Eastern rockhopper penguins *Eudyptes filholi* as biological samplers of juvenile and sub-adult cephalopods around Campbell Island, New Zealand

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Abstract

Early life-history stages of cephalopods are known to play an important role as prey in food webs of the Southern Ocean, but little information is available about their biology and availability to predators. Top predators, such as penguins, are known to feed regularly on coastal juvenile/sub-adult cephalopods. Using eastern rockhopper penguins *Eudyptes filholi* as coastal biological samplers, we examined in detail the cephalopod component of their diet in Campbell Island (New Zealand) during two consecutive breeding seasons in order to evaluate: i) the relative importance of cephalopods (by frequency of occurrence, by number and by mass) to the diet of both adult and chick penguins, ii) the habitat and trophic levels of the cephalopods in the region, and iii) the status of the juvenile/sub-adult cephalopod community in the waters around Campbell Island. Our results show that eastern rockhopper penguins feed on eight species of juvenile and sub-adult cephalopods, with *Onykia ingens*, *Martialia hyadesi* and *Octopus campbelli* being the most important species by frequency of occurrence, number and mass. Differences between the diets of adult and chick penguins and between breeding seasons were found. Habitat (*δ*13C) and trophic level (*δ*15N) information also showed that all cephalopod species (and all studied stages) occupy similar habitat on the Campbell shelf, with *M. hyadesi* showing lower *δ*15N values than *O. ingens* and *O. campbelli*. This study indicates that eastern rockhopper penguins can be valuable biological samplers of local juvenile/sub-adult cephalopods (including poorly known cephalopod species) around Campbell Island when breeding, that these cephalopods were likely to be caught naturally (not from fisheries), providing relevant information for the conservation of these penguins.

**Keywords: eastern rockhopper penguins, diet, *Martialia hyadesi*, *Onykia ingens*, *Octopus campbelli*, pelagic squid**

**Introduction**

Coastal marine zones are complex ecosystems influenced by physical, chemical and biological processes under anthropogenic pressure ([Halpern et al. 2008](#_ENREF_34)), with resources (e.g. exploitable marine organisms) that require careful management ([Ngoile and Horrill 1993](#_ENREF_58)). It is increasingly important to have a regional and global effort to identify resources that require conservation, to develop and implement ecosystem-based management and to carry out marine basic research to address gaps in ecosystem knowledge ([Halpern et al. 2008](#_ENREF_34)). Within the Southern Ocean, the current or most immediate threats to the conservation of species, ecosystems and resources are regional ocean warming, acidification, changes in sea-ice distribution and human activities (such as fishing) ([Anderson et al. 2011](#_ENREF_2); [Kennicutt II et al. 2014](#_ENREF_51); [Tuck et al. 2011](#_ENREF_82); [Xavier et al. 2016a](#_ENREF_87)).

New Zealand and its adjacent sub-Antarctic islands, are strongly influenced by the Southern Ocean ([Gordon et al. 2010](#_ENREF_32)), and some marine keystone species of this region, including fish and seabirds, have already been affected by climate change and by fisheries ([Cunningham and Moors 1994](#_ENREF_30); [Gordon et al. 2010](#_ENREF_32); [Hilton et al. 2006b](#_ENREF_37); [Last et al. 2011](#_ENREF_53); [Morrison et al. 2015](#_ENREF_55); [Scott et al. 2008](#_ENREF_77)). Yet, there is a paucity of information on how cephalopods might be affected despite an increase of basic ecological and taxonomic knowledge of cephalopods from the Southern Ocean recently ([Cherel and Duhamel 2003b](#_ENREF_11); [Collins and Rodhouse 2006b](#_ENREF_27); [Guerreiro et al. 2015](#_ENREF_33); [Jackson et al. 1998b](#_ENREF_48); [Xavier et al. 2015](#_ENREF_86); [Xavier et al. 2016c](#_ENREF_91)). Cephalopods are known to be an important prey of numerous predators, to have a large biomass and may be affected by environmental change ([Clarke 1983](#_ENREF_24); [Rodhouse 2013](#_ENREF_69); [Xavier and Cherel 2009](#_ENREF_88)). Indeed, the majority of cephalopod species exhibit a “live fast and die young” life cycle which can result in either positive or negative effects in their response to environmental change (e.g. warming may increase cephalopod growth rate but shorten lifespan that will have implications for their demography and life history) ([Pecl and Jackson 2008](#_ENREF_60); [Xavier et al. 2015](#_ENREF_86)). As cephalopod fauna from the Pacific sector in the Southern Ocean is still poorly known ([Xavier et al. 2014b](#_ENREF_95)) , top predators (including penguins) can be used as biological samplers to provide valuable information about local cephalopod fauna ([Cherel et al. 1999](#_ENREF_17); [Cherel and Weimerskirch 1995](#_ENREF_18); [Cherel and Weimerskirch 1999b](#_ENREF_20); [Clarke 1980](#_ENREF_23); [Rodhouse et al. 1992](#_ENREF_70); [Seco et al. 2016](#_ENREF_78); [Xavier and Cherel 2009](#_ENREF_88)), complementing with scientific surveys ([Gordon et al. 2010](#_ENREF_32)).

 Among top predators, albatrosses, petrels, whales and numerous penguin species are known to feed on cephalopods, consuming significant quantities of squid in some regions and seasons ([Croxall and Prince 1996](#_ENREF_29); [Xavier and Cherel 2009](#_ENREF_88)). The eastern rockhopper penguin *Eudyptes filholi* ([Banks et al. 2006](#_ENREF_3); [De Dinechin et al. 2009](#_ENREF_31)), breeds in the southern Indian Ocean (Marion, Crozet, Kerguelen and Heard islands) and in the south Pacific Ocean (Auckland, Antipodes, Campbell and Macquarie islands) (([BirdLifeInternational 2012](#_ENREF_5); [Cunningham and Moors 1994](#_ENREF_30); [Heather and Robertson 1996](#_ENREF_35); [Morrison et al. 2015](#_ENREF_55)), and feeds predominantly on fish, crustaceans and cephalopods ([Crawford et al. 2003](#_ENREF_28); [Hull 1999](#_ENREF_38); [Marchant and Higgins 1990](#_ENREF_54); [Morrison et al. 2014](#_ENREF_56); [Sagar et al. 2005](#_ENREF_76); [Williams and Laycock 1981](#_ENREF_84); [Williams 1995](#_ENREF_85)). Regarding the latter group, the work by P.J. Moors and D. M. Cunningham ([Marchant and Higgins 1990](#_ENREF_54)) at Campbell Island provided the first preliminary analyses of the cephalopods ingested by eastern rockhopper penguins, but presented no information of estimated body size and mass, and maturity stage. This information is important, not only to the biology/ecology of cephalopods but also for management and conservation of local/regional marine ecosystems. Moreover, cephalopod species may have been misidentified and a review of these identifications may be required.

Rockhopper penguins are known to forage up to 120 km from their breeding island during the breeding season ([Sagar et al. 2005](#_ENREF_76)) and consequently may allow inferences on spawning grounds, biology and ecology of neritic and oceanic cephalopods and fish ([Tremblay and Cherel 2000](#_ENREF_80); [Tremblay and Cherel 2003](#_ENREF_81)). Moreover, the use of stable isotope values of beaks of cephalopods from the diet of their predators can provide valuable information on cephalopod habitat (via *δ*13C) and trophic level (via *δ*15N) ([Cherel and Hobson 2005](#_ENREF_15)) as well as for other prey taxa, such as fish and crustaceans ([Barrett et al. 2007](#_ENREF_4); [Karnovsky et al. 2012](#_ENREF_50); [Stowasser et al. 2012](#_ENREF_79)).

The objectives of our study are to use eastern rockhopper penguins as biological samplers of cephalopods around sub-Antarctic islands, because cephalopod-related information is very scarce. More specifically, we aim to:

a) Characterize the cephalopod component of the diet of eastern rockhopper penguins at Campbell Island during breeding in two consecutive breeding seasons (1985/86 and 1986/87)

b) Study the habitat and trophic level characteristics of cephalopods in the Campbell Island region through stable isotopic analysis and

c) Assess how such information is relevant to the ecology and biodiversity studies of juvenile/sub-adult cephalopods in coastal waters around Campbell Island.

**Material and Methods**

Fieldwork

A total of 122 diet samples were obtained from adults (n=98, via stomach pump lavage, individuals captured *en route* to the breeding colonies from shoreline landing areas, 1-200m from colonies, samples collected in approximately 5 minutes per bird using with seawater) and chicks (n=24, via autopsy, chicks sampled opportunistically from fresh carcasses encountered in the colonies) of eastern rockhopper penguins from Penguin Bay, Campbell Island (52° 32´ 24”S, 169° 8´ 42” E), New Zealand (Figure 1), during the breeding seasons of 1985-86 (adults=29 (23-12-1985 to 24-01-1986); chicks=24 (18-12-1985 to 27-01-1986) and 1986-87 (adults=69 (13-01-1987 to 07-02-1987), collected randomly each year by P. J. Moors and D. M. Cunningham ([Marchant and Higgins 1990](#_ENREF_54)). All adult birds (each sampled once only) were released in apparently good condition and all returned to their nesting colony, with the exception of one casualty across the two years. Chick carcasses were not collected in the second (1986-87) breeding season due to fieldwork constraints.

After collecting the samples, liquid was removed from each sample by straining through a muslin bag. Samples were frozen until analysis in mainland New Zealand. Here, we re-examined the cephalopod component of the diet of eastern rockhopper penguins because the taxonomy of cephalopods has undergone considerable revision since the 1980s (e.g. with new species being described or taxonomy updated ([Collins and Rodhouse 2006a](#_ENREF_26); O’Shea 1999)), additional information on the sizes and maturity stage of some of the cephalopod species caught is now available ([Jackson et al. 2000a](#_ENREF_41); [Jackson 1997a](#_ENREF_45); [Phillips et al. 2003](#_ENREF_61)) and our ability to use stable isotopic analyses to assess the habitat and trophic levels of the main cephalopod species has become well established ([Cherel and Hobson 2005](#_ENREF_15); [Xavier et al. 2016b](#_ENREF_90)). Furthermore, our study incorporates previously unpublished data from adults and analyses of cephalopods in the diet of eastern rockhopper penguins between seasons, information not presented in Marchant & Higgins (1990). The fish and crustacean components in the diet of eastern rockhopper penguins have been published ([Marchant and Higgins 1990](#_ENREF_54)).

Laboratory work

Cephalopod beaks were identified to species level wherever possible, following Xavier & Cherel (2009) and beak reference collections at the National Institute of Water and Atmospheric Research (NIWA, see below) and at the Marine and Environmental Sciences Centre, University of Coimbra (Portugal). The lower rostral length (LRL; for squid) and lower hood length (LHL; for octopods) were measured using a stereo microscope (Wild Heerbrugg, objective: 1.5x; ocular: 15x) and maturity stage estimated (according to the darkening of the beaks: juvenile beaks were undarkened or with only the tip of the rostrum darkened; sub-adult beaks exhibited darkened tip and wings; or adult beaks with wings totally darkened) ([Clarke 1986](#_ENREF_25); [Xavier and Cherel 2009](#_ENREF_88)). Allometric equations from published sources were used to convert LRL/LHL to mantle length (ML, in mm) and mass (M, in grams (g)) ([Brown and Klages 1987](#_ENREF_7); [Clarke 1986](#_ENREF_25); [Jackson 1995](#_ENREF_44); [Lalas and McConnell 2012](#_ENREF_52); [Piatkowski et al. 2001](#_ENREF_62); [Roberts and Lalas 2015b](#_ENREF_67); [Rodhouse and Yeatman 1990](#_ENREF_73)). Octopod beaks were identified using a reference collection (that includes beaks from known species in the region, including the octopod *Enteroctopus zealandicus*) held at NIWA in Wellington, New Zealand. For the most important cephalopod species (i.e. by frequency of occurrence, by number and/or by mass)that allowed comparisons of sizes (i.e. LRL/LHL; see below) between years, statistical tests were also performed to assess differences between old and fresh beaks.

For each cephalopod species, frequency of occurrence (F: number of samples with a certain cephalopod species present divided by the total number of samples analysed), number (N: number of lower beaks of a certain species divided by the total number of lower beaks) and contribution to mass (estimated mass of all individuals of a certain cephalopod species divided by the total estimated mass for all cephalopod individuals) were estimated following previous diet studies ([Xavier et al. 2002](#_ENREF_92)). To complement these analyses, a re-calculation of the contribution of each species by mass was carried out, by adding the mean mass of all individuals of a given species to each beak unmeasured of that species (e.g. the mean mass of 13.5 g was multiplied by the 18 unmeasurable lower beaks of *O. ingens* in 1985-86).

For the most numerous species, after double-checking identifications, all lower beaks for each species that were in good condition (i.e. fresh, with no signs of erosion) were stored in a glass jar containing 75% ethanol, and beaks were randomly selected for stable isotopic analysis (*O. ingens:* 16 lower beaks; *M. hyadesi:* 10 lower beaks; *O. campbelli:* 20 lower beaks) following previous studies ([Seco et al. 2016](#_ENREF_78); [Xavier et al. 2016b](#_ENREF_90)). After taking measurements (see above), beaks were prepared for stable isotopic analysis after the beaks being transported from New Zealand to Portugal by plane (kept in 70% ethanol). Each beak was cleaned with 80% ethanol, stored in a microtube and dried in an oven at 60oC. Dried beaks were smashed into very small fragments inside the microtube using a cleaned tweezer to decrease the chance of contamination. Using a Mettler Toledo® UMX2 ultra-microbalance, samples were weighed, approximately 0.35 mg (0.27 ± 0.10 mg, mean ± standard deviation) into a tin capsule. Values of *δ*13C and *δ*15N were obtained using a Continuous Flow Isotope Ratio Mass Spectrometer (Delta V™ Advantage - Thermo Scientific®) interfaced with an organic elemental analyser (Flash™ EA 1112 – Thermo Scientific®) at MARE – Figueira da Foz, following previous published work ([Seco et al. 2016](#_ENREF_78)). We present the results in standard *δ* notation in ‰ (parts per thousand), following the equation *δ*X = [(Rsample -Rstandard -1) x 1000], were X represents 13C or 15N and R the 13C/12C and 15N/14N ratios, respectively. Data are presented relative to Vienna-Pee Dee Belemnite (V-PDB) limestone and Atmospheric N2 for C and N, respectively. Reference material [acetanilide (Thermo Scientific®)] was measured to determine machine internal analytical errors (< 0.2 ‰ both for δ13C and δ15N). Carbon-to-Nitrogen (C:N) ratios of the samples were estimated to assess the potential effect of lipids on the results (i.e. C:N < 3.5) ([Post et al. 2007](#_ENREF_63)).

Statistical analyses

 All statistics were carried out using STAT PLUS. In order to maximize the number of beaks analyzed to assess the widest range of cephalopods ingested (rather than fresh items only), accumulated old and fresh beaks were combined for statistical analyses (Tables 1, 2), following previous studies ([Cherel and Weimerskirch 1999b](#_ENREF_20); [Xavier et al. 2003](#_ENREF_89)). Comparisons between the beak sizes were performed (i.e. between adults and chicks; between seasons for species; between habitat and trophic levels between the most numerous cephalopod species (i.e. *O. ingens*, *M. hyadesi* and *O. campbelli*) using non-parametric tests (Mann-Whitney U test, between two species/samples; Kruskal-Wallis, between more than two species/samples), after confirming that the data was not normally distributed (using STAT PLUS normality test). Result are provided as mean ± standard deviation , unless stated otherwise. A result was significant when P< 0.05.

**Results**

**Cephalopod component of the diet of eastern rockhopper penguins**

 A total of 939 lower beaks were identified and measured from the 122 samples of eastern rockhopper penguins at Campbell Island in the 1985-86 and 1986-87 breeding seasons (Table 1). All the beaks were from juveniles for the squid species and from juveniles/sub-adults for the octopus *O. campbelli*. Photographs were taken of the key cephalopod species that haven´t been described before (Figure 2). No adult beaks (i.e. with wings fully darkened) were encountered in the samples. Eight species of cephalopods were recorded, with *O. ingens*, *M. hyadesi* and *O. campbelli* being the most important species by frequency of occurrence, number and by mass (Table 1). The cephalopods consumed ranged from <1 g (an individual of *O. campbelli*) to 129 g (an individual of *O. ingens*) of estimated mass (Table 2).

**Adults versus chicks**

Comparing diet data between adults and chicks was only possible in the 1985-86 breeding season. *O. ingens* was the most important cephalopod in the diet of adult eastern rockhopper penguins (F: 36.6%; N = 64.9%; M = 50.0-64.6%), whereas *M. hyadesi* was the most important species in the diet of chicks (F: 58.3%; N = 49.5%; M = 55.6-59.6%: Table 1).The sizes of lower beaks of *O. ingens* (the only species with sufficient beaks for a statistical test) were different between adults and chicks, with beaks from penguin chicks slightly larger than those from adults (Mann-Whitney U test; U(33,27) = 595.50; p = 0.025; *O. ingens* from adults: 1.3 ± 0.5 mm (n= 33); from chicks: 1.5 ± 0.4 mm (n=27)).

**Inter-annual variations**

Using only comparable adult diet data between years, *O. ingens* was the most important cephalopod species in the diet(by F, N and M) in both 1985-86 and 1986-87 breeding seasons (Table 1). Even when recalculating the mass (by the inclusion of a mean mass for unmeasurable lower beaks), *O. ingens* continued to be the most important species (by F, N and M) but its relative contribution to the diet by mass in the 1986-87 season decreased (from 49.6% to 39.7%), while the contribution to the diet by mass of *M. hyadesi* increased (from 13.0% to 38.0%: Table 1).

 A comparison of beak sizes between the main cephalopod species and between seasons (combining all measurements from adults and chicks between years) revealed that there was a significant difference between the beak sizes of *O. campbelli* between the two seasons (but still juvenile/sub-adult specimens), with larger beaks in the 1985-86 season (Mann-Whitney U test; U(63,248) = 11062.00; p < 0.0001; Table 2; Figure 3).

**Habitat and trophic levels of cephalopods around Campbell Island**

Carbon-to-nitrogen (C:N) ratios of beaks showed no effect of lipids on the results, with values ranging from 3.2 to 4.0 across all beaks analysed (Table 3). All the main species (*O. ingens*, *O. campbelli* and *M. hyadesi*) occupy a very similar habitat based on *δ*13C values (Kruskall-Wallis test H(2,46)= 0.08; p=0.962), with *O. ingens* showing a wider range of values compared to the other two species (Table 3; Figure 4). *δ*15N values of *M. hyadesi* were significantly lower than those of *O. ingens* (Kruskall-Wallis test H(1,24)= 10.95; p=0.0009) and *O. campbelli* (Kruskall-Wallis test H(1,32)= 9.09; p=0.002). The latter two species had similar *δ*15N values (Kruskall-Wallis test H(1,36) = 0.23; p=0.633).

**Discussion**

**Cephalopod component of penguins’ diet**

This studyaimed, in part, to compare our results with those obtained by D.M. Cunningham and P.J. Moors from the same diet samples ([Marchant and Higgins 1990](#_ENREF_54)) collected in the mid-1980s. Cephalopods previously reported comprised a total of three species (*Moroteuthis ingens*, *Alluroteuthis* *antarcticus* and *Octopus dofleini*) in adult penguins and four species (*M. ingens*, *Kondakovia longimana*, *M. hyadesi* and *O. dofleini)* in chicks ([Marchant and Higgins 1990](#_ENREF_54)).

*O. dofleini* [now known as the giant Pacific octopus *E. dofleini* ([Bouchet 2010](#_ENREF_6))] was misidentified in Marchant and Higgins (1990) with the small octopod *O. campbelli*, which was the most important (and only) octopod species in the diet of eastern rockhopper penguins in our study (Table 1). *E. dofleini* is only known to be distributed in the northern Pacific Ocean from Japan to southern California ([Nesis 1987](#_ENREF_57)). Within the genus *Enteroctopus,* the octopus *E. zealandicus* occurs in New Zealand sub-Antarctic waters ([Bouchet 2010](#_ENREF_6)), and has been recorded in the diet of New Zealand sea lions *Phocarctos hookeri* at Campbell Island ([Roberts and Lalas 2015b](#_ENREF_67)), but beaks of *E. zealandicus* are considerably larger than the beaks from *O. campbelli* for the same maturity stage (i.e. small beaks from juvenile/sub-adult of *O. campbelli* were already considerably more darkened than would be the case for undarkened juvenile/sub-adult beaks from *E. zealandicus*).

Our study also identified *O. ingens* (previously known as *Moroteuthis ingens*) as the most important species (see Tables 1, 2, and Marchant and Higgins 1990) but also identified other cephalopod species in the diet of eastern rockhopper penguin adults (namely *A. antarcticus*, *Gonatus antarcticus*, *M. hyadesi, Moroteuthis* sp. B ([Imber 1992](#_ENREF_40)) and Oegopsida sp. A([Cherel et al. 2004b](#_ENREF_14))) and chicks (*G. antarcticus*), which may be attributed to better reference collections and cephalopod identification guides ([Cherel et al. 2004b](#_ENREF_14); [Imber 1992](#_ENREF_40); O’Shea 1999; [Xavier and Cherel 2009](#_ENREF_88)), information not available in the 1980s. Consequently, this study identified four additional squid species from the waters around Campbell Island that were present in the 1980s (see Table 1), providing valuable information on their circumpolar distribution and the use of the Campbell shelf area for their early life stages in the 1980s. *G. antarcticus* and *K. longimana* have a circumpolar distribution reaching as far south as the Antarctic continent ([Xavier et al. 2016c](#_ENREF_91)), with our study providing evidence of their distribution extending also to the Campbell Island shelf (at least in the 1980s), in South Pacific waters. *M.* sp. B ([Imber 1992](#_ENREF_40)) is a poorly-known species previously reported from New Zealand waters ([Imber 1992](#_ENREF_40); [West and Imber 1986](#_ENREF_83)), and occurring in the diet of predators around the Southern Ocean: i) Patagonian toothfish *Dissostichus eleginoides*, ii) wandering albatross *Diomedea exulans* and king penguin *Aptenodytes patagonicus* in the south Indian Ocean (Crozet and Kerguelen islands) ([Cherel et al. 2004a](#_ENREF_13); [Cherel et al. 2017](#_ENREF_21); [Xavier and Cherel 2009](#_ENREF_88)) and iii) Antipodean *albatross Diomedea antipodensis antipodensis* and Gibson’s albatross *D. a. gibsoni* from south Pacific waters ([Xavier et al. 2014a](#_ENREF_94)). Similarly, *Oegopsida* sp. A (Cherel) is a rare species that occurs infrequently in the diet of Patagonian toothfish and wandering albatrosses in the south Indian Ocean (at Crozet Island) ([Cherel et al. 2004a](#_ENREF_13); [Cherel et al. 2017](#_ENREF_21)) and South Georgia ([Imber 1992](#_ENREF_40)), and in Antipodean and Gibson’s albatross diets from south Pacific waters ([Xavier et al. 2014a](#_ENREF_94)).

Overall, our results show that out of the eight species of cephalopods found in the diet of eastern rockhopper penguins, *O. ingens*, *M. hyadesi* and *O. campbelli* were the most important species by frequency of occurrence, number and by mass (Table 1). Surprisingly, there were differences between adults and chicks, not only in terms of the most important cephalopod species (*O. ingens* in adults and *M. hyadesi* in chicks; Table 1) but also chicks being fed slightly larger *O. ingens* than those taken by adults (although the differences in size are unlikely to be ecologically relevant). As prey found in the diet of chicks is provided by adults, adult eastern rockhopper penguins perhaps preferentially feed their chicks slightly larger squid.

**Differences in cephalopod component of penguins’ diet between breeding seasons**

Other than comparing the biodiversity of cephalopods occurring in the diet of eastern rockhopper penguins at Campbell island ([Marchant and Higgins 1990](#_ENREF_54)), our detailed study allowed us to compare penguin diet across two consecutive breeding seasons. *O. ingens* was the most frequent cephalopod species in both breeding seasons (1985-86 and 1986-87), with both *O. ingens* and *M. hyadesi* also playing an important role by mass. *O. ingens* and *M. hyadesi* are known to occur in the diet of a wide range of predators across the Southern Ocean ([Xavier and Cherel 2009](#_ENREF_88)), with penguins (and some smaller albatross species; e.g. black-browed albatross *Thalassarche* *melanophrys* and grey-headed albatross *T. chrysostoma*) generally taking juveniles (examples across the Southern Ocean in Appendix 1) and larger predators, such as whales, great albatrosses (e.g. wandering albatross) and toothfish generally taking sub-adults and adults ([Cherel and Duhamel 2004](#_ENREF_12); [Cherel et al. 2004a](#_ENREF_13); [Cherel et al. 1996](#_ENREF_16); [Cherel and Weimerskirch 1999b](#_ENREF_20); [Cherel et al. 2017](#_ENREF_21); [Clarke 1980](#_ENREF_23); [Xavier et al. 2014a](#_ENREF_94)), with larger predators consuming correspondingly larger squid specimens. The most important (and only) octopod species found in the diet of eastern rockhopper penguins was *O. campbelli,* exhibiting significantly larger sizes in the 1985-86 breeding season than in the 1986-87 breeding season (Table 2), despite all beaks being representative of a similar life stage (i.e. juvenile/sub-adult specimens; Figure 2). As warmer waters are most likely to affect primary productivity negatively, and affect the population of eastern rockhopper penguins through changes in the distribution, availability or abundance of their food supply ([Cunningham and Moors 1994](#_ENREF_30); [Hilton et al. 2006a](#_ENREF_36)), sea temperatures may have potentially affected the sizes of *O. campbelli*. With cooler sea surface temperature conditions in 1985-86 than in 1986-87 ([Morrison et al. 2015](#_ENREF_55)), *O. campbelli* may have exhibited higher growth in 1985-86. Indeed with cooler sea surface temperature waters stay more oxygenated, allowing animals to grow bigger ([Chapelle and Peck 1999](#_ENREF_9)). However, as the dataset is only from the diet of a selective predator (i.e. rockhopper penguins) that may not represent the overall size distribution of *O. campbelli* around Campbell Island, and there was no independent evaluation of the abundance of *O. campbelli* in the area in the breeding seasons included here, it is difficult to draw any firm conclusions.

*O. campbelli* and *O. ingens* also occurred as relatively minor components in the diet of *P. hookeri* breeding at Campbell Island ([Roberts and Lalas 2015b](#_ENREF_67)) and at the Auckland Islands ([Childerhouse et al. 2001](#_ENREF_22)). New Zealand sea lions also feed on *E. zealandicus* and the pelagic squid *Todarodes filippovae* ([Roberts and Lalas 2015a](#_ENREF_66)), both of which are absent in rockhopper penguin diets (Table 1). Such different diets could be attributed to *E. zealandicus* being able to reach large sizes (and therefore hard to handle by the penguins), with larger, mature individuals occurring in deeper waters (up to *c*. 530 m deep) where this octopus could be caught by New Zealand sea lions (O’Shea 1999). Surprisingly, juvenile *E. zealandicus* are common in littoral sub-Antarctic waters (including Campbell Island) but do not occur in the diet of eastern rockhopper penguins, which could be attributed to temporal (e.g. juveniles of *E. zealandicus* are only available during other periods of the year outside the rockhopper penguin breeding season), spatial (e.g. juveniles of *E. zealandicus* are distributed outside the penguins’ foraging area) or ecological (e.g. juveniles of *E. zealandicus* are capable of avoiding capture by rockhopper penguins) reasons but more research is needed to address this issue.

**Biodiversity, habitat and trophic level of cephalopods in the Campbell Island region through stable isotopic analysis**

As biological samplers of juvenile and sub-adult cephalopods around Campbell Island, eastern rockhopper penguins fed on early stages of eight different cephalopod species, demonstrating the importance of the waters around Campbell Island for the life cycle of both benthic and pelagic cephalopods. Although the spawning area of these species is unknown, the presence of juvenile cephalopods in the diet of eastern rockhopper penguins, supported with the habitat (*δ* 13C) stable isotopic values of sub-Antarctic/subtropical waters ([Ceia et al. 2015](#_ENREF_8); [Guerreiro et al. 2015](#_ENREF_33); [Jaeger et al. 2010](#_ENREF_49)) and of the studied cephalopods (see Figure 4), suggests that some spawning may occur relatively near to Campbell Island in shelf or slope waters. Moreover, these results also suggest that eastern rockhopper penguins are foraging (at least, during the breeding season in the 1980s) close to Campbell Island at a specific or similar isotopic related-habitat type and feeding on some early stages of cephalopods (as supported by the stable isotopic values of the cephalopod prey; see below).

*O. campbelli* occurs locally to Campbell Island (O’Shea 1999), so the isotopic proxy values of habitat (*δ* 13C) reflect the waters around Campbell Island. Furthermore, our study provides isotopic evidence that juvenile and pelagic *O. ingens* and *M. hyadesi* occur in similar habitat as benthic *Octopus campbelli* around Campbell Island during the breeding season of eastern rockhopper penguins. Consequently, our results suggest eastern rockhopper penguins at Campbell Island perform benthic dives, as already described at Kerguelen Islands ([Tremblay and Cherel 2000](#_ENREF_80)), although elsewhere they perform mostly pelagic dives ([Hull 2000](#_ENREF_39)). Onychoteuthids (such as *O. ingens*) and Ommastrephids (such as *M. hyadesi*) are known to occur in oceanic waters but can also be found in shelf/slope waters in Antarctic and sub-Antarctic regions ([Cherel and Duhamel 2003a](#_ENREF_10); [Jackson et al. 2000b](#_ENREF_42); [Jackson et al. 1998a](#_ENREF_47); [Rodhouse et al. 2014](#_ENREF_71); [Roper et al. 1985](#_ENREF_74); [Rosas-Luis et al. 2016](#_ENREF_75); [Xavier et al. 2016c](#_ENREF_91); [Xavier et al. 1999](#_ENREF_93)). As large juvenile and immature *O. ingens* occur near the seabed in shelf environments before gradually moving into deeper waters as adults ([Cherel and Duhamel 2003a](#_ENREF_10); [Cherel and Weimerskirch 1999a](#_ENREF_19); [Jackson 1993](#_ENREF_43)), it is likely that eastern rockhopper penguins fed on this species close to the bottom, where *O. campbelli* is also likely to be encountered. While eastern rockhopper penguins appear to perform mainly pelagic dives (perhaps foraging on fish and crustaceans) (Thompson, unp. data from Campbell Island during the chick-rearing phase) ([Hull 2000](#_ENREF_39)), our data is indicative that some cephalopod prey are taken at or near the bottom, as well as pelagically.

Juvenile *M. hyadesi* is more likely to have been caught in the water column within the pelagic environment ([Rodhouse 1997](#_ENREF_68)). *O. ingens* and *M. hyadesi* appear to have a life span of 1-2 years ([Jackson 1997b](#_ENREF_46); [Rodhouse et al. 1994](#_ENREF_72)) and juveniles may be a regular annual prey for eastern rockhopper penguins, at least during the breeding season.

The trophic level (*δ*15N) of the most important cephalopod species show that *M. hyadesi* has a lower trophic value than *O. ingens* and *O. campbelli* (Figure 4). Consequently, it can be hypothesized that *M. hyadesi* could form part of the diet of *O. ingens* and *O. campbelli*, and that beaks of *M. hyadesi* found in the diet of rockhopper penguins are a result of being initially captured by *O. ingens* or *O. Campbelli*, rather than rockhopper penguins preying directly on *M. hyadesi*. However, there are no records of *M. hyadesi* in the diet of *O. ingens* (see below) ([Cherel and Duhamel 2003a](#_ENREF_10)), nor is it likely that a benthic octopod such as *O. campbelli* would venture into the water column to feed on a mobile, pelagic squid such as *M. hyadesi* (however, studies on the diet of *O. campbelli* would be required to clarify this statement).

 In terms of conservation, it is worth noting that the cephalopod prey of breeding eastern rockhopper penguins is likely to be naturally caught as there are no cephalopod fisheries around Campbell Island and the cephalopod bycatch/discards from the southern blue whiting *Micromesistius australis* trawl fishery around Campbell Island are relatively small (squid and octopods are < 0.01% of the total catch ([Anderson 2009](#_ENREF_1))). Furthermore, *O. ingens* collected from New Zealand waters, including specimens collected from around Campbell Island, revealed a diet that is mostly composed of small teleost fish (< 100 mm standard length; principally: *Lampanyctodes hectoris* (59% F), *Stomias boa/Chauliodus sloani* (46% F) ([Jackson et al. 1998b](#_ENREF_48))), with no presence of southern blue whiting. Therefore, the cephalopods preyed upon by breeding eastern rockhopper penguins at Campbell Island are unlikely to have been obtained through local commercial fishing activities , although overlap with commercial fishing may occur outside the breeding season, as rockhopper (and other) penguins are known to disperse during the non-breeding period ([Pütz et al. 2006](#_ENREF_64); [Ratcliffe et al. 2014](#_ENREF_65)).

 This study was conducted in the 1980s, and it would be interesting to investigate the current diet of eastern rockhopper penguins, to assess temporal dietary differences across several decades. Rockhopper penguin population fluctuations at Campbell Island appear follow trends in local sea surface temperature, with declining population trajectories during periods of relatively warm water and recovering populations during relatively cooler periods (Morrison et al. 2015). This thermally dynamic marine system is additionally very likely to affect the cephalopod prey of rockhopper penguins. Indeed, with stable isotope analyses used in this study, it would be possible to evaluate whether there have been changes in habitat and trophic levels of the cephalopod community present in the diet of eastern rockhopper penguins around Campbell Island. As cephalopods may be sensitive to environmental change, which may in turn impact their distribution and abundance (see introduction), such research could provide valuable information on changes in sub-Antarctic marine ecosystems, and contribute information towards the conservation of this penguin species.

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**Compliance with Ethical Standards**

 **The authors have no conflict of interests**

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Table 1. Frequency of occurrence (F and F%), number of lower beaks (N and N%; measured beaks with unmeasurable beaks in parentheses) and mass (M% = mass estimated (in grams) through allometric equations; M%\* = mass estimated based on adding the mean known mass value to unmeasurable beaks of a given species) of cephalopods found in the diet of eastern rockhopper penguins *Eudyptes filholi* at Campbell Island during the seasons 1985-86 (Dec. 1985 to Jan. 1986) and 1986-87 (Jan. to Feb. 1987).

|  |  |  |  |
| --- | --- | --- | --- |
|  | Frequency of occurrence | Number of lower beaks | Mass |
| Overall 1985-86 season (N=53) |  |  |  |  |  |  |
| Species |  F |  F% | N | N% | M% | M%\* |
| Gonatidae |  |  |  |  |  |  |
| *Gonatus antarcticus* | 1 | 1.9 | 1 (1) | 0.4 | <0.1 | <0.1 |
| Onychoteuthidae |  |  |  |  |  |  |
| *Kondakovia longimana* | 2 | 3.8 | 7 (3) | 2.6 | 2.1 | 4.7 |
| *Onykia ingens* | 27 | 50.9 | 78 (60) | 29.1 | 20.9 | 22.6 |
| Ommastrephidae |  |  |  |  |  |  |
| *Martialia hyadesi* | 16 | 30.2 | 108 (42) | 40.3 | 52.6 | 63.3 |
| Octopodidae |  |  |  |  |  |  |
| *Octopus campbelli* | 18 | 34.0 | 63 (63) | 23.5 | 24.3 | 23.7 |
| Unknown/eroded lower beaks | 9 | 17.0 | 11 (0) | 4.1 | <0.1 | <0.1 |
|  |  |  |  |  |  |  |
| Adult penguins (N=29) |  |  |  |  |  |  |
| Onychoteuthidae |  |  |  |  |  |  |
| *Onykia ingens* | 15 | 51.7 | 37 (33) | 64.9 | 64.6 | 65.0 |
| Ommastrephidae |  |  |  |  |  |  |
| *Martialia hyadesi* | 2 | 6.9 | 3 (2) | 5.3 | 14.6 | 15.1 |
| Octopodidae |  |  |  |  |  |  |
| *Octopus campbelli* | 9 | 31.0 | 9 (9) | 15.8 | 20.8 | 19.9 |
| Unknown/eroded lower beaks | 6 | 20.7 | 8 (0) | 14.0 |  |  |
|  |  |  |  |  |  |  |
| Chick penguins (N=24) |  |  |  |  |  |  |
| Gonatidae |  |  |  |  |  |  |
| *Gonatus antarcticus* | 1 | 4.2 | 1 (1) | 0.5 | <0.1 | <0.1 |
| Onychoteuthidae |  |  |  |  |  |  |
| *Kondakovia longimana* | 2 | 8.3 | 7 (3) | 3.3 | 2.5 | 4.3 |
| *Onykia ingens* | 12 | 50.0 | 42 (27) | 19.8 | 13.0 | 21.2 |
| Ommastrephidae |  |  |  |  |  |  |
| *Martialia hyadesi* | 14 | 58.3 | 105 (40) | 49.5 | 59.6 | 55.6 |
| Octopodidae |  |  |  |  |  |  |
| *Octopus campbelli* | 9 | 37.5 | 54 (54) | 25.5 | 25.0 | 18.8 |
| Unknown/eroded lower beaks | 3 | 12.5 | 3 (0) | 1.4 | <0.1 | <0.1 |
|  |  |  |  |  |  |  |
| Overall 1986-87 season |  |  |  |  |  |  |
| Adult penguins (N=69) |  |  |  |  |  |  |
| Gonatidae |  |  |  |  |  |  |
| *Gonatus antarcticus* | 4 | 5.8 | 4 (4) | 0.6 | 0.1 | <0.1 |
| Neoteuthidae |  |  |  |  |  |  |
| *Alluroteuthis antarcticus* | 2 | 2.9 | 2 (2) | 0.3 | <0.1 | <0.1 |
| Onychoteutyhidae |  |  |  |  |  |  |
| *Moroteuthis* sp. B (Imber) | 6 | 8.7 | 16 (15) | 2.4 | 2.4 | 1.5 |
| *Onykia ingens* | 40 | 58.0 | 300 (190) | 44.7 | 49.6 | 39.7 |
| Ommastrephidae |  |  |  |  |  |  |
| *Martialia hyadesi* | 18 | 26.1 | 86 (21) | 12.8 | 13.0 | 38.0 |
| Octopodidae |  |  |  |  |  |  |
| *Octopus campbelli* | 36 | 52.2 | 252 (248) | 37.6 | 34.9 | 20.8 |
| Oegopsida sp. A (Cherel) | 2 | 2.9 | 3 (3) | 0.4 | <0.1 | <0.1 |
| Unknown/eroded lower beaks | 5 | 7.2 | 8 (0) | 1.2 | <0.1 | <0.1 |

Table 2. Measurement of lower rostral length (LRL, mm), mantle length (ML, mm) and mass (M, g) from cephalopods found in the diet of eastern rockhopper penguins *Eudyptes filholi* at Campbell Island. Values are means ± SD with ranges in parentheses. Mann-Whitney non-parametric tests were applied to lower beaks measurements (combining old and fresh beaks) to species that occurred in both years, with at least 10 measurements. Values are means ± SD with ranges in parentheses.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Overall 1985-86 season | Overall 1986-87 |  |
| Species | LRL  | ML | M | LRL  | ML | M | Statistical test |
| Species |  |  |  |  |  |  |  |
| Neoteuthidae |  |  |  |  |  |  |  |
| *Alluroteuthis antarcticus* | - | - | - | 0.8  | 23 | 2.0 |  |
| *Gonatidae* |  |  |  |  |  |  |  |
| *Gonatus antarcticus* | 1.2 | 8.9 | 1 | 1.2 ± 0.1(1.1-1.3) | 8.4 ± 3.0(4.2-11.5) | 1.0 ± 0.2(1) |  |
| Onychoteuthidae |  |  |  |  |  |  |  |
| *Kondakovia longimana* | 3.1 ± 0.3(2.8-3.3) | 95 ± 12(81-102) | 27 ± 8(18-32) | - | - | - |  |
| *Moroteuthis* sp. B (Imber) | - | - | - | 1.8 ± 0.7(1.2-3.3) | - | 12 ± 18(2-63) |  |
| *Onykia ingens* | 1.4 ± 0.4 (0.7-2.7) | 84 ± 15 (58-128) | 13 ± 14(2-58) | 1.5 ± 0.8(0.6-3.7) | 86 ± 27(54-162) | 20 ± 25(1-129) | U(63,248)=11062p=0.195 |
| Ommastrephidae |  |  |  |  |  |  |  |
| *Martialia hyadesi* | 2.3 ± 0.3 (1.4-3.1) | 159 ± 9(133-183) | 49 ± 14(16-92) | 2.3 ± 0.4(1.6-3.4) | 158 ± 11(137-193) | 47 ± 20(20-116) | U(42,21)=382p=0.394 |
| Octopodidae |  |  |  |  |  |  |  |
| *Octopus campbelli* | 1.5 ± 0.3 (0.2-2.4) | 3.0 ± 0.7(0.3-0.1) | 15 ± 6(<1-39) | 1.3 ± 0.3(0.4-1.9) | 2.4 ± 0.7(0.7-3.8) | 11 ± 5(1-23) | U(60,191)=6365p<0.0001 |
| Oegopsida sp. A (Cherel) | - | - | - | 1.3 ± 0.3(1.1-1.6) | - | - |  |

Table 3. Measurement of lower rostral length (LRL, mm), and the isotopic values of the most important cephalopod species found in the diet of eastern rockhopper penguins *Eudyptes filholi* at Campbell Island.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Species | Number of lower beaks | LRL | *δ* 13C | *δ* 15N | C:N mass ratio |
| Onychoteuthidae |  |  |  |  |  |
| *Onykia ingens* | 16 | 2.2 ± 1.0(0.9-3.7) | –18.8 ± 0.8(–20.5 - –17.7) | +4.4 ± 0.6(3.1- 5.2) | 3.5 ± 0.2(3.2-4.0) |
| Ommastrephidae |  |  |  |  |  |
| *Martialia hyadesi* | 10 | 2.3 ± 0.1(2.1 – 2.7) | –18.8 ± 0.5(–19.8 - –18.3) | +3.3 ± 0.6(2.1 - 4.0) | 3.4 ± 0.2(3.3 – 3.9) |
| Octopodidae |  |  |  |  |  |
| *Octopus campbelli* | 20 | 1.1 ± 0.2(0.6-1.7) | –18.8 ± 0.6(–19.9 - –17.8) | +4.4 ± 0.8(3.3 - 6.0) | 3.4 ± 0.2(3.2 – 3.9) |

Legends of Figures

Figure 1. Geographic location of Penguin Bay, Campbell Island in relation to New Zealand.

Figure 2. Photographs of lower beaks of *Octopus campbelli* (above; includes also an upper beak (on the far right)) and *Onykia ingens* (below) from the diet of eastern rockhopper penguins *Eudyptes filholi* at Campbell Island. (Scale in *O. ingens* equals 1 cm)

Figure 3. Frequency of occurrence of the squid *Onykia ingens* (family Onychoteuthidae), *Martialia hyadesi* (family Ommastrephidae) and the octopod *Octopus campbelli* (family Octopodidae) and by lower rostral length in the diet of eastern rockhopper penguins *Eudyptes filholi* at Campbell island in seasons 1985-86 (white bars) and 1986-87 (black bars).

Figure 4. *δ* 13C and *δ* 15N values from lower beaks for main cephalopod

species found in the diet of eastern rockhopper penguins *Eudyptes filholi* from Campbell island (Mean ± SD), from combined beaks of samples collected from 1985-86 and 1986-87 breeding seasons.