**Distribution of short-finned squid** ***Illex argentinus* (Cephalopoda: Ommastrephidae)inferred from the diets of Southern Ocean albatrosses using stable isotope analyses**

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Corresponding author: José Seco - joses.seco@gmail.com***Abstract***

*The diets of marine predators are a potential source of information about range shifts in their prey. For example, the short-finned squid Illex argentinus, a commercially fished species on the Patagonian Shelf in the South Atlantic, has been reported in the diet of grey-headed, Thalassarche chrysostoma; black-browed, T. melanophris; and wandering, Diomedea exulans, albatrosses breeding at Bird Island, South Georgia (54****°****S 28****°****W) in the Southern Ocean. Tracking data suggests that these birds may feed on I. argentinus while foraging in Southern Ocean waters during their breeding season. This led to the hypothesis that I. argentinus may occur south the Antarctic Polar Front. To test this hypothesis, we used stable isotope analyses to assess the origin of I. argentinus. We compared I. argentinus beaks from the diets of the three albatross species with beaks of cephalopod species endemic to the Patagonian Shelf and others from the Southern Ocean. Our results show that I. argentinus from the diet of albatrosses at Bird Island have δ13C values in the range -18.77 to -15.28 ‰. This is consistent with δ13C values for Octopus tehuelchus, a typical species from the Patagonian Shelf. In contrast, Alluroteuthis antarcticus, a Southern Ocean squid, has typically Antarctic δ13C in the range -25.46 to -18.61. This suggests that I. argentinus originated from warmer waters of the Patagonian Shelf region. It is more likely that the albatross species obtained I. argentinus by foraging in the Patagonian Shelf region than that I. argentinus naturally occurs south of the Antarctic Polar Front.*

Key words: Trophic interactions, δ13C, *Thalassarche chrysostoma, Thalassarche melanophris, Diomeda exulans*, Patagonian Self, Bird Island.

INTRODUCTION

In the last half century some populations of ommastrephid squids have been responsible for invasion and increasing range into new areas (Rodhouse, 2008). Such can be particularly dramatic when species cross ecological barriers to colonise new habitats (Chown et al., 2012).

Range expansion and contraction in ommastrephid squid is associated with characteristically high variability in their population dynamics (Rodhouse, 2008) . These range shifts can be difficult to observe directly. It is therefore necessary to make use of additional sources of information, such as the diets of predators, and to test hypotheses arising from the use of such information.

Examples of range shifts in ommastrephid squid include the expansion of the normally tropical to sub-tropical jumbo flying squid *Dosidicus gigas* into Alaskan waters (Field *et al.*, 2007), the increased abundance of Japanese flying squid *Todarodes pacificus* in Japanese waters during warmer years (Rodhouse, 2008; Sakurai *et al.*, 2000) and the inter-annual variability in the distribution of the short-finned *Illex argentinus* along the Patagonian Shelf (Waluda *et al*, 2008).

*I. argentinus* is a Sub-Antarctic and temperate water squid (Arkhipkin, 2013; Roper *et al.*, 1984) which occurs predominantly in the southwest Atlantic, specifically on the Patagonian Shelf where it is commercially exploited by a major international fishery (Rodhouse, 2013; Rodhouse *et al.*, 1998; Sacau *et al*., 2005). However, *I. argentinus* has also been observed in the Antarctic Polar Frontal Zone (APFZ) (Rodhouse, 1991; Rodhouse *et al.*, 1998). This region is north of the Antarctic Polar Front (APF), which is a significant ecological barrier between the southwest Atlantic and the colder waters of the Southern Ocean (Collins & Rodhouse, 2006). Furthermore, *I. argentinus* beaks have been observed in the diets of grey-headed, *Thalassarche chrysostoma*; black-browed, *T. melanophris*; and wandering, *Diomedea exulans*, albatrosses during the breeding season at Bird Island, South Georgia (Rodhouse, 1991; Rodhouse *et al.*, 1987; Xavier *et al.*, 2006; Xavier *et al.*, 2002). Bird Island is located south of the APF and the available tracking data suggested that these albatross species caught *I. argentinus* while foraging in the Southern Ocean during their breeding season (Xavier *et al*., 2002; 2006). These observations led to the hypothesis that albatrosses catch *I. argentinus* in the Southern Ocean and that *I. argentinus* might be transported across the APF in gyres (e.g. subtropical gyres originating in the warmer waters of the Brazilian current) or core rings (Xavier et al., 2006).

Stable isotope analysis has been successfully used to study the spatial distribution of cephalopods in the Southern Ocean (Cherel & Hobson, 2005; 2007; Cherel *et al.*, 2011). We used this approach to evaluate the hypothesis that albatrosses at Bird Island catch *I. argentinus* in the Southern Ocean (i.e. that *I. argentinus* can occur in Southern Ocean waters). We analysed stable isotopes values of carbon (δ13C) in *I. argentinus* beaks obtained from the diets of the three albatross species and compared these values with those for the beaks of cephalopod species endemic to the Patagonian Shelf (*Octopus tehuelchus*) and the Southern Ocean (*Alluroteuthis antarcticus*). These reference beaks were also obtained from the diets of predators.

MA TER I A L S A ND MET HOD S

The lower beaks of *I. argentinus* were collected from the stomach contents of black-browed, grey-headed, and wanderingalbatrosses chicks at Bird Island, South Georgia (Xavier *et al.*, 2002; 2004). Immediately after a chick had been fed by a returning parent, the chick was inverted over a bucket and its stomach contents collected (Xavier *et al.,* 2003a). The beaks were collected from black-browed (n≈30) and grey-headed (n≈40) albatross chicks at the end of the chick-rearing period in 1999 and from wandering albatrosses chicks during the chick-rearing periods in 2007, 2008 and 2009 (Table 1). The beaks were kept in 70% ethanol until further analyses.

Beaks were also collected for *O. tehuelchus*,a reference cephalopod species for the Patagonian Shelf (Norman *et al.*, 2014; Storero *et al.*, 2012) and *A. antarcticus* a reference species for the Southern Ocean (Rodhouse *et al.*, 2014). *O. tehuelchus* beaks were collected from fresh scats of the South American sea lion (*Otaria flavescens*) from the rookery at Punta Bermeja, Rio Negro Province, Argentina (41°S 63°W) (Bustos *et al.*, 2014) in November of 2005. *A. antarcticus* beaks were collected from the stomach contents of adult wandering albatrosses (subjected to stomach lavage) at Bird Island in 2009 (Xavier *et al.*, 2003b) and from the stomach contents of Southern elephant seals (*Mirounga leonina*) (immobilized by injection of ketamine hydrochloride and subjected to stomach lavage following (Antonelis *et al.* 1987) at Stranger Point, Isla 25 de Mayo/King George Island, South Shetlands (62°S 58° W) during the moulting season of 1995/96 (Daneri *et al.*, 2000).

Cephalopods were identified from the morphology of their beaks following Xavier and Cherel (2009).

Whole lower beaks were cleaned, dried and milled to a fine powder. The ratio of stable isotopes of carbon was measured using a Continuous Flow Isotope Ratio Mass Spectrometer (CFIRMS).The results are presented in δ notation as deviations in the proportion of 13C from the standard reference in parts per thousand (‰), calculated using the equation:

δ13C =[(Rsample / Rstandard) -1]×1000

where Rsample is the ratio 13C/12C in the sample and Rstandard the ratio 13C/12C in the international reference standard, Vienna Pee-Dee Belemnite (0.0112372). Replicate measurements of internal laboratory standards (acetanilide) indicate measurement errors < 0.1 ‰. Data were statistically analysed using R (R Core Team, 2013).

RE SU L T S

The δ13C values for *I. argentinus* from albatrosses at Bird Island ranged from -18.77 to -15.28‰ (Table 1; Figure 1). These values were similar to those from *O. tehuelchus* (-18.76 to -16.50‰), our reference species from the Patagonian Shelf. In contrast, the mean values for our Southern Ocean reference species, *A. antarcticus*, were more negative with specimens from Antarctic Peninsula (*A. antarcticus* AP; -25.46 to -23.99‰) more negative than those from Bird Island (*A. antarcticus* BI; -22.95 to -18.16‰). Only a single δ13C value from *A. antarcticus* overlapped with some samples of *I. argentinus* and analysis of variance confirmed statistically significant differences in δ13C values between groups (F7, 69= 5.005; p < 0.001).

*I. argentinus* δ13C values were similar to those for *O. tehuelchus* with the exception of *I. argentinus* caught in 2007 (Tukey’s pairwise comparisons, p = 0.04). These 2007 values were the also significantly different from those for *I. argentinus* caught in 1999 from black-browed albatrosses (BBA) and 2008 (Tukey’s pairwise comparisons between: 2007 and 2008, p= 0.008; 2007 and 1999 BBA, p= 0.002;). *A. antarcticus* δ13C values were significantly different from all of those for the other two cephalopod species. *A. antarcticus* values were also significantly different between sampling locations (Tukey’s pairwise comparisons between *A. antarcticus* obtained

from the Antarctic peninsula predators(*A. antarcticus* AP values) and *A. antarcticus* obtained from Bird island predators (*A. antarcticus* BI) (p < 0.01).

Table 1. Stable isotope values of carbon (δ13C), of *Illex argentinus*, *Alluroteuthis antarcticus and Octopus tehuelchus* beaks sampled from Antarctic predators. *A. antarcticus* and *O. tehuelchus* as reference values for Antarctic waters and Patagonian Shelf, respectively (99 – 1999, 07 – 2007, 08 – 2008, 09 – 2009, sampling years; AP – Antarctic Peninsula; BI – Bird Island), number of lower beaks analyzed (n), predator where the beaks were found (BBA – Black-browed albatross; GHA – Grey-headed albatross; WA – Wandering albatross; SL – South American sea lion; ES – Elephant seal) (SE = standard error).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | δ13C | | |
|  |  |  | mean | ± | SE |
| Taxa | n | Predator | (range) | | |
| *Illex argentinus* 99 | 10 | BBA | -18.06 | ± | 0.13 |
| (-18.66 | - | -17.41) |
| *Illex argentinus* 99 | 10 | GHA | -17.52 | ± | 0.31 |
| (-18.67 | - | -15.89) |
| *Illex argentinus* 07 | 10 | WA | -16.45 | ± | 0.23 |
| (-17.30 | - | -15.28) |
| *Illex argentinus* 08 | 10 | WA | -17.89 | ± | 0.14 |
| (-18.77 | - | -17.29) |
| *Illex argentinus* 09 | 8 | WA | -17.31 | ± | 0.20 |
| (-18.18 | - | -16.36) |
| *Illex argentinus* (All) | 48 |  | -17.45 | ± | 0.12 |
| (-18.77 | - | -15.28) |
| *Octopus tehuelchus* | 10 | SL | -17.68 | ± | 0.28 |
| (-18.76 | - | -16.50) |
| *Alluroteuthis antarcticus* AP | 9 | ES | -24.52 | ± | 0.14 |
| (-25.46 | - | -23.99) |
| *Alluroteuthis antarcticus* BI | 20 | WA | -20.95 | ± | 0.50 |
| (-22.95 | - | -18.61) |



Figure 1. Mean (± SE, standard error) stable carbon isotope values of *Illex argentinus* beaks found in the diet of Antarctic predators (BBA - Black-browed albatrosses, and GHA - Grey-headed albatrosses, the other *I. argentinus* were caught by wandering albatrosses) in different years (99 – 1999, 07 – 2007, 08 – 2008, 09 – 2009), *Alluroteuthis antarcticus* (AP, Antarctic Peninsula and BI, Bird Island)and *Octopus tehuelchus* as reference values for Antarctic waters and Patagonian Shelf, respectively. (Figure also in .pdf in the other attach)

DISCUSSION

The hypothesis that albatrosses at Bird Island catch *I. argentinus* in the Southern Ocean (i.e. that *I. argentinus* can occur naturally in Southern Ocean waters) is not supported by our data. Our results show a clear distinction between the mean δ13C values for *I. argentinus* and those for the Southern Ocean reference species, *A. antarcticus*. Conversely, there was considerable overlap between the mean values for *I. argentinus* and those for the Patagonian Shelf reference species, *O. tehuelchus.* Therefore*,* although *I. argentinus* often occurs in the diets of Southern Ocean predators (Rodhouse *et al.*, 1987; Xavier *et al*., 2006; Xavier & Cherel, 2009), our stable isotope values from the beaks of *I. argentinus* collected from albatross stomach samples suggest that the albatrosses forage for *I. argentinus* on the Patagonian Shelf. This is consistent with the known foraging ecology of wandering albatrosses breeding at Bird Island, which regularly forage at the Patagonian Shelf and feed on *I. argentinus* (Xavier *et al.,* 2004)*.*

A previous study, combining satellite tracking and stomach sampling data, suggested that wandering albatrosses might have caught *I. argentinus* in Southern Ocean waters during some short trips around South Georgia (Xavier *et al.,* 2004). However, the tracked wandering albatrosses were not stomach washed prior to these foraging trips and it is possible that they already had *I. argentinus* their stomachs. It is also possible that the albatrosses could have consumed *I. argentinus* used as bait by fishing vessels that operate in the South Georgia region (Xavier *et al.,* 2006). Stable isotope analysis is a useful tool for assessing the origin of the *I. argentinus* beaks, confirming that they originate from warmer waters.

The presence of *I. argentinus* in the diets of grey-headed and black-browed albatrosses is more puzzling as tracking data suggest that, during their breeding period at Bird Island, they generally forage in Southern Ocean waters south of the Antarctic Polar Front (APF), except during the incubation period, when they may extend their foraging range to the Patagonian Shelf and slope (Phillips *et al.*, 2004). It is unlikely that grey-headed and black-browed albatrosses consumed bait from fishing vessels in the Southern Ocean because licensed long-line vessels do not operate in the South Georgia area during chick rearing (Phillips *et al*., 2010). It is therefore likely that grey-headed and black-browed albatrosses may also extend their foraging range to warmer waters during the late chick-rearing period in some breeding seasons. Further tracking studies, concentrating on this period would be useful to test this conclusion.

In conclusion, our study shows that *I. argentinus* found in the diets of albatrosses at Bird Island originated from warmer waters of the Patagonian Shelf. Therefore, our results suggest that it might be relatively common for albatrosses that breed at Bird Island to forage on the Patagonian Shelf where *I. argentinus* is most abundant. Consequently, this region is likely to be more important to albatrosses breeding at South Georgia than previously thought and this may have conservation implications. Numerous fisheries operate on the Patagonian Shelf, including longline fisheries known to be responsible for incidental mortality to albatrosses (Favero *et al*., 2003). A detailed evaluation of how dependent Southern Ocean seabird populations are on that region for food is therefore recommended. Although our analysis confirms the Patagonian Shelf origin of *I. argentinus* collected from albatrosses at Bird Island in multiple years, it does not exclude the possibility that some of these specimens might have been caught in colder waters (e.g. APFZ waters). Future studies analysing the stable isotope signature of the most recent deposition of keratin in the beaks (from the edges of the beaks) would be able to assess this.

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