 | 7/21 | 7/21 | 7/31 | 7/31 | 8/06 |
| Deployment duration (days) | 8 | 5 | 3 | 4 | 4 | 3 | 3 | 3 |
| Chick weight (g) | 230 | 205 | 235 | 315 | 215 | 365 | 415 | 240 |
| Daylight during tagging period ^a | 61% | 61% | 61% | 60% | 60% | 59% | 59% | 59% |
| Moon illumination during tagging period ^a | 25% | 25% | 75% | 10% | 10% | 50% | 50% | 100% |
| TDR no. (1 or 2) | 1 | 2 | 1 | 2 | 1 | 1 | 2 | 1 |
| Tag drift regression ^b (<i>n</i> , adjusted <i>R</i> ² , slope ± 2 SE) | 387, 0.01, −0.00005 ± −0.38 | 245, 0.05, 0.0002 ± 0.23 | 145, −0.007, −0.00001 ± −0.44 | 221, −0.005, 0.000002 ± 0.19 | 221, 0.02, −0.00008 ± −0.49 | 144, 0.54, −0.0017 ± −0.46 | 146, 0.03, 0.0002 ± 0.20 | 146, 0.06, 0.0003 ± 0.67 |

^a Data retrieved from Time & Date (<https://www.timeanddate.com/>) on 18 July 2019.

^b Total *df* = *n*−1

each night until tags were retrieved. Additionally, nest sites were searched from 07h00 to 08h30 if the tagged bird was not located the night before. If a bird voluntarily regurgitated during tag retrieval, the sample was collected and stored in a freezer for genetic analysis.

Statistical analysis

Dive summaries

Pressure values from TDRs were recorded in decibars and converted to meters of depth. Dive profiles from the TDRs, covering the 33 days during which tags were deployed, were extracted and summarized using the package “diveMove” (Luque 2007) in R (R Core Team 2013). We assessed all dive events individually for accuracy. We augmented the “diveMove” output summaries with hand calculations for any erroneously calculated dives and reinstated short-duration (< 3 s) dives. To account for potential differences in the behavior of the pressure transducers, we calculated a surface offset for each bird. First, a linear regression was used to determine if the pressure sensors had drifted during the tracking period by quantifying the trends in the pressure from dry 30-minute records, which indicated non-diving activity (Table 1). Additionally, offset values were calculated per tag by applying the largest pressure value of the background logging data when the bird was not diving (dry activity logger) to all values recorded underwater from that tag. These zero-offset values represent the potential systematic bias of each TDR and provided a conservative correction for dive depth. Individual dive events were identified as deviations from 0 m, greater than a threshold of 0.5 m, and lasting for ≥ 1 s.

All summary statistics and tests were performed in R using the R Commander interface (version 2.5-1; Fox 2005), and significance was assessed using $\alpha = 0.05$. Unless stated otherwise, all means are stated with SD. Dive parameters were summarized to allow comparisons to other shearwater TDR studies (e.g., Shoji *et al.* 2016). For each dive, we calculated the maximum depth reached (m), dive duration (s), and post-dive interval (s). Because the deployment duration and the number of dives executed per day varied across individuals, averages were first calculated for individual birds and combined across birds.

To analyze temporal variability in diving behavior, the data were binned using hourly increments. Daylight hours were defined using civil twilight, which occurred at 06h35 \pm 0h08m through 21h06 \pm 0h02m during the 29 June–09 August tagging study. Descriptive statistics (frequency, mean, median, and maximum) of the dive depths and durations were calculated for each hour of the day. Chi-squared tests were used to discern temporal variation among individuals.

To assess the behavioral aerobic dive limit (bADL) and dive bouts, the post-dive interval (PDI) was calculated for each dive. PDIs suggest the efficiency of dive activity with regards to anaerobic metabolism (Butler 2001). Dives longer than the bADL should be increasingly anaerobic, while dives shorter than the bADL should be aerobic (Dean 2012). To distinguish a threshold between short and long dives relative to a PDI, we plotted PDI against dive duration. The slope of the line joining the lowest PDI values theoretically defines the bADL (Kooyman & Kooyman 1995, Tremblay *et al.* 2005, Dean 2012). PDI was truncated at 300 seconds. Dive bouts were defined as intervals of sequential dive activity separated by rest periods (no diving) on the surface or in flight. A log-

survivorship curve was used to identify bout intervals in CHSH. The bADL and dive bouts were estimated using all the dives recorded, across multiple individuals.

We compared the maximum dive depth of CHSH to that of other *Puffinus* and *Ardenna* shearwaters tagged with TDRs using an allometric regression of maximum dive depth as a function of mean body mass, including published data from other species during the breeding season (incubating to early chick-rearing; Shaffer *et al.* 2009, Rayner *et al.* 2011, Hyrenbach *et al.* 2013, Péron *et al.* 2013, Meier *et al.* 2015, Shoji *et al.* 2016). Whenever a species had been tagged using different instruments, only the maximum depth from TDR tags was used in the analysis. The Pink-footed Shearwater *A. creatopus* was not included in the analysis because its diving behavior grouped with *Calonectris* spp., which are not considered to be aquatic shearwaters (Kuroda 1954, Adams *et al.* 2019).

Minimum foraging distance

Because the TDRs do not include geo-locating records, we estimated the minimum foraging distance using a non-directional radius outward from Kure Atoll by expressing distance as a function of flying time \times flying speed (Matthews 1953). The time was calculated for each bird using the lag between the time the last dive was recorded and the time the tag was retrieved at night in the colony ($n = 5$). Flying speed was estimated using airspeeds from a representative glide-flapper (Manx Shearwater) in a headwind and a tailwind (Spear & Ainley 1997b). This approach yielded two maximum distance estimates, based on the assumption of continuous directional flight back to the colony.

Diet analysis

Sample storage

Regurgitations were opportunistically collected when tagged birds voluntarily regurgitated. Prey items were collected in a plastic bag filled with ~200 mL of freshwater. Samples were immediately frozen at approximately -18 °C and kept frozen during transport back to Honolulu.

DNA extraction, PCR, and sequencing

Muscle tissue samples (< 2 mm³) were collected from individual prey specimens from underneath the skin whenever possible, using sterilized forceps. The tissue samples were placed in 1.5-mL tubes with 500 μ L of Longmire's buffer (Longmire *et al.* 1997) and 400 μ g of Proteinase-K, then incubated overnight at 37 °C. Following the incubation, genomic DNA was extracted from the tissue samples using chloroform–isoamyl alcohol (24:1) phase separations (Renshaw *et al.* 2015), with slight modifications as described in Appendix 1.

Each DNA extract was amplified via polymerase chain reaction (PCR) at a fragment of the mitochondrial cytochrome *c* oxidase subunit I gene (COI) using the primers mCOIintF (Leray *et al.* 2013) and jgHCOI2198 (Geller *et al.* 2013). The 40 μ L PCR mix included 8 μ L of 5X GoTaq Flexi Buffer (Promega, Wisconsin, USA), 0.8 μ L of 10 mM dNTPs, 3.2 μ L of 25 mM MgCl₂, 2 μ L of 10 μ M forward primer, 2 μ L of 10 μ M reverse primer, 4 μ L of bovine serum albumin (from 4 mg/mL concentrate, VWR, Pennsylvania, USA), 0.2 μ L of GoTaq G2 Flexi DNA Polymerase

TABLE 2
Summary of dives from TDRs deployed on eight CHSH on Kure Atoll, 29 June–09 August 2017

| Bird | Julian day deployed | No. days | No. dives | Overall depth max (m) | Dive depth mean max (m) | Dive depth median max (m) | Dive depth max IQR ^a (m) | Dive max duration (s) | Dive mean duration (s) | Dive median duration (s) |
|------|---------------------|----------|-----------|-----------------------|-------------------------|---------------------------|-------------------------------------|-----------------------|------------------------|--------------------------|
| 1 | 180 | 8 | 306 | 13.5 | 3.2 | 2.3 | 1.6–4.0 | 18 | 4 | 3 |
| 2 | 180 | 5 | 195 | 24.1 | 2.6 | 1.2 | 0.4–3.0 | 31 | 4 | 2 |
| 3 | 195 | 3 | 130 | 15.4 | 2.7 | 2.1 | 1.4–3.3 | 19 | 4 | 3 |
| 4 | 203 | 4 | 319 | 12.7 | 2.1 | 1.4 | 0.7–2.7 | 19 | 4 | 3 |
| 5 | 203 | 4 | 109 | 10.7 | 3.7 | 2.9 | 1.8–5.4 | 16 | 5 | 4 |
| 6 | 213 | 3 | 122 | 13.0 | 4.0 | 3.0 | 1.9–5.1 | 18 | 5 | 3 |
| 7 | 213 | 3 | 187 | 18.5 | 4.1 | 2.7 | 1.0–5.6 | 23 | 6 | 4 |
| 8 | 219 | 3 | 154 | 15.8 | 4.2 | 3.3 | 1.9–5.3 | 27 | 6 | 4 |

^a Interquartile range for all maximum depths per dive

(Promega, Wisconsin, USA), 4 μ L of DNA extract, and 15.8 μ L of sterile water. The thermocycling protocol involved an initial denaturing step of 95 °C for 3 min; 16 cycles of denaturation at 95 °C for 30 s, annealing at 62 °C (–1 °C per cycle) for 45 s, and extension at 72 °C for 1 min; 25 cycles of denaturation at 95 °C for 30 s, annealing at 46 °C for 45 s, and extension at 72 °C; and a final extension step at 72 °C for 10 min.

PCR products were visualized using a 1% agarose gel, stained with ethidium bromide (MP Biomedicals, California, USA). Successful amplifications were cleaned with 2 μ L of ExoSAP-IT (Affymetrix, California, USA) per 5 μ L of PCR product and incubated at 37 °C for 15 min, followed by deactivation at 85 °C for 15 min. Purified PCR products were sequenced unidirectionally with the mlCOIntF primer on an ABI 3130XL capillary sequencer (Life Technologies, California, USA). Chromatograms were checked by eye with MEGA7 (Kumar *et al.* 2016). Curated amplicons were queried against the National Center for Biotechnology Information (NCBI) nucleotide database (<https://www.ncbi.nlm.nih.gov>) for prey taxa identification using BLASTN (Altschul *et al.* 1990). Species-level taxonomic identification was assigned based on a >97% match and whether the distribution for the species matched known records of species in North Pacific waters. Otherwise, the sequence was assigned to the genus level or higher.

RESULTS

Diving behavior

In total, 1521 dives were recorded from eight adult birds of unknown sex during 33 days of tag deployment spanning 29 June to 09 August 2017. The duration of TDR deployments ranged from three to eight days (median = 4 d, interquartile range (IQR) = 3–4 d). We found no significant influence of the deployment duration on the maximum depth recorded ($r = -0.004$; $df = 6$, $P = 0.99$). While there was a moderately strong positive correlation between the number of deployment days and the total number of dives recorded ($r = +0.65$, $df = 6$, $P = 0.08$); the number of daily dives per bird ranged from 19 to 86 (mean = 45 ± 19 dives, median = 39 dives, IQR = 33–54 dives, $n = 33$).

The maximum depths attained by individual birds were 10.7–24.1 m (Table 2; mean = 15.4 ± 4.2 m). The mean (3.34 ± 0.78 m) and median (2.35 ± 0.79 m) maximum depths per dive were considerably shallower than the range of individual maximum depths. The range of maximum durations was 16–31 s (mean = 21 ± 5 s). Likewise, the mean (5 ± 1 s) and median (4 ± 1 s) durations per dive were considerably shorter than the range of individual maximum durations.

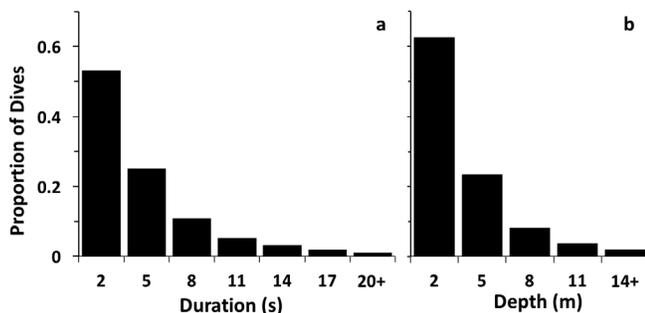


Fig. 1. Histogram of binned (a) dive durations and (b) dive depths recorded in this study ($n = 1521$).

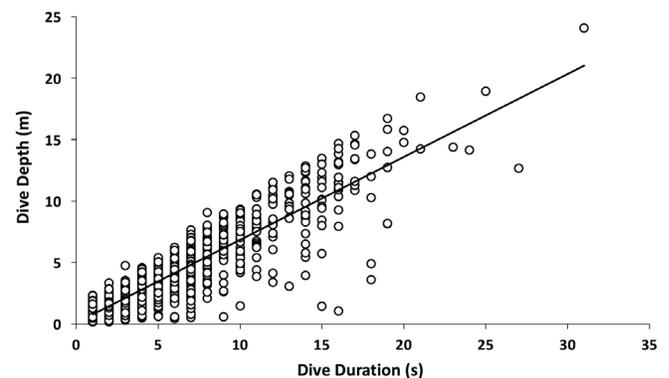


Fig. 2. Scatter plot of dive depth and dive duration for all recorded dives in this study ($n = 1521$). Adjusted $R^2 = 0.82$.

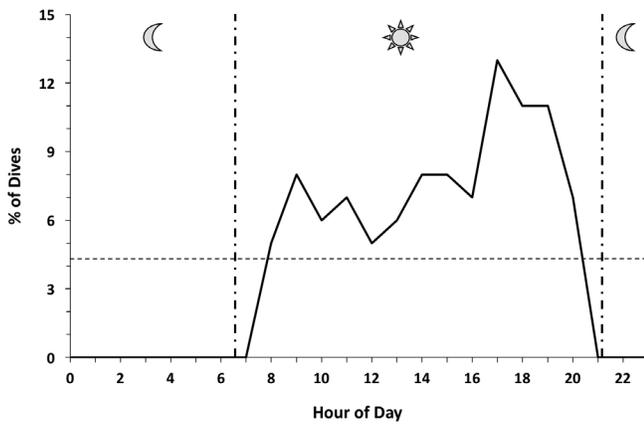


Fig. 3. Percent of dives that occurred in each hour of the day ($n = 1521$). Vertical dashed and dotted lines indicate average civil twilight throughout the study period (29 June–09 August 2017). The horizontal dashed line represents expected percent of dives per hour, per 24 h (4.2%).

The frequency of dive depths and durations were skewed towards the shallower (skewness = 1.7) and shorter (skewness = 2.1) end of each bird's recorded range. More than half of the dives lasted < 3 s (53%; Fig. 1A) and were < 3 m deep (63%; Fig. 1B). Only 1% of the dives were deeper than 15 m and longer than 19 s, with a maximum depth of 24.1 m and a maximum duration of 31 s. Overall, when all dives were considered, the duration increased linearly with the depth attained ($R^2 = 0.83$; $F = 7258.77$; $df = 1, 1519$; $P < 0.01$; see Fig. 2).

Diel diving pattern

On average, throughout the 39-day summer tagging period (29 June–09 August), there were approximately 14.5 h of daylight (52200 s), from 06h35 ± 00h08 to 21h06 ± 00h02. Diving was restricted to daylight (Fig. 3), as defined by the times of civil twilight. Only one bird (CHSH 2) had two dives outside civil twilight (at 05h47 and 21h09), and the remaining 1519 recorded dives (99.87%) occurred during daylight hours. On average, birds spent 209 ± 99 s (range = 76–482 s) per day underwater.

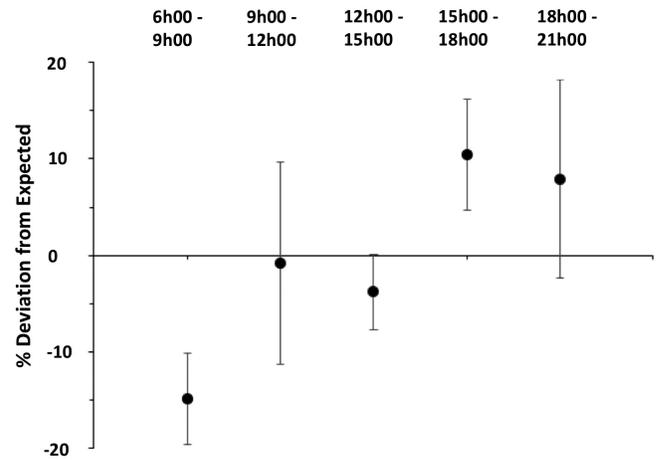


Fig. 4. Mean (± SD) percent deviation from the expected values (20%) per three-hour time block. Daily values are calculated by individual and averaged across all tagged birds ($n = 8$).

Overall, diving activity did not commence at first light (07h05 ± 00h09) but began during 08h00–09h00 (5% of dives). Dive activity was lowest throughout the middle of the day (10h00–13h00), with 6%–7% of all dives occurring hourly. Dive activity increased slightly (7%–8% hourly) in the afternoon (14h00–16h00). Peak diving occurred in the evening, with 12% of all dives occurring between 17h00 and 18h00; diving rapidly decreased around sunset (20h41 ± 00h09).

Dive frequency throughout the day varied by individual when the following three-hour time blocks were compared (see Fig. 4): early morning (06h00–09h00), late morning (09h00–12h00), early afternoon (12h00–15h00), late afternoon (15h00–18h00), and evening (18h00–21h00). Of the eight tagged CHSH, only one showed a uniform diel pattern, with the others showing significant deviations from uniform expectations (chi-squared tests, $df = 4$, $P < 0.01$; Table 3).

Dive bouts and behavioral aerobic dive limit

We used PDI to assess bADL and dive bouts. Theoretically, the changing slope of the line joining the lowest PDI values defines the

TABLE 3
Chi-squared test results, comparing the observed number of individual dives during 5 three-hour periods (06h00–21h00) to an expectation of uniform diving effort during daylight hours^a

| Bird no. | X ² | P value | 06h00 to 09h00 | 09h00 to 12h00 | 12h00 to 15h00 | 15h00 to 18h00 | 18h00 to 21h00 |
|----------|----------------|------------|----------------|----------------|----------------|----------------|----------------|
| 1 | 36.13 | P < 0.0001 | -3.23 | -0.21 | -0.34 | -0.08 | 2.73 |
| 2 | 31.71 | P < 0.0001 | -3.61 | -0.06 | 0.55 | 0.48 | 1.51 |
| 3 | 76.46 | P < 0.0001 | -3.61 | -3.61 | -0.97 | 2.28 | 2.47 |
| 4 | 69.33 | P < 0.0001 | -5.26 | 0.01 | -0.37 | 1.85 | 1.85 |
| 5 | 38.35 | P < 0.0001 | -3.30 | 0.81 | 2.08 | -1.79 | 0.33 |
| 6 | 7.67 | P = 0.1000 | -1.19 | 0.35 | -1.06 | 0.89 | 0.63 |
| 7 | 16.94 | P = 0.0020 | -1.52 | -0.73 | -1.21 | 1.81 | 0.95 |
| 8 | 25.31 | P < 0.0001 | -2.18 | 1.95 | -1.34 | 1.28 | -0.81 |

^a Bolded Z-scores highlight significant deviations, with positive and negative values indicating an excess and a deficit of dives, respectively; for all tests $df = 4$ and $\alpha = 0.05$.

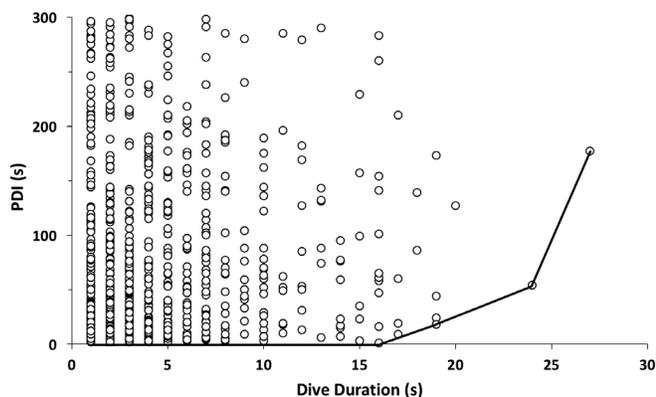


Fig. 5. The relationship between dive duration and post-dive interval (PDI) for all dives recorded. Theoretically, the behavioral aerobic dive limit (BADL) is defined by the slope change in the line joining the lowest values of PDI (approximately 16–19 s).

bADL (Gentry & Kooyman 1986). While this limit was estimated at 16–19 s when all dives were combined ($n = 1521$; Fig. 5), individual values were 6–19 s (mean = 11 ± 4 s, SD). Accordingly, only 2.8% of all dives exceeded the maximum estimated bADL of 19 s. A log-survivorship curve of the PDIs revealed that the first change in slope, which is indicative of the diving-bout duration, occurs near 900 s (15 min, Fig. 6).

Interspecific shearwater comparison

An allometric regression of four *Puffinus* and three *Ardenna* species predicted the maximum dive depth of CHSH based on mean body mass (324 g) would be 23.4 m ($y = 0.0925x - 6.6196$, $R^2 = 0.61$). The observed maximum depth for CHSH was slightly deeper (24.3 m). Among this group of shearwaters, including CHSH, the maximum depth recorded increased significantly with mass (adjusted $R^2 = 0.53$; $df = 1, 5$; $P < 0.001$; Fig. 7). CHSH had the lowest mass (324 g) of the species tagged with TDRs (species range = 324–850 g).

Minimum foraging distance

The estimated flight time, based on the time elapsed between the last dive recorded and the time of tag retrieval at the colony, was 1.4–3.1 h ($n = 5$). Flight speed for Manx-type shearwaters was estimated at 40 ± 7 km·hr⁻¹ in a tailwind and 63 ± 6 km·hr⁻¹ in a headwind (Spear & Ainley 1997b). Accordingly, the CHSH minimum foraging range from Kure Atoll was estimated at 44–147 km with a tailwind and 82–212 km with a headwind (Fig. 8). Both ranges fell within the 322-km boundary of PMNM.

Diet

Of the eight tagged birds, five regurgitated when tags were retrieved. A total of 48 prey items were found, with the number of prey items regurgitated per bird ranging from 2–22 (median = 10). We were unable to sequence three prey items (two from CHSH 6, one from CHSH 7), and we included the remaining 45 usable sequences in the analysis.

Overall, nine fish species and two squid species were identified from eight families, with 27 (60%) of the prey items positively

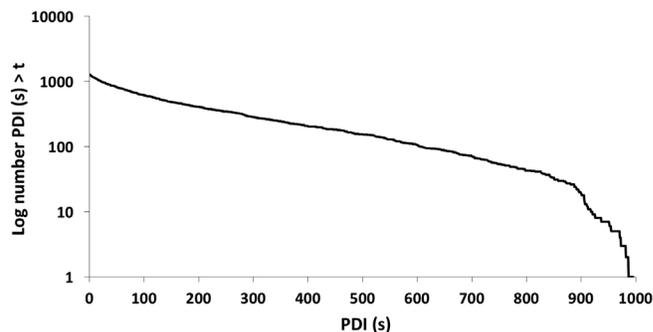


Fig. 6. Log survivorship curve of the post-dive intervals (PDI) for all dives recorded.

identified (> 97% match to the NCBI database) using a 313-bp sequence region of COI (Table 4). In addition, 16 prey sequences had a 90%–96% match to the oceanic lightfish *Vinciguerria nimbaria* (asterisks in Table 4). This species does not have a North Pacific reference sample in the NCBI database, and our samples grouped most closely with a reference from the Tasman Sea. Therefore, including *V. nimbaria*, we confidently identified 96% (43 specimens) of successfully sequenced prey items (45 specimens). Two prey items with a 90%–96% match to species in the reference database (asterisks in Table 4) were not positively identified to the species level.

Genetic identification worked well for digested food items recovered from tagged birds, spanning a diverse range of families involving reef-associated (Holocentridae, Mullidae, Carangidae), pelagic-oceanic (Ommastrephidae, Exocoetidae, Hemiramphidae, Gempylidae), and meso-pelagic (Phosichthyidae) prey. These families were previously documented in CHSH diets from the Northwestern Hawaiian Islands during the 1970s (Harrison *et al.* 1983).

The maximum individual dive depths from the days of tag retrieval were 7.9–18.9 m (Table 5), and the minimum depths were 0.2–1.3 m. While the mean time spent underwater by individual birds during the last day of diving varied widely (233 ± 144 s), the number of prey items did not correlate with the time spent underwater ($r = 0.08$, $df = 3$, $P = 0.90$) nor with the number of dives conducted that day ($r = 0.02$, $df = 3$, $P = 0.97$).

DISCUSSION

This study provides several new insights into the foraging behavior of CHSH, based on quantitative observations from two different complementary approaches: TDRs and genetic analysis of prey. The CHSH maximum dive depth (24.3 m) was close to—though slightly deeper (0.9 m) than—what was predicted allometrically, compared with TDR-tagged *Puffinus* and *Ardenna* aquatic shearwaters.

Despite the small sample size ($n = 8$ birds over 33 days), there was no relationship between the duration of the TDR deployments and the maximum diving depths recorded. This result indicates that the data from these eight birds can be compared, even though the duration of the TDR deployments was variable (range: 3–8 days). Overall, most dives were much shallower than the maximum diving depth, with 81% of dives less than 5 m deep. Other Manx-type shearwaters followed this pattern, with a skew towards frequent shallow dives (45% of dives by Manx Shearwaters and 63%–93%

TABLE 4

Family, genus, and species of prey species identified using a portion of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene^a

| Family | Genus | Species | Common name | % of samples | % of identified items |
|----------------|--------------------------|-------------------------|-------------------------|--------------|-----------------------|
| Holocentridae | <i>Sargocentron</i> | <i>xantherythrum</i> | Hawaiian squirrelfish | 40 | 24 |
| Holocentridae | <i>Sargocentron</i> | <i>punctatissimum</i> | Speckled squirrelfish | 20 | 2 |
| Phosichthyidae | * <i>Vinciguerria</i> | <i>nimbaria</i> | Oceanic lightfish | 60 | 33 |
| Carangidae | <i>Decapturus</i> | <i>macarellus</i> | Mackerel scad | 40 | 7 |
| Carangidae | <i>Selar</i> | <i>crumenophthalmus</i> | Bigeye scad | 40 | 9 |
| Exocoetidae | <i>Exocoetus</i> | <i>monocirrhus</i> | Barbel flyingfish | 40 | 4 |
| Mullidae | <i>Mulloidichthys</i> | <i>vanicolensis</i> | Yellowfin goatfish | 20 | 2 |
| Gempylidae | <i>Gempylus</i> | <i>serpens</i> | Snake mackerel | 20 | 2 |
| Hemiramphidae | ** <i>Oxyporhampus</i> | <i>micropterus</i> | Bigwing halfbeak | 20 | 4 |
| Ommastrephidae | *** <i>Stethoteuthis</i> | <i>oualaniensis</i> | Purpleback flying squid | 20 | 2 |
| Ommastrephidae | <i>Ommastrephes</i> | <i>bartramii</i> | Neon flying squid | 80 | 11 |

^a Sample size: 5 regurgitation samples and 45 successfully sequenced prey items, > 97% match to NCBI database unless marked *

*16 sequences 90-96% match to NCBI

**1 sequence 90% match to NCBI

***1 sequence 96% match to NCBI

by Balearic Shearwaters *P. mauretanicus* were < 5 m; Dean 2012, Meier *et al.* 2015). The high frequency of short-duration dives may indicate that CHSH are using pursuit plunging and dipping as foraging techniques more frequently than pursuit diving. CHSH may be using these techniques to avoid contact with the large, fast-moving tuna in near-surface waters.

Underwater activity

Despite most dives being shallow, the maximum dive depths (> 10 m) attained by all birds and the observed variability in individual diel dive patterns indicate that this species uses multiple foraging strategies. The linear relationship between diving depth and duration indicates that CHSH are spending more time underwater to reach deeper depths, rather than remaining

shallower for a longer time. When birds submerge, the amount of oxygen stored in their circulatory, muscle, and respiratory systems determines their aerobic diving limit. Dives beyond the aerobic limit require extended surface recovery time to metabolize the build-up of lactate in their blood (Boyd 1997). While extended PDIs following long-duration dives relate to threshold recovery time, they can also occur when birds remain at the surface to rest, even though it is not physiologically necessary. Subsequently, this leads to wide individual variability of threshold ranges (Kooymann & Kooymann 1995) and requires a pooled assessment to reduce individual noise. Given the pooled bADL estimate of 16 s, 97% of the recorded dives could be considered aerobic. From this result, we concluded that CHSH are conducting highly efficient shallow dives, rather than using anaerobic metabolism to seek out deep prey. This diving behavior is consistent with CHSH foraging alongside sub-surface predators, which drive prey closer to the surface and make deep diving unnecessary (see also Hyrenbach *et al.* 2013). In this scenario, we would expect the birds to avoid the rapid charging of tuna pursuing prey to the surface. Accordingly, deep diving may

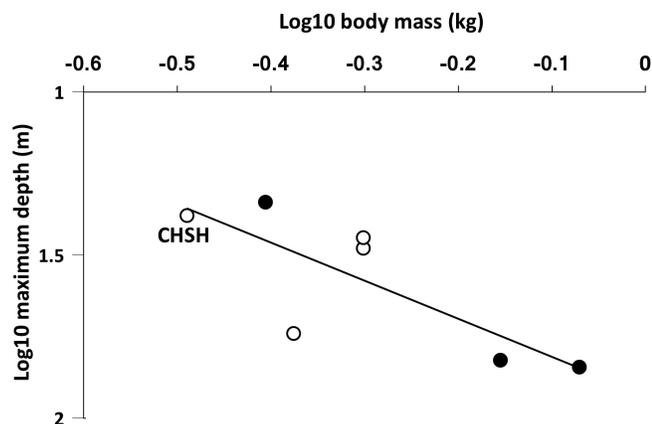


Fig. 7. Allometric regression of Log10 body mass (kg) and Log10 maximum depth (m) for *Puffinus* (white symbols) and *Ardenna* (black symbols) shearwaters. Adjusted $R^2 = 0.53$.

TABLE 5

Summary of dive activity during the day when birds voluntarily regurgitated during tag retrieval

| Bird # | No. dives | Total time underwater (s) | Maximum depth (m) | Total prey items | No. families |
|--------|-----------|---------------------------|-------------------|------------------|--------------|
| CHSH 1 | 38 | 143 | 12.9 | 2 | 2 |
| CHSH 2 | 45 | 187 | 18.9 | 2 | 1 |
| CHSH 3 | 35 | 131 | 7.9 | 22 | 7 |
| CHSH 6 | 48 | 221 | 12.4 | 10 | 3 |
| CHSH 7 | 80 | 482 | 14.8 | 12 | 4 |

occur as the feeding flock breaks up and the sub-surface predators follow the prey into deeper water. Alternatively, deep diving may be more favorable when foraging occurs in a flock dominated by larger-bodied surface-feeding or plunging species (e.g., boobies *Sula* spp.). In this scenario, CHSH are likely able to target shallow schools of prey both from above (shorter, shallower aerobic dives) and from below (longer, deeper dives; occasionally anaerobic).

CHSH did not appear to engage in short diving bouts. Thus, their PDI (900 s) could indicate how long CHSH associate with feeding flocks and/or sub-surface predators. However, because this represents the period during which diving takes place, CHSH may associate with these feeding flocks for longer and alter diving activity only as surface prey patches form or disperse. As prey schools and foraging flocks break up, CHSH may be able to continue foraging by pursuing any remaining solitary fish that submerge to depths to which other guild members are incapable of diving (Hoffman *et al.* 1981). While near-surface foraging by tuna likely instigates seabird foraging, it is unknown if CHSH are the initial spotters of tuna or if they arrive to the feeding flock following other birds. Given their relative numbers (i.e., the CHSH population is about a tenth of the WTSH population at Kure Atoll) and that they contribute little to the composition of mixed-species foraging flocks in the equatorial Pacific (Spear *et al.* 2007), they most likely follow other birds. Ballance *et al.* (1997) indicate that CHSH are associated with WTSH-dominated flocks.

Timing

The diel timing of diving indicates that CHSH rely on visual pursuit of prey. Visual predation could occur by detecting either prey beneath

the surface or already-formed flocks associated with sub-surface predators. Foraging constrained to daylight is further supported by a report of low rhodopsin density in CHSH eyes, indicating they are not well adapted for nocturnal foraging (Harrison *et al.* 1983). The results of Spear *et al.* (2007) indicate that CHSH are daytime foragers, compared to some other Hawaiian seabirds that can feed at night.

Even though the tagging duration for two of the birds overlapped with a moon illumination greater than 75%, this did not affect their timing of diving. While CHSH dove throughout the day, they spent most of their time in the air or sitting on the water, with only 0.4% of the available daylight hours spent underwater. Though there was individual variation in temporal diving patterns, the birds disproportionately dove in the late afternoon and avoided diving in the early morning. A relationship between the mean (or median) depth and daylight hour was not apparent, indicating that there was no systematic change in foraging depth during the day. Yet, a weak positive relationship between time of day and the maximum depth indicates that CHSH may dive deeper later in the day. Delayed diving behavior in the morning is consistent with behavioral observations of pairs circling the colony and calling in the morning until around 09h00 (IN pers. obs.). Afternoon and evening dives may reflect prey density distributions, timing of tuna foraging, or a strategy to reduce the cost of provisioning chicks at the colony on Kure Atoll. Furthermore, a shorter duration in the bird's stomach means prey will be less digested and therefore more nutritious for chicks. While we expected prey items to be relatively undigested because foraging occurred in the evening, all recovered prey items were of digestion grade 2 (half-digested) or 3 (severely digested and unrecognizable; see Harrison *et al.* 1983).

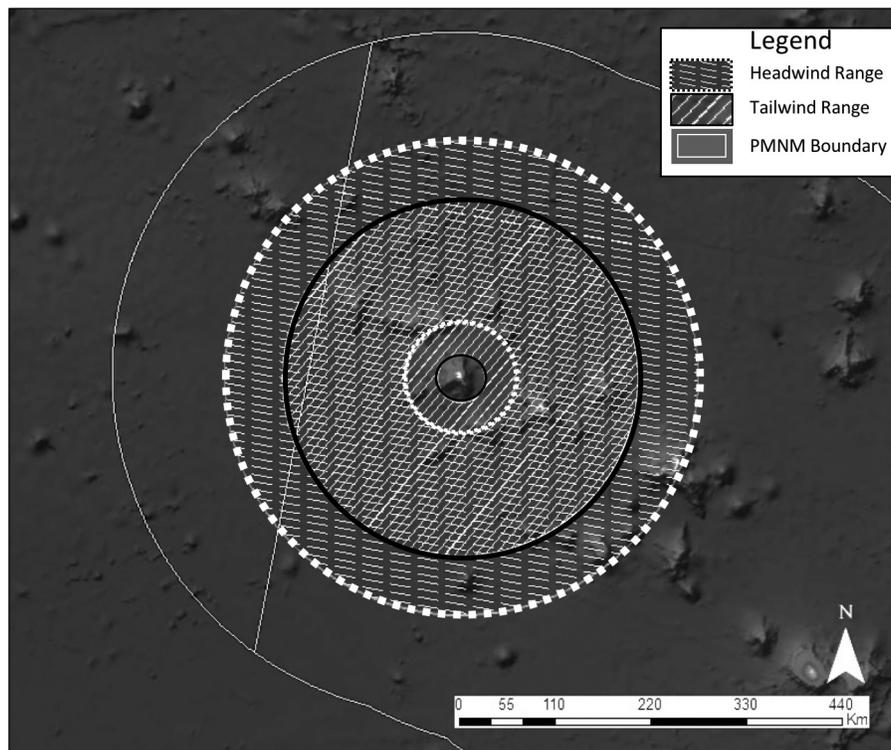


Fig. 8. Minimum foraging range of CHSH ($n = 5$) from Kure Atoll (center). White dotted outlined circles with dashed hatching represent range (± 1 SD) in a headwind, and solid black outlined circles with diagonal lines represent range (± 1 SD) in a tailwind. The solid white outline represents the 322-km PMNM boundary.

Diet

Most specimens (93%) in one of the diet samples (from CHSH 3) were identified as the oceanic lightfish (family Phosichthyidae). Overall, oceanic lightfish accounted for 33% of the CHSH diet from five birds. Our low sample size may be over-representing the importance of this fish, as indicated by its low relative abundance (2% of all prey items) in the historical CHSH diet samples (Harrison *et al.* 1983, $n = 189$). The 14 oceanic lightfish within the diet sample from CHSH 3 were juveniles and adults (length range: 3.1–5.4 cm; standard length at maturity: 3.4 cm; Stequert *et al.* 2003). We noted minimum length due to the digested nature of the prey items. CHSH 3 reached a maximum depth of 7.9 m and did not dive at night, indicating that this non-migrating midwater fish species was captured close to the surface during daylight, likely driven there by tuna. Though not known to be epipelagic, there are circumstantial reports of *Vinciguerria* spp. being consumed by seabirds at the surface during daylight hours in the eastern tropical Pacific (Pitman & Ballance 1990). Pitman & Ballance attributed the rare swarms of midwater *Vinciguerria* to the presence of surface convergence zones, which aggregated their (copepod) prey to the surface and subsequently attracted the foraging seabirds. Many tropical seabird species consume this fish (Harrison *et al.* 1983, Spear *et al.* 2007).

Aside from the oceanic lightfish, all other species were epipelagic, in accordance with the findings of Spear *et al.* (2007). Many species within the Holocentridae, Mullidae, and Carangidae families are broadcast spawners that have pelagic larval and juvenile life histories before transitioning to reef-associated adults. Adult squirrelfish (genus *Sargocentron*) in the Holocentridae family are nocturnal on reefs, but little is known about their pelagic juvenile stage. Considering that two *Sargocentron* species were found in the diet of daylight-feeding seabirds, pelagic juveniles are likely not nocturnal. The pelagic duration of larval goatfish (Mullidae) is typically a month, but juveniles are subject to oceanographic conditions and density-dependent mechanisms that cue recruitment to nearshore environments (Kamikawa 2016). In Hawai‘i, juvenile goatfish are often found in the stomachs of pelagic predators, including other seabirds, dolphinfish, and tunas (Kamikawa 2016). Ommastrephidae (flying squid) and Exocoetidae (flyingfish) are aerial and were likely captured above the surface.

Biases

Although we did not attempt to measure the stress caused by handling and the attachment of the TDR to the underside of the bird’s tail, this impact was probably inconsequential, since the light weight and small cross-sectional area of the tags (0.03% of the birds’ cross-sectional area) meant that they likely caused little drag during diving (Kay *et al.* 2019). One study that tested diving alcids for the effects of tail-mounted devices found no statistically significant differences between control and tagged birds, but tagged birds were observed to perform at a slightly lower level with regard to colony attendance and food delivery (Wanless *et al.* 1989). Therefore, it is possible that CHSH could have experienced some stress from being tagged, including reduced diving ability and increased foraging or commuting effort (Burger & Shaffer 2008).

Only two loggers were available for this study, so tag redeployment over the 39-day period may have resulted in temporal variation.

Specifically, the diving patterns observed throughout the deployment duration may have been impacted by changing ocean conditions and chick-rearing status. The summer of 2017 was an ENSO-neutral season, with the Multivariate El Niño Index between -0.3 and -0.8 during the deployment period (MEI 2019). Therefore, ocean conditions during 2017 were not particularly anomalous.

The energetic demands of chicks of different ages (and sizes) could have also influenced the diving patterns of the adult birds during our study; CHSH have been observed to hatch chicks over a wide range of dates (May–August, range: 51–112 d; Seto 2001). To avoid this bias, we chose adults provisioning downy chicks (range: 200–415 g, mean \pm SD: 277 ± 78 g) to increase the chances of tag retrieval. While the chick hatching dates of the selected nest sites were unknown, chick size was moderately correlated with Julian day ($r = 0.58$, $df = 6$, $P = 0.13$), indicating that chicks became progressively larger between early June and August. Our TDR data could not discern differences in foraging distance relative to chick size, nor use of dual-foraging strategies for self-provisioning vs. chick-provisioning, as used by WTSH (McDuie *et al.* 2015). Future tagging studies should consider these potential biases.

Finally, estimated foraging ranges indicated that CHSH provisioning chicks on Kure Atoll foraged within PMNM waters. Namely, foraging behavior in the evening prior to returning to their nest site underscores that PMNM is an important resource for chick-provisioning CHSH. These findings also raise new questions about the foraging ecology and the diet of this poorly studied species. Though concern is justified that larger satellite-tracking or geolocating tags could encumber CHSH, such tags might better identify the direction of travel and how CHSH use ephemeral and permanent oceanographic features. Long-term tracking after breeding could further delineate the range and foraging locations of this species, both within and outside PMNM, and identify ocean hotspots targeted by CHSH.

The distribution and behavior of CHSH prey is another critical knowledge gap. Thus, we recommend more research on the understudied juvenile stages of reef fish, as they are important to CHSH diet. Though there is much to learn about the foraging habitat and prey of CHSH, our findings suggest that maintaining the PMNM as a protected area that is closed to fisheries will benefit this species by minimizing fishery interactions and enhancing prey populations, especially when CHSH foraging is mediated by subsurface predators.

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Appendix 1

PCR cleanup protocol:

- 1) Remove ExoSAP-IT reagent from -20°C freezer and keep on ice throughout this procedure.
- 2) Mix 5µl of a post-PCR reaction product with 2µl of ExoSAP-IT reagent for a combined 7µl reaction volume. Note: When treating PCR product volumes greater than 5µl, simply increase the amount of ExoSAP-IT reagent proportionally.
- 3) Seal plate securely with self-adhesive film; centrifuge sample briefly to make sure everything is at the bottom of the wells.
- 4) Incubate at 37°C for 15 minutes to degrade remaining primers and nucleotides.
- 5) Incubate at 80°C for 15 minutes to inactivate ExoSAP-IT reagent.
- 6) The PCR product is now ready for use in DNA sequencing, SNP analyses, or other primer-extension applications. Treated PCR products may be stored at -20°C until required.

Big Dye Terminator Cycle Sequencing:

- 1) Generate a master mix based on the following per sample requirements (taking into account the total number of reactions needed; numbers are µl volumes)

UltraPure Water = 3.75

5X Sequencing Buffer = 1.75

F primer (0.8µmol/µl) = 2

Big Dye = 0.5

DNA = 2

Total = 10

- 2) Seal plate securely with self-adhesive film; centrifuge sample briefly to make sure everything is at the bottom of the wells.
- 3) Perform cycle sequencing in thermocyclers using the following protocol:

i – 96°C for 5 minutes

ii – 96°C for 10 seconds

iii – 50°C for 5 seconds

iv – 60°C for 4 minutes

****repeat steps ii-iv an ADDITIONAL 24 times**

v – hold at 4°C indefinitely

Purification by Ethanol Precipitation:

- 1)** Centrifuge briefly to make sure contents of wells are at the bottom; remove self-adhesive film.
- 2)** Add 5µl of 125mM EDTA (pH 8.0) to each sample. Make sure the EDTA reaches the bottom of the wells.
- 3)** Add 30µl of 100% ethanol to each sample and pipet up and down 5 times to thoroughly mix contents of each well.
- 4)** Incubate at room temperature for 15 minutes. Keep in a dark place (i.e. a drawer) to protect the samples.
*****use this time to chill the centrifuge down to 4°C**
- 5)** Centrifuge at max speed (2250g [rcf]) at 4°C for 30 minutes.
*****proceed to next step immediately; if this is not possible, continue centrifuging the plate until you are ready to perform the next step**
- 6)** Gently, remove plate from centrifuge, lay down paper towels in centrifuge, and invert plate onto the paper towels. Centrifuge with plate inverted at 180g [rcf] for 1 minute.
- 7)** Add 30µl of 70% ethanol to each sample.
- 8)** Centrifuge plate at 1650g [rcf] for 15 minutes.
- 9)** Gently, remove plate from centrifuge, lay down paper towels in centrifuge, and invert plate onto the paper towels. Centrifuge with plate inverted at 180g [rcf] for 1 minute.
- 10)** Continue to air dry until all visible ethanol has evaporated.
- 11)** Add 20µl formamide to each sample and load on the sequencer