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RESEARCH

Evidence of eastern rockhopper penguin feeding on a key commercial arrow squid species

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Abstract

Cephalopods are crucial to the Southern Ocean ecosystem, connecting top predators with mid-trophic organisms, yet their ecology in the Pacific sector is not well understood. This research used stable isotope analysis to explore the habitat and trophic ecology of cephalopods found in the diet of eastern rockhopper penguins (*Eudyptes chrysocome filholi*) around Campbell Island, a New Zealand sub-Antarctic island. Eastern rockhopper penguins were used as biologic samplers, revealing some differences in cephalopod diversity and ecology between two breeding seasons—1986–87 and 2012–13—, and the squid *Nototodarus sloanii*, a commercially valuable species, was described for the first time in the 2012–13 season. Stable isotope values (δ^{13}C and δ^{15}N) for the squid species *Moroteuthopsis ingens* were consistent between seasons, indicating ecological stability, whereas the octopod species *Octopus campbelli* showed changes, suggesting shifts in habitat and feeding. Warmer oceanic temperatures in 2012–13 may have facilitated the emergence of *N. sloanii* on the diet of the eastern rockhopper penguins. These findings highlight potential changes in cephalopod biodiversity in the Pacific sector of the Southern Ocean, emphasizing the need for further research on ecological dynamics of this region.

Keywords Arrow squid · Campbell Island · Cephalopods · *Eudyptes chrysocome filholi* · Stable isotope analyses

Introduction

Marine ecosystems are increasingly under pressure from human activities, leading to significant impacts on natural resources, particularly in the Southern Ocean (Halpern et al. 2008; Cavanagh et al. 2021). Cephalopods, which play a pivotal role in these ecosystems by linking lower and higher trophic levels (Cherel 2020), are also affected, with shifts in distribution and abundance due to climate change and other anthropogenic factors (Rodhouse 2013; Abreu et al. 2020). Despite their ecological importance, there is limited information on cephalopod ecology and distribution, particularly in the Pacific sector of the Southern Ocean (Xavier et al. 2014). This is due in part to the capacity of larger cephalopods to evade scientific nets (Clarke 1996; Boyle & Rodhouse 2007).

To address this, researchers have used stomach content analysis of cephalopod predators, which retain cephalopod beaks in their stomachs, allowing for the identification of species and insights into their ecology (Cherel 2020; Xavier et al. 2022). Among top predators, penguins are predators of cephalopods (Xavier & Cherel 2021), which eastern

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rockhopper penguin *Eudyptes chrysocome filholi* plays a key role as a biologic sampler of cephalopod diversity (Sagar et al. 2005) around Campbell Island, New Zealand.

The aim of this study was to assess the biogeography of cephalopods around Campbell Island using stable isotope analysis (δ^{13}C and δ^{15}N) of beaks collected from eastern rockhopper penguins during the 1986–87 and 2012–13 breeding seasons. Stable isotope analysis provides insights into habitat preferences—higher vs. lower latitude and inshore vs. offshore—from δ^{13}C values and as well as provide information on the trophic niche through δ^{15}N values (Cherel & Hobson 2005; Guerreiro et al. 2015). More specifically, we aimed to i) identify the habitat and trophic level characteristics of the cephalopod species through stable isotope analysis and to ii) assess the biodiversity of cephalopods in the eastern rockhopper penguin diet across two breeding seasons.

Materials and methods

Dietary sampling and stable isotope analysis

Fieldwork was conducted on Campbell Island (52°32′24″ S 169°8′42″ E), New Zealand, during the 1986–87 and 2012–13 breeding seasons (Fig. 1). Cephalopod beaks were collected from eastern rockhopper penguin regurgitations. Species identification was done using lower beak morphology, with measurements of the lower rostral length (LRL) for squid and the lower hood length (LHL) for octopods (Xavier & Cherel 2021). Measurements were taken with a Leica Wild M8 Stereo Microscope at 50× magnification, and species-specific allometric equations were used to estimate cephalopod mass (M) and mantle length (ML) (Xavier & Cherel 2021). All procedures were performed at MARE-UC, University of Coimbra, Portugal.

Stable isotope analysis (SIA) was applied to assess habitat and trophic levels of key cephalopod species: *Moroteuthopsis ingens*, *Octopus campbelli*, and *Nototodarus sloanii*. Beaks were pooled to obtain sufficient material for SIA, increasing accuracy by combining individuals of the same life stage (Guerra et al. 2010; Queirós et al. 2018; Guímaro et al. 2021). Beaks were cleaned with 70% ethanol, dried at 60 °C for 24 h, and ground to a fine powder using a ball mill (Mixed Mill MM 400 (Retsch, Haan, Germany)). Approximately, 0.30 mg of the homogenized sample was weighed (Mettler Toledo® UMX2 ultra-microbalance) into a tin capsule for analysis. SIA was conducted using a continuous flow Isotope Ratio Mass Spectrometer (Delta VTM Advantage, Thermo Scientific®) interfaced with an elemental analyser

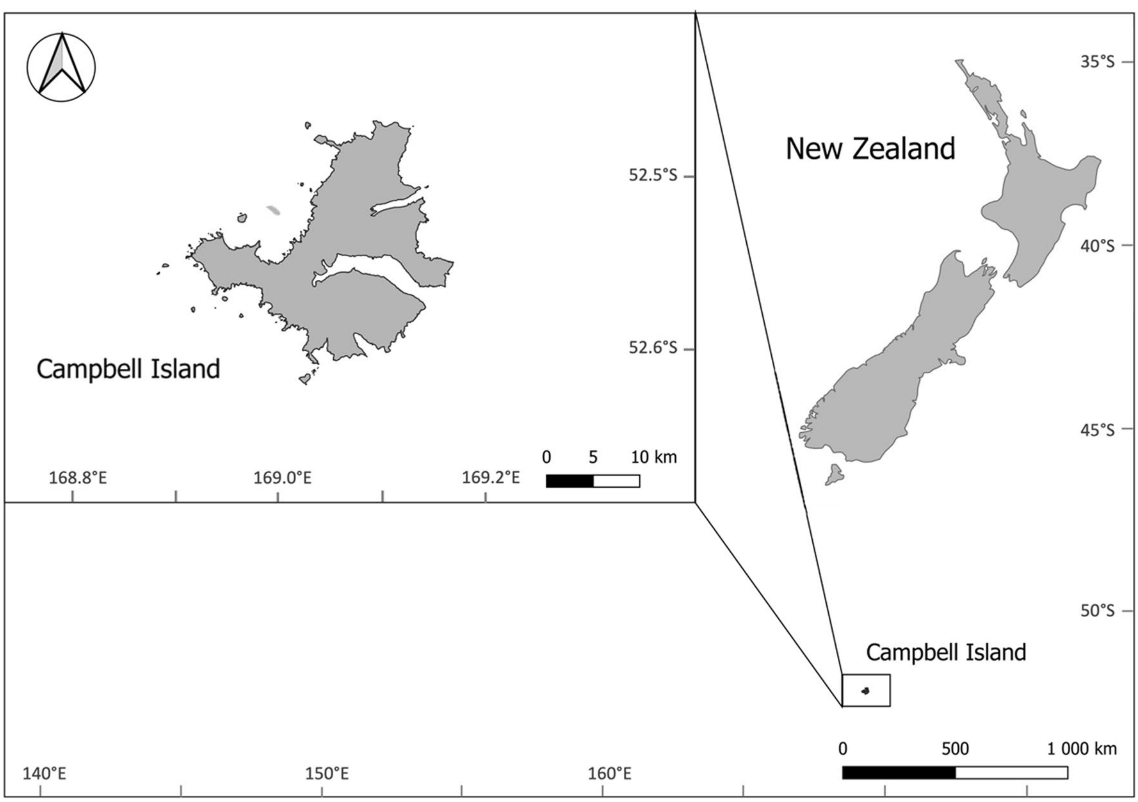

Fig. 1 Geographic location of the New Zealand sub-Antarctic island—Campbell Island

(FlashTM EA 1112, Thermo Scientific®). Results were presented in standard δ notation (‰), following the equation $\delta X = [(Rsample - Rstandard - 1) \times 1000]$. In the equation, X represents ^{13}C or ^{15}N, Rsample represents the ratios $^{13}C/^{12}C$ ($\delta^{13}C$) and $^{15}N/^{14}N$ ($\delta^{15}N$) and Rstandard represents the Vienna Pee Dee Belemnite (V-PDB) and Atmospheric N2 (AIR) for carbon and nitrogen, respectively. In every batch, replicate measurements of a secondary isotopic reference material (acetanilide STD, Thermo scientific-PN 338 36,700) indicated a precision of < 0.2 ‰ for both $\delta^{13}C$ and $\delta^{15}N$ values.

Data analysis

To compare cephalopod species between seasons, Shapiro–Wilk normality tests were performed followed by Bartlett's test for homogeneity, and Mann–Whitney and Kruskal–Wallis tests were performed when the data were non-parametric. For differences in $\delta^{13}C$ and δ ^{15}N values among cephalopod species, a MANOVA followed by ANOVAs were performed, as well as multiple comparisons run with post hoc Tukey tests. Bayesian-mixed models (SIBER, Jackson et al. 2011) were used to estimate and plot isotopic niches, comparing niche width among species and testing for temporal variation using SEA_C and SEA_B. All analyses were performed in R 4.3.0 (R Core Team 2023).

Results and discussion

Cephalopod biodiversity between breeding seasons

This study provides new insights of cephalopod biodiversity in the diet of eastern rockhopper penguins by comparing two breeding seasons. While our 2012–13 data were based on only 11 individuals, compared to 69 individuals from the 1986 to 87 season (Xavier et al. 2018a, b), the 321 beaks collected in 2012–13 yielded significant new information on cephalopod species around Campbell Island. To the best of our knowledge, this is the first time that the presence of the arrow squid *Nototodarus sloanii* has been documented in the diet of eastern rockhopper penguin, with 103 beaks recorded.

In 1986–87, the diet comprised seven cephalopod species, including six squid and one octopod species (Xavier et al. 2018a, b). In contrast, the 2012–13 diet included only three species: *Moroteuthopsis ingens*, *N. sloanii*, and *Octopus campbelli* (Table 1). The overall diversity seemed to decrease from seven species to three, likely due

Table 1 Values of the frequency of occurrence (F and F%), number of lower beaks (N and N%), mass in the samples (M and M%), values of lower beak's rostral length (LRL) for squid species and lower hood length (LHL) for octopod species, and estimated values for the total mass (TM) and mantle length (ML) of the cephalopod component of the diet of eastern rockhopper penguin at Campbell island during the breeding seasons 2012–13 and 1986–87

Species	Frequency of occurrence		Number of lower beaks		Mass		Lower rostral/hood length	Mantle length	Total mass
	F	F%	N	N%	M	M%	LRL/LHL	ML	TM
2012–13 season (n = 11)									
Moroteuthopsis ingens	10	90.9	23	7.2	158.1	64.3	2.2 ± 0.4 (1.1–2.7)	158.3 ± 88.5 (12.7–358.0)	109.7 ± 12.3 (73.1–127.0)
Octopus campbelli	6	55.4	195	60.7	12.1	4.9	1.4 ± 0.2 (0.8–1.7)	12.1 ± 3.5 (3.8–19.1)	2.7 ± 0.4 (1.4–3.4)
Nototodarus sloanii	10	90.9	103	32.1	75.8	30.8	2.2 ± 0.2 (1.4–2.7)	75.8 ± 8.8 (51.3–96.3)	132.8 ± 12.3 (92.4–157.7)
1986–87 season (n = 69)									
Moroteuthopsis ingens	40	58.0	300	44.7	49.6	39.7	1.5 ± 0.8 (0.6–3.7)	86.0 ± 27.0 (54.0–162.0)	20.0 ± 25.0 (1.0–129.0)
Octopus campbelli	36	52.2	252	37.6	34.9	20.8	1.3 ± 0.3 (0.4–1.9)	11.0 ± 5.0 (1.0–23.0)	2.4 ± 0.7 (0.7–3.8)
Gonatus antarcticus	4	5.8	4	0.6	0.1	< 0.1	1.2 ± 0.1 (1.1–1.3)	8.4 ± 3.0 (4.2–11.5)	1.0 ± 0.2 (1.0)
Alluroteuthis antarcticus	2	2.9	2	0.3	< 0.1	< 0.1	0.8	23.0	2.0
Martialia hyadesi	18	26.1	86	12.8	13.0	38.0	2.3 ± 0.4 (1.6–3.4)	158.0 ± 11.0 (137.0–193.0)	47.0 ± 20.0 (20.0–116.0)
Moroteuthopsis sp. B (Imber)	6	8.7	16	2.4	2.4	1.5	1.8 ± 0.7 (1.2–3.3)	–	12.0 ± 18.0 (2.0–63.0)
Gonatopsis octopedatus	2	2.9	3	0.4	< 0.1	< 0.1	1.3 ± 0.3 (1.1–1.6)	–	–

The data from 1986 to 87 was adapted from Xavier et al. (2018a, b) and the TM and ML were estimated by allometric equations given by Xavier and Cherel (2021), Lalas and McConnell (2012), Roberts and Lalas (2015), and Northern (2017). Values are Mean ± SD (Min–Max) to the smaller sample size in 2012–13, although *M. ingens* and *O. campbelli* were present in both years.

There is a need to be conservative when considering the overall changes in the cephalopod component of rockhopper penguin diet. Nevertheless, changes in temperature of waters around Campbell Island during recent last decades (Cunningham & Moors 1994; Pütz et al. 2006; Morrison et al. 2015), could reflect real changes in the distribution and relative abundance of cephalopods in this region. The identification of *N. sloanii* may indicate a southward range expansion, as this species is more common in warmer waters (> 12 °C) of New Zealand (Jackson et al. 2000).

Stable isotope analyses: habitat and trophic niche of cephalopods

Stable isotope analysis (SIA) can provide valuable insights into the habitat and trophic niches of cephalopods (Cherel & Hobson 2005; Guerreiro et al. 2015). The 2012–13 SIA data (Table 2) suggest that the cephalopods' spawning areas are likely near Campbell Island, aligning with sub-Antarctic water characteristics (Jaeger et al. 2010; Guerreiro et al. 2015).

For *M. ingens*, no significant differences were observed in $\delta^{13}C$ or $\delta^{15}N$ values between years (Table 2). However, isotopic niche areas differed significantly (SEA_B: $p = 0.042$) with moderate overlap (SEA_C: overlap = 0.65) (Fig. 2). This stability in isotope values suggests consistent habitat use and

Table 2 Stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of the cephalopod component of the diet of eastern rockhopper penguin at Campbell Island, during the breeding seasons 2012–13 and 1986–87

Species	n ind	n cap	LRL/LHL (mm)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:N mass ratio
2012–13 season						
Moroteuthopsis ingens	11	10	2.2 ± 0.3 (1.6–2.7)	− 19.6 ± 0.6 (− 20.6 to − 18.4)	4.6 ± 1.2 (1.9–6.4)	3.7 ± 0.2 (3.2–3.9)
Octopus campbelli	19	10	1.6 ± 0.5 (0.9–2.5)	− 21.2 ± 0.3 (− 21.6 to − 20.8)	2.7 ± 0.4 (2.3–3.7)	3.8 ± 0.1 (3.7–3.9)
Nototodarus sloanii	12	10	2.0 ± 0.4 (1.4–2.7)	− 20.0 ± 0.6 (− 21.5 to − 19.0)	2.3 ± 0.6 (1.8–3.8)	3.9 ± 0.3 (3.5–4.2)
1986–87 season						
Moroteuthopsis ingens	16	10	2.1 ± 1.0 (1.1–3.6)	− 19.2 ± 0.6 (− 20.5 to − 18.3)	4.4 ± 0.6 (3.3–5.2)	3.6 ± 0.3 (3.1–3.9)
Octopus campbelli	20	10	1.4 ± 0.3 (0.9–1.7)	− 18.8 ± 0.4 (− 19.3 to − 18.0)	4.3 ± 0.7 (3.3–5.3)	3.4 ± 0.2 (3.2–3.7)

Values are Mean ± SD (Min–Max)

The data from 1986 to 87 was adapted from Xavier et al. (2018a, b). *n ind* number of individuals aggregated in capsules, *n cap* Number of capsules used

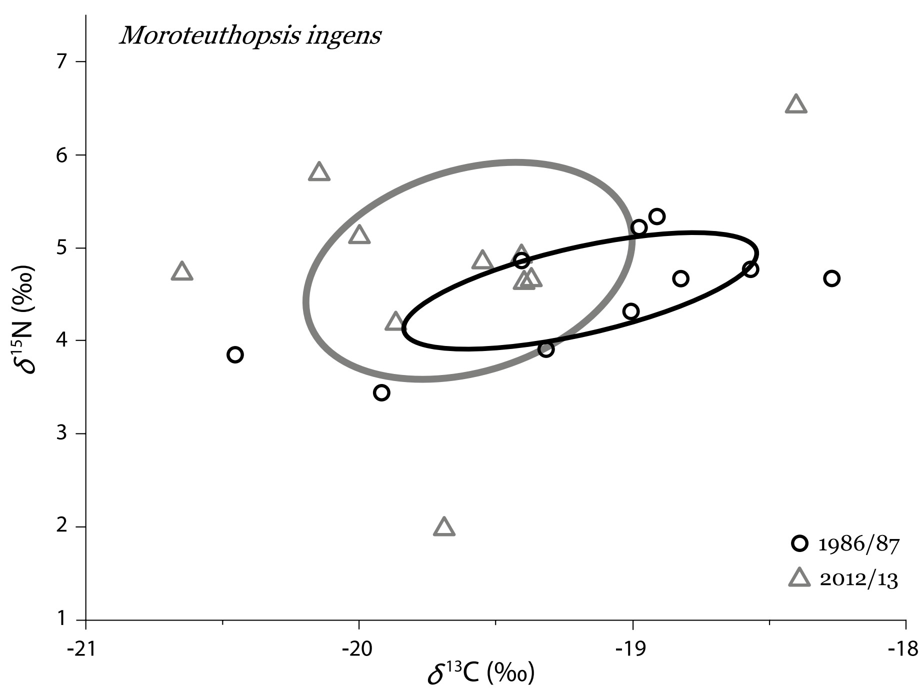

Fig. 2 Stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) from lower beaks of *Moroteuthopsis ingens* comparing breeding seasons 1986–87 (n ind = 16, n cap = 10) and 2012–13 (n ind = 11, N cap = 10) (*n ind* number of individuals aggregated in capsules; *n cap* number of capsules used). Values are Mean ± SD

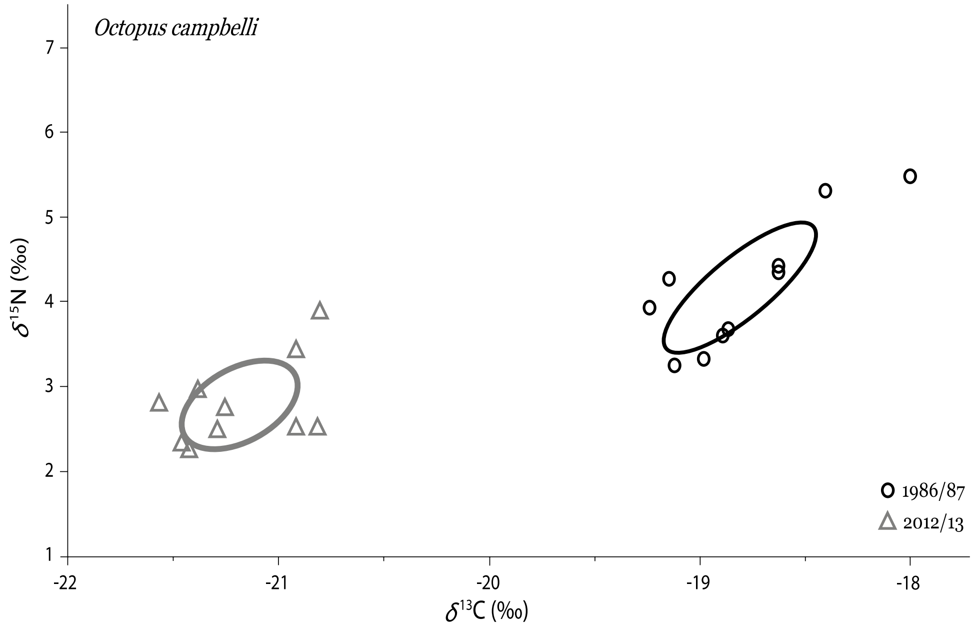

Fig. 3 Stable isotope values (δ^{13}C and δ^{15}N) from lower beaks of *Octopus campbelli* comparing breeding seasons 1986–87 (n ind = 19, n cap = 10) and 2012–13 (n ind = 20, n cap = 10) (*n ind* number of individuals aggregated in capsules, *n cap* number of capsules used). Values are Mean ± SD

diet preferences, although variability within samples may reflect a broader ecological niche or variability in prey items. Moreover, juveniles/ sub-adults of this cephalopod species are pelagic (Jereb & Roper 2010), foraging on myctophid, crustacean species and even cannibalizing their own species (Jackson et al. 1998; McBride et al. 2022).

For the octopod species that occurs locally at Campbell Island waters (O'Shea 1999)—*O. campbelli*—δ^{13}C values were significantly lower in 2012–13 compared to 1986–87 (ANOVA: $F_{1,18} = 216.800$, $p < 0.001$, Tukey's multiple comparison test: 1986–87—2012–13: $p < 0.001$: Table 2) (Fig. 3). The lack of overlap in isotopic niche areas (SEA_B: $p = 0.803$) and the significant differences in δ^{13}C and δ^{15}N values suggest changes in foraging location and possibly a shift to lower trophic levels. This could be due to warmer water conditions driving the species to migrate to cooler, offshore waters (Morrison et al. 2015; Bereiter et al. 2018). The observed changes in δ^{13}C and δ^{15}N values might also indicate shifts in prey preferences or feeding strategies (Bearhop et al. 2004), but there is a lack of knowledge that needs to be addressed by future studies.

The new squid species observed, *N. sloanii*, presented δ^{13}C values in accordance with the values of sub-Antarctic waters (Jaeger et al. 2010; Guerreiro et al. 2015) and lower δ^{15}N values indicative of foraging at lower trophic levels (Dunn 2009). The presence of this typical arrow squid from warmer waters of New Zealand (Jackson et al. 2000) in 2012–13 and their absence from 1986 to 87 can be supported by the changes in the oceanographic conditions from cooler in 1986–87 to warmer waters in 2012–13 (Morrison et al. 2015; Bereiter et al. 2018).

Considerations of the presence of the arrow squid on the diet of eastern rockhopper penguin

Despite the limited data available, the discovery of *N. sloanii* in the diet of eastern rockhopper penguins adds valuable information to the understanding of penguin foraging ecology. The presence of this squid, a significant species in New Zealand fisheries (Uozumi 1998), highlights potential interactions between the penguins and local fisheries, although the risk of fisheries impact on this penguin's species is very low (Trathan et al. 2014). Moreover, there is a temporal overlap between the chick rearing and the beginning of the squid fisheries season in December/January (MPI 2022), but tracking data suggest that penguins move to deeper waters, away from main fishing grounds (Green et al. 2022). While cephalopods constitute a small portion of the eastern rockhopper penguin diet, the finding of a key commercial arrow squid species such as *N. sloanii* may have implications for future interactions with New Zealand squid fisheries, highlighting the need for further research to provide valuable insights into the conservation of the eastern rockhopper penguin species.

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Author contributions HRG, DRT and JCX contributed to the study conception and design. Sampling was carried out by KWM; data collection and analysis was performed by HRG, and cephalopod beaks identification was double- checked by JCX. All authors contributed to the drafting of the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval The methods employed in this research received approval from the Massey University Animal Ethics Committee under protocol number 10/90.

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