

MS. JULIE C MCINNES (Orcid ID : 0000-0001-8902-5199)

DR. BRUCE E DEAGLE (Orcid ID : 0000-0001-7651-3687)

DR. MICHAËL GRAS (Orcid ID : 0000-0001-9572-8456)

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High occurrence of jellyfish predation by black-browed and Campbell albatross identified by DNA metabarcoding

Julie C McInnes^{1,2}, Rachael Alderman³, Mary-Anne Lea¹, Ben Raymond^{2,1}, Bruce E Deagle², Richard A Phillips⁴, Andrew Stanworth⁵, David R Thompson⁶, Paulo Catry⁷, Henri Weimerskirch⁸, Cristián G Suazo⁹, Michaël Gras¹⁰ and Simon N Jarman¹¹

¹ Institute for Marine and Antarctic Studies, University of Tasmania, Private Bag 129, Hobart, TAS 7001, Australia

² Australian Antarctic Division, 203 Channel Highway, Kingston, TAS 7050, Australia

³ Department of Primary Industries, Parks, Water and Environment, 134 Macquarie Street Hobart TAS, 7000, Australia

⁴ British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge CB3 0ET, United Kingdom

⁵ Falklands Conservation, 41 Ross Rd, Stanley FIQQ1ZZ, Falkland Islands

⁶ National Institute for Water and Atmospheric Research, 301 Evans Bay Parade, Hataitai, Wellington 6021, New Zealand

⁷ Marine and Environmental Sciences Centre (MARE), ISPA-Instituto Universitário, Rua Jardim do Tabaco 34, 1149-041, Lisbon, Portugal

⁸ Centre d'Etudes Biologiques de Chizé, UMR 7372 CNRS/ULR, 79360 Villiers-en-Bois, France

⁹ Department of Animal Ecology and Systematics, Justus Liebig University Giessen, Heinrich-Buff-Ring 38, 35392 Giessen, Germany

¹⁰ Directorate of Natural Resources- Fisheries of the Falkland Islands Government, Stanley Falkland Islands

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¹¹CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, 4485-661 Vairão, Portugal

⁵Corresponding author:

Julie McInnes, Institute for Marine and Antarctic Studies, University of Tasmania, Private Bag 129, Hobart, TAS 7001, Australia. +61 3 6226 6379. Email: julie.mcinnnes@utas.edu.au

RA: rachael.alderman@dpipwe.tas.gov.au

MAL: marianne.lea@utas.edu.au

BR: ben.raymond@aad.gov.au

BD: bruce.deagle@aad.gov.au

RAP: raphil@bas.ac.uk

AS: CO@conservation.org.fk

DT: david.thompson@niwa.co.nz

PC: paulo.catry@gmail.com

HW: henriw@cebc.cnrs.fr

CGS: biosuazo@gmail.com

MG: michael.gras@ymail.com

SJ: simon.jarman@gmail.com

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Abstract

Gelatinous zooplankton are a large component of the animal biomass in all marine environments, but are considered to be uncommon in the diet of most marine top predators. However, the diets of key predator groups like seabirds have conventionally been assessed from stomach content analyses, which cannot detect most gelatinous prey. As marine top predators are used to identify changes in the overall species composition of marine ecosystems, such biases in dietary assessment may impact our detection of important ecosystem regime shifts. We investigated albatross diet using DNA metabarcoding of scats to assess the prevalence of gelatinous zooplankton consumption by two albatross species, one of which is used as an indicator species for ecosystem monitoring. Black-browed and Campbell albatross scats were collected from eight breeding colonies covering the

circumpolar range of these birds over two consecutive breeding seasons. Fish was the main dietary item at most sites, however cnidarian DNA, primarily from scyphozoan jellyfish was present in 42% of samples overall and up to 80% of samples at some sites. Jellyfish was detected during all breeding stages and consumed by adults and chicks. Trawl fishery catches of jellyfish near the Falkland Islands indicate a similar frequency of jellyfish occurrence in albatross diets in years of high and low jellyfish availability, suggesting jellyfish consumption may be selective rather than opportunistic. Warmer oceans and overfishing of finfish are predicted to favour jellyfish population increases and we demonstrate here that dietary DNA metabarcoding enables measurements of the contribution of gelatinous zooplankton to the diet of marine predators.

Introduction

Gelatinous zooplankton (including scyphozoans, salps, ctenophores and hydrozoans) form a large biomass component of marine ecosystems and are thought to be increasing in abundance in some areas (Brodeur *et al.* 2002; Brotz *et al.* 2012; Richardson *et al.* 2009). Jellyfish have traditionally been regarded as an unlikely primary prey source because of their very low energy density, especially in comparison to common alternative prey groups like fish (Doyle *et al.* 2007). There is growing evidence that these gelatinous animals are consumed by many larger animals either through predation or scavenging (Cardona *et al.* 2012; Houghton *et al.* 2006; Milisenda *et al.* 2014; Sweetman *et al.* 2014). However, consumption by seabirds has only been observed intermittently, involving direct observations of predation (Arai 2005; Fraser 1939; McCanch & McCanch 1996; Suazo 2008; Weimerskirch *et al.* 1986) or analysis of stomach contents of birds caught or killed at sea, rather than at breeding colonies (Arai 2005; Harrison 1984; Schneider *et al.* 1986). Gelatinous organisms are difficult to identify in stomach contents samples using visual identification of prey remains because they lack robust diagnostic morphological features and are rapidly digested (Arai *et al.* 2003). Consequently, hard parts of animals such as cephalopod beaks, fish bones and crustacean carapaces are more likely to be represented in stomach content samples (Barrett *et al.* 2007). This issue is compounded by the retention of some prey parts in the stomach, for example squid beaks can be retained for up to 50 days in albatrosses (Furness *et al.* 1984).

In recent years, the ability to detect gelatinous prey consumption by seabirds has improved through the use of animal-borne cameras (Sutton *et al.* 2015; Thiebot *et al.* 2016) and DNA metabarcoding of scat samples (Jarman *et al.* 2013; McInnes *et al.* 2016a). DNA dietary metabarcoding can identify prey DNA in predator scats without biases from retention of hard-parts and can detect soft-bodied

prey (O'Rorke *et al.* 2012; Pompanon *et al.* 2012). Using these methods, scyphozoan jellyfish have been detected frequently in the diet of Adélie penguins (Jarman *et al.* 2013; McInnes *et al.* 2016a). However, the role of jellyfish as a prey item remains unclear for many seabird predators. It is not known, for example, whether jellyfish are taken opportunistically or as a targeted prey; or if they are more important as a prey item during certain times of the year. If consumption of gelatinous prey is opportunistic, it might be expected that their prevalence in the diet would follow prevalence in the foraging region. Higher jellyfish abundances would lead to more frequent encounters and therefore higher occurrence in the diet. To subsist largely on jellyfish requires predators to consume large volumes (Duron 1978), which may be possible when jellyfish occur in large groups or hotspots (Houghton *et al.* 2006).

The duration of seabird foraging trips is constrained during the breeding season by the need to return to the nest to provision chicks. These constraints can be met using a variety of foraging strategies, including parents minimising energy expenditure by selecting higher quality prey for provisioning compared to self-feeding (Ydenberg *et al.* 1994). Thus, it is possible that gelatinous prey might typically be consumed during adult self-feeding, rather than for provisioning chicks. Since the majority of seabird diet studies are conducted during chick rearing and represent the provisioning diet (Barrett *et al.* 2007; McInnes *et al.* 2016b), the prevalence of gelatinous prey would naturally be low in these studies.

Understanding the full spectrum of seabird diets is important not only to investigate the foraging ecology of the bird, but also to assess the potential impacts of threats such as climate change and fishing, and thus has implications for the way we undertake ecosystem monitoring. The hierarchical nature of food-webs means that the diets of top order predators such as seabirds are responsive and reflective of overall change in availability of lower trophic levels (Boyd & Murray 2001). Marine ecosystems are difficult to study due to their relative inaccessibility and therefore top predator diet is often used to identify changes in the overall species composition of an ecosystem, including the availability of different prey groups (Chiaradia *et al.* 2010; Croxall *et al.* 1999). However, if the dietary methods used to assess these changes cannot accurately identify all trophic connections then the interpretation of dietary results could be misleading.

Albatrosses are one of the most threatened seabird groups because they are incidentally killed (bycaught) by commercial fisheries and affected by environmental change (Phillips *et al.* 2016). The black-browed albatross (*Thalassarche melanophris*) is one of the most numerous albatross species

and breeds on 14 island groups, with a circumpolar distribution (ACAP 2010). Black-browed albatross diet has been well studied compared to that of other albatross species (McInnes *et al.* 2016b), and they are used as an indicator species in ecosystem monitoring (SC-CCAMLR 1997). The Campbell albatross (*Thalassarche impavida*) is closely related and is endemic to Campbell Island, New Zealand. There have been 12 papers reporting the complete diet from black-browed albatross stomach contents, which equates to 18 studies when stratified by year and site. The main prey groups identified from stomach contents are fish and cephalopods and gelatinous prey have only been recorded in 8% of published papers (n=1) and 16% of studies (n=3), all from the Falkland Islands. In these studies, jellyfish were detected infrequently (<20% of samples) and in low volumes (< 5.3% by mass; Thompson 1992). The single study on Campbell albatross diet also reported gelatinous organisms as a minor prey item (< 2.3% by prey mass; Cherel *et al.* 1999). Despite the rare occurrence in stomach contents predation of scyphozoan jellyfish has been observed visually in black-browed albatross (Suazo 2008; Weimerskirch *et al.* 1986), and stomach temperature loggers and stable isotopes used on species in the same genus indicate their consumption may be more common (Catry *et al.* 2004; Connan *et al.* 2014).

In this study, we examined the prevalence of gelatinous prey in the diet of black-browed and Campbell albatross. We also estimated the relative availability of jellyfish from net catches by fishery vessels near two of the sites where we sampled albatross scats. We hypothesise that gelatinous prey commonly occur in the diets of albatross but that consumption is likely to be opportunistic and reflect prey availability. We used DNA metabarcoding of albatross scat samples collected from eight colonies across their breeding range and spanning two breeding seasons to assess the prey groups consumed. We also assessed dietary differences between years, breeding sites and breeding stages.

Materials and Methods

Study sites and sample collection

A total of 1460 fresh black-browed albatross scat samples were collected from seven breeding colonies and Campbell albatross from one colony, over multiple seasons: in 2013/14 and 2014/15 at New Island and Steeple Jason Island (Falkland Islands), Macquarie Island (Australia), Campbell Island (New Zealand), and Bird Island (South Georgia); in 2013/14 and 2015/16 at Canyon des Sourcils Noirs (Kerguelen Island); in 2014/15 and 2015/16 at Albatross Islet (Chile); and in 2013/14 at Diego Ramírez (Chile; Figure 1). Most samples (n=1185) were collected during the chick-rearing period with 718 during early chick-rearing (early December to end of January) and 467 during late chick-rearing (February and March), an additional 275 samples were collected during incubation (October to early

December, Appendix S1). Samples from chicks and adults were identified where possible. Due to the availability of birds at the colony, samples were predominantly collected from adults during incubation and early chick-rearing and chicks during late chick rearing. As such, samples sizes were too low during this study to directly compare dietary differences between chicks and adults; however, dietary comparisons between breeding stages were examined for sites where samples were collected during multiple breeding stages.

DNA metabarcoding

DNA was extracted using a Promega 'Maxwell 16' instrument and a Maxwell® 16 Tissue DNA Purification Kit. Samples were vortexed prior to extraction and ~ 30mg of each sample was used. PCR inhibitor concentrations were reduced in the DNA by mixing this sub-sample in 250uL of STAR buffer (Roche Diagnostics) prior to extraction. Samples were PCR amplified with a universal metazoan primer set that is highly conserved and amplifies a region of the nuclear small subunit ribosomal DNA gene (18S rDNA; McInnes *et al.* 2016c). Sequencing of PCR products was performed over two runs with a MiSeq genome sequencer, using the MiSeq V2 reagent kits (300 cycles). DNA extractions, PCR amplification and sequencing followed the methods used in McInnes *et al.* (2016c). A two-stage PCR process was used to enable amplification of the DNA region and attachment of unique 'tag' sequences to each sample which allows amplified samples to be pooled (Binladen *et al.* 2007). Stage one PCR reactions (10 µL) were performed with 5 µL 2x Phusion HF (NEB), 1 µL 100x Bovine Serum Albumin (NEB), 0.1 µL 5uM of each 18S_SSU amplification primer (Table 1), 0.5 µL of Evagreen, 2 µL faecal DNA and 1.3 µL of water. Thermal cycling conditions were 98°C, for 2 mins; followed by 35 cycles of 98°C for 5s, 67°C for 20s, 72°C for 20s, with an extension of 72°C for 1 min. Each sample was run in triplicate on a LightCycler 480 (Roche Diagnostics). A negative control containing no template DNA and positive control containing fish DNA were included in each PCR amplification run. If either the negative amplified or the positive failed to amplify, the PCR was re-run. If ≥2 replicates of each sample had a 'crossing threshold' (ct) score < 30 they were combined to reduce biases produced by amplification from low template concentration samples (Murray, Coghlan & Bunce 2015). Pooled samples were diluted 1:10 for the second stage PCR. A unique tag was attached to each sample in 10 µL PCR reactions with 5 µL 2x Phusion HF (NEB), 1 µL 100x Bovine Serum Albumin (NEB), 1 µL of 1 µM of each tag primer, and 2 µL of diluted PCR product from stage one. Thermal cycling conditions were 98°C, for 2 mins; followed by 10 cycles of 98°C for 5s, 55°C for 20s, 72°C for 20s, with an extension of 72°C for 1 min. Four microlitres of PCR product from each sample and the negative controls were pooled and purified from unincorporated reaction components by washing utilising reversible binding to Ampure (Agencourt) magnetic beads, with 1.8 µL of Ampure per

microlitre of DNA product. Sequencing of PCR products was performed with a MiSeq genome sequencer, using the MiSeq reagent kit V2 (300 cycles) with paired-end reads. Samples were split over two sequencing runs. A blocking primer was not used in this study as they may inadvertently block similar groups such as other vertebrates like fish (Piñol *et al.* 2015). This likely reduced the samples size, but provided more reliable results from higher quality samples containing more food DNA. A breakdown of the proportion of DNA sequences originating from non-food groups for each site can be found in Appendix S1.

Bioinformatics

Amplicon pools were de-multiplexed based on unique 10 bp Multiplex IDentifiers (MIDs) incorporated in the Illumina two-step MID protocol using our custom R script (Appendix S2). Fastq files were processed using USEARCH v8.0.1623 (Edgar 2010). Reads R1 and R2 from the paired end sequencing were merged using the `fastq_mergepairs` function, retaining only merged reads flanked by exact matches to the 18S_SSU primers and primer sequences were trimmed. Reads from all samples were pooled and dereplicated using full length matching (`-derep_fulllength`), then clustered into Operational Taxonomic Units (OTUs) using the `cluster_otus` command (`-otu_radius_pct = 10`). Potentially chimeric reads are discarded during this step. Reads for each sample were assigned to these OTUs (`usearch_global -id 0.97`) and a summary table generated using a custom R script that is included in the supplementary material (Appendix S2). Each OTU was identified by BLAST and categorised to closest match using MEGAN 5 and the Lowest Common Ancestor (LCA) assignment algorithm (Huson *et al.* 2007). LCA parameters were set at a minimum score of 250 and a top-percent of 5%. These cut-offs were determined by manually checking a sub-set of samples against BLAST. OTUs from the 18S primers were categorised into food or non-food items based on their taxonomy, so that, for example, obligate parasites and groups highly unlikely to be food such as land plants were 'non-food' (Jarman *et al.* 2013; McInnes *et al.* 2016c).

Analysis

Samples were included in the final analysis if they contained at least 100 sequences that could be assigned to a food group (Jarman *et al.* 2013). The diet data were presented using two dietary metrics to reduce any biases caused by reporting one alone. The frequency of occurrence (FOO) was calculated as the total number of samples at each site-year combination containing a given food group. FOO calculations were based on food items which comprised >1% of food sequences for that sample. The second metric used was the proportion of sequences in a sample, or relative read abundance (RRA). This was calculated as the total sequence reads for each prey group divided by the

total food sequences in that sample. The mean RRA was calculated for each site-year combination. Both metrics have inherent biases. FOO can overestimate the importance of common prey groups eaten only in small amounts, including secondary ingestion (the food consumed by the prey species). The RRA may not accurately reflect the exact proportion of each prey group consumed, however, has been shown to be representative of the relative diet proportion of prey items in feeding trails (Deagle *et al.* 2010; Willerslev *et al.* 2014) and using stable isotope analysis (Kartzinel *et al.* 2015). The RRA provides a viable option for dietary studies, particularly as a way to distinguish between primary and secondary prey items. To achieve the latter, samples were categorised according to the prey group represented by >70% of the sequences. This enabled assessment of the relative contribution of each prey group. If no major group dominated, the sample was classified as “mixed”. As samples were collected from both chicks and adults, the mixed category could represent an adult feeding on multiple prey groups or a chick fed from different parents.

Statistical analyses were carried out using R software (R Core Team 2015). Poisson generalised linear models (GLM) with a log link function were used to test for differences in prey groups between breeding colonies and years, and between years and breeding stages at each colony. The model included the count of samples (n) as the dependant variable and predictor variables included prey group (P), year (Y) and breeding stage (S), or colony (C). The base model included the sample size as a function of the main effects (prey group, year, breeding stage or colony) as well as the year:stage or year:colony interaction. These terms effectively describe the patterns in the data arising from the experimental sampling process (e.g. total number of samples within a given year). The interaction terms, prey:year, prey:stage or prey:colony were added to the base model to test the effect of year or stage (or colony for the pooled data) on diet composition. The analysis of deviance (with Chi-squared test) and Akaike’s information criterion (AIC) were used to compare fitted models and test the significance of predictor terms (Burnham & Anderson 2002). Dissimilarity indices were calculated with the Manhattan method using the command ‘vegdist’ in the package ‘Vegan’ (Oksanen *et al.* 2016). From these indices, a hierarchical clustering was then constructed using the average agglomeration method. The command ‘simprof’ from the package ‘clustsig’ was used on FOO and RRA data to assess if any significantly different site clusters were present, with a significance of $p < 0.05$ (Whitaker & Christman 2014).

Fishery catch data

It is difficult to determine the availability of prey within the marine environment due to its relative inaccessibility. However, an approximation of jellyfish abundance can be assessed from trawl fishery

catch data. Trawl fisheries operate in waters adjacent to the Falkland Islands year-round, where jellyfish are caught as bycatch during fishing operations. Weights of the jellyfish portion of catch are reported daily by captains to the Directorate of Natural Resources – Fisheries (DNRF) of the Falkland Islands Government. Monthly and annual jellyfish catch data were obtained for trawl fishing vessels operating in the Falkland Islands Interim and Outer Conservation Zones (FICZ/FOCZ) between 2011-2016. Data were provided by the Directorate of Natural Resources of the Falkland Islands Government. The total fishing effort (measured in fishing days) and the amount of jellyfish caught per fishing day each month (total tonnes jellyfish/ fishing day) were calculated. There are up to 44 vessels operating in the fishing ground during a given month. Fishing activity is typically low in January and there were no trawl operations in January 2014

Results

Amplification success

A total of 1460 scat samples were collected across all islands and years. DNA was amplified in 1039 samples, and 449 samples provided >100 food sequences. The prevalence of non-food DNA (i.e. from the bird, parasites, etc.) in many samples is typical when using “universal” eukaryote PCR primers (McInnes *et al.* 2016c). Only two samples from Albatross Islet in 2016 contained food DNA, therefore that year of data was not included, resulting in 447 samples used in subsequent analyses (see Appendix S1). Of these samples, 61 were from incubation, 240 from early chick rearing and 146 from late chick-rearing.

Overall diet composition

Actinopterygii (bony fish) were found to be the most abundant prey group overall, present in 86% of samples (FOO) and comprising 66% of food DNA sequences (RRA). Scyphozoa (true jellyfish) were present in 37% of samples and comprising 20% of food DNA sequences. Other prey items included Crustacea 30% FOO (8% RRA), Cephalopoda 10% FOO (3% RRA), Hydrozoa 6% FOO (2% RRA), Chondrichthyes (skates, sharks, rays) 5% FOO (2% RRA); and Anthozoa, Ctenophora and Tunicata with 2%, 1% and 3% FOO respectively (< 1% RRA; Table 1, Figure 2+3).

There was a significant difference in the frequency of occurrence of prey groups detected between years (base model AIC 636.6, P:Y AIC =615.6; $\chi^2_{16} = 53.03$, $p < 0.001$) and breeding colonies (P:C AIC =490.6; $\chi^2_{56} = 258$, $p < 0.001$), however, the inclusion of colony alone provided the best model fit.

There was no significant improvement to the model when year and colony were both included (P:Y and P:C AIC =503; $\chi^2_{118} = 258$, $p < 0.276$), which suggests that any year differences were likely to be

an artefact of different colonies sampled. Although there was some variation in the prey detected between breeding stages (Appendix S3), there was no significant effect of breeding stage or year on the frequency of prey groups detected when each colony was tested individually. At each site the base model provided the best fit of the data (see Appendix S4).

When each sample was classified according to the dominant prey group (> 70% of sequences), samples fell into the six main prey groups listed above. Anthozoa, Ctenophora and Tunicata were present in samples in low proportions, and always co-occurred with other prey items, suggesting they may have represented secondary ingestion. A small percentage of samples (10%) were classified as 'mixed' (Figure 3). Using FOO data, there was no significant site clusters identified, whereas using RRA, sites were clustered into two significantly different groups ($p < 0.05$; Figure 4). Cluster 1 included Campbell, Steeple Jason and Macquarie islands, and cluster 2 included the remainder. The main differences were the ratio of Actinopterygii to Scyphozoa. In both clusters these two prey groups together contributed 85% of the sequences; however, in group 1 the ratio of Actinopterygii to Scyphozoa was 1.3 : 1 compared to 13.7 : 1 for group 2 (Figure 4).

Cnidarians

Cnidarian DNA occurred in a large proportion of samples and comprised a high proportion of sequence reads at several black-browed albatross sites and at Campbell Island (Fig 2, Table 1). Scyphozoan jellyfish from the orders Coronatae (crown jellyfish) and Semaestomeae were the main gelatinous prey DNA detected. The occurrence of these two orders differed between sites; Semaestomeae were detected at all sites, although only in large proportions at Steeple Jason Island (53 – 78% FOO, 30 – 50% RRA) and Campbell Island (23 – 44% FOO, 13 – 21% RRA), whereas Coronatae was detected mostly at Macquarie Island (50 – 64% FOO; 35 – 41% RRA; Table 1). Hydrozoans from the order Siphonophorae were found in albatross scat samples from Campbell Island in 2014 (31.1% FOO, 17.5% RRA). Anthozoa from the order Actiniaria (sea anemone) occurred in relatively low proportions, the highest in samples from Campbell Island in 2015 (7.7% FOO and 6.9% RRA); all co-occurred with Semaestomeae.

Crustaceans

Crustacean DNA occurred in greater than 10% of samples at each site, however constituted less than 5% of prey sequences at most sites. The exceptions to the latter were New Island in both years (60 – 68% FOO, 20 – 24% RRA), Campbell Island in 2015 (35 – 46% FOO, 6 – 23% RRA), Bird Island in 2014 (12% FOO, 7% RRA) and Diego Ramírez in 2014 (21% FOO, 12% RRA). Crustaceans in the diet at New

Island were mostly from the family Munidae (lobster krill), at Campbell Island from the sub-order Lepadomorpha (goose barnacles), at Bird Island from the order Euphausiacea (krill) and at Diego Ramírez from the order Podoplea, although the last all co-occurred with fish and so may represent secondary ingestion.

Cephalopoda

Cephalopoda DNA occurred in greater than 5% of samples at all sites, and greater than 10% of samples at Macquarie Island, Steeple Jason Island, Bird Island (2015), Diego Ramirez and Campbell Island (2014). However, at only three sites was the RRA of cephalopod sequences > 5%. These were Bird Island in 2015 (14% FOO, 8% RRA), Steeple Jason Island in 2015 (15% FOO, 8% RRA) and Diego Ramírez in 2014 (11% FOO, 5% RRA) and in each case, were almost all from the order Teuthida (squids).

Jellyfish abundance at the Falkland Islands

Between 2011 and 2016, there have been variable amounts of jellyfish caught in the trawl fishery at the Falkland Islands, with large jellyfish blooms evident in 2014 and 2016 (Figure 5). There is also a seasonal pattern of abundance evident with higher jellyfish catches per fishing day from February – April, which overlaps temporally with the albatross chick rearing period. There was no fishing activity in January 2014, therefore no jellyfish catch data. The two seasons that albatross diet sampling occurred corresponded with a year of high jellyfish catch reported in 2014 (~ 3800 tonnes) and a year of low catch reported in 2015 (~330 tonnes; Figure 5), with a ten-fold difference in reported catch between years. Overall, there was no statistical difference in albatross diets between years at each site, or between breeding stages (Appendix S4). However, at Steeple Jason Island a higher proportion of samples contained jellyfish DNA during late chick-rearing (80-100% FOO) compared to incubation (40% FOO) and early chick-rearing (20-56% FOO; Figure 5, Appendix S3). Even though there was large differences in the fishery catch in March of each year, this was not reflected in the diet (100% FOO, 60% RRA in March 2014 and 88% FOO and 53% RRA in March 2015). The breeding success at New Island and Steeple Jason were similarly high in both years of the study, irrespective of higher prevalence of jellyfish in the diet at Steeple Jason (Breeding success: New Island 84.3% and 80.8% and Steeple Jason 60.1 and 81.8% in 2014 and 2015 respectively).

Discussion

This is the first study of albatross diets covering the same or sibling species at such a wide geographic scale and employing synchronous sampling at multiple sites. Our results confirm the

hypothesis that gelatinous prey, specifically scyphozoan jellyfish (hereafter termed jellyfish), are a common prey of black-browed and Campbell albatross. We also show that the frequency of jellyfish occurrence in the diet was similar in years of high and low relative jellyfish abundance, suggesting that consumption is not purely opportunistic.

Our hypothesis that gelatinous animals are a common prey item in albatross diet was motivated by the apparent discrepancy between at-sea observations of albatross foraging on jellyfish, yet low detection rates in stomach contents (11% of black browed studies and < 5% of meal mass when present). Additionally, previous DNA metabarcoding of penguin scats has identified frequent occurrence of jellyfish in the diets which has not been detected often using stomach content analyses (Jarman *et al.* 2013; McInnes *et al.* 2016a). We found an even higher frequency of occurrence of gelatinous prey in albatross diets than the penguin studies and much higher than studies on albatross using conventional methods. Jellyfish were present at seven of the eight sampled breeding sites and were a common prey item at three of these sites with up to 80% of samples from Steeple Jason Island containing jellyfish and comprising 50% of DNA sequences. hydrozoans, anthozoans and ctenophores were also detected during this study, though with the latter two in low proportions.

High rates of jellyfish ingestion have not been detected in previous albatross studies (Cherel & Klages 1998), which is likely explained by the limitations of stomach content analyses. Predation on jellyfish by black-browed albatross has been observed previously at sea (Suazo 2008; Weimerskirch *et al.* 1986) and at Beauchêne Island in the Falkland Islands where jellyfish was found in 20% of samples (< 10% mass; Thompson 1992). Our suggestion that they may be consumed during self-feeding rather than provisioning was not supported, with jellyfish detected in the diets of chicks during late chick-rearing and adults during incubation and early-chick rearing. This finding provides further evidence that low detection rates reported in previous studies were not purely the result of sampling timing.

The frequency of jellyfish occurrence varied extensively between colonies. Almost no jellyfish were found in the diet at the Chilean sites or at Kerguelen in 2014. Although at Diego Ramirez jellyfish occurred in 30% of samples, the RRA was only 1% and no samples contained jellyfish as the main prey item. When sites were clustered into groups by diet, the main difference was the RRA of fish and jellyfish sequences. This division had no relationship with site proximity suggesting this is not just a localised occurrence. Albatross at Steeple Jason and New Island had very different diets in

both years and clustered into separate groups, even though these sites are only 70km apart. This is consistent with previous dietary work at the Falkland Islands that found large differences between colonies (Thompson 1992), suggesting birds from the two colonies use distinct foraging grounds, which has been confirmed by subsequent tracking studies (Catry *et al.* 2013). Across all sites, spatial differences in diet were greater than temporal differences, suggesting that the site-by-site differences relate to site-specific factors such as local prey abundance or learned foraging preferences. However, jellyfish availability estimates from the Falkland Islands indicate that the consumption of jellyfish is not based purely on availability of prey. During this study, the frequency of jellyfish occurrence in the scats of black-browed albatross was similar in years of high and low availability, which suggests that they may actively be targeting jellyfish.

Seabirds have been found to target jellyfish aggregations to forage on juvenile fish that associate with jellyfish for food or protection (Sato *et al.* 2015), and therefore consumption of jellyfish could be accidental or secondary in such cases where jellyfish are predated by fish (Milisenda *et al.* 2014). DNA metabarcoding can detect DNA from secondary ingestion (Jarman *et al.* 2013). In our study, the detection of anthozoans, ctenophores and tunicates were likely to be through secondary ingestion as they occurred only in low abundance and always co-occurred with other prey items. However, this was unlikely to be the case for hydrozoans and Scyphozoan jellyfish. The FOO and RRA from both prey groups were high for sites where they were detected regularly, whereas we would expect the RRA to be much lower if predation was secondary. When these hydrozoans and scyphozoans were consumed by an individual, they were often the dominant item (> 70% RRA). This was further confirmed by some samples where they were the only food DNA present in the sample.

During this study, the breeding success was similarly high at Steeple Jason and New Island in 2015 even though jellyfish occurred more frequently in the diet at Steeple Jason Island. The breeding success was slightly lower at Steeple Jason in 2014, however, was still higher than the long-term average at the Falkland Islands (New Island 56% from 2004-2009), and higher than conspecifics at other island groups (ACAP 2010; Catry *et al.* 2011). This suggests that the consumption of jellyfish by albatross may not be impacting breeding success at the population level. However, the consequences of choosing this prey at the individual level and the effect on chick fledging mass is unknown. An increase in easily accessible but energetically poor food may be a good short-term solution when higher energy prey is scarce, but over the long-term the impacts of low nutritional prey in albatross diets are unknown. For other marine predators low nutritional prey has reduced

body condition, breeding success, and ultimately survival (Grémillet *et al.* 2008; Kitaysky *et al.* 2006; Rosen & Trites 2000).

A challenge of seabird dietary studies is the inability to accurately quantify the available biomass of potential prey species. The majority of marine ecosystem monitoring studies measure from the top-down rather than bottom-up, which makes it difficult to determine the reasons for prey selection. This is especially the case in the Southern Ocean, which is one of the most inaccessible places on earth. The Falkland Islands in the South Atlantic provided a unique opportunity to gain an insight into the relative occurrence of jellyfish across multiple years through catch data. These catch amounts do not provide a definitive biomass of jellyfish, but instead give an indication of relative jellyfish prevalence in the sea across years. There are several factors that should be considered when interpreting this data. Jellyfish are caught as bycatch rather than targeted by the fishery and are likely to be actively avoided where possible, including making shorter trawls to avoid damage to fishing gear (FIG 2015). The jellyfish catch data in this study is also over a broad scale around the Falkland Islands rather than specifically relating to the albatross foraging area, therefore does not allow interpretation of fine-scale changes in jellyfish abundance. However, the ten-fold increase in the jellyfish catch in 2014 from 2013 and then back to similarly low levels in 2015, is large enough to give an indication of major differences in the jellyfish prevalence between years. More in-depth studies using finer-scale jellyfish biomass estimates around both New Island and Steeple Jason colonies would provide a more robust estimate of jellyfish abundance and albatross prey choices. This could also be studied across multiple breeding stages. Although there was no statistical difference between breeding stages at Steeple Jason, there was a trend for a higher occurrence of jellyfish in the diet during late chick-rearing (80-100%) compared to incubation (40%) and early chick-rearing (20-56%) and a similar trend at Macquarie Island and Bird Island (Appendix S3). This pattern is consistent with a switch to low-energy prey late in the breeding season when high nutrient food near colonies can be depleted (Ashmole 1963). Black-browed albatross are known to consistently return to the same foraging sites (Weimerskirch *et al.* 1997), therefore the ability to switch prey would be advantageous as it allows for more flexibility, especially when resources are scarce.

Ongoing monitoring of diet and foraging ecology of top predators will help characterise the impacts of environmental change and fisheries on breeding populations (Constable 2001; Croxall *et al.* 1999; Furness 1982). Climate change is predicted to cause major changes in the abundance and distribution of marine species (Constable *et al.* 2014). Jellyfish typically benefit from perturbations to

the marine environment (Purcell 2012), such as ocean warming (Purcell 2005; Quiñones *et al.* 2015) overfishing (Daskalov *et al.* 2007), and the increasing number of coastal anthropogenic structures which promote the settlement of early larval stages (Duarte *et al.* 2013). Population increases are therefore predicted under current climate change scenarios and global trends show a slight increase over the long-term, but show significant oscillations in blooms over shorter time scales (Condon *et al.* 2013). At the Falkland Islands, there have been two obvious jellyfish blooms in the last six years, however, long-term data are not available. Continued collection of jellyfish catch data would be valuable to understand if these blooms are increasing in prevalence and what impacts this may have on seabird diet.

Cephalopods were only a minor prey item for both species in this study with DNA present in 10% of samples and comprising 2.8% of sequences overall. Although there was up to 50% FOO at Macquarie Island (5% RRA) and 27% FOO (20% RRA) at Steeple Jason Island during early-chick rearing 2015, the overall contribution in this study was much lower than previous stomach content studies. Low squid occurrence has also been inferred from stable isotope analysis of black-browed albatross (Granadeiro *et al.* 2013), however, cephalopods are typically detected in high proportions in stomach contents. Previous black-browed albatross diet studies report that cephalopods occur in 50% of samples on average and 27% of the diet by mass (see Appendix S5), although this varied among sites (Cherel *et al.* 2000; Thompson 1992) and between years (Arata & Xavier 2003; Xavier *et al.* 2003). Although a large number of samples were collected earlier in our study (during incubation and early chick-rearing) than previous studies, cephalopod occurrence was still low in our study during late chick-rearing. Cephalopods do undertake vertical and horizontal migrations during their lifecycles (Arkhipkin *et al.* 2004), so temporal changes in their availability should be expected. Indeed there have been years where less than 10% of samples from these sites contained cephalopods (Arata & Xavier 2003; Cherel *et al.* 2000). Nevertheless, consistently low cephalopod abundance using such a large-scale sampling scheme is unusual, especially as globally, cephalopod populations are increasing (Doubleday *et al.* 2016).

The low rate of cephalopod DNA in the scats that we observed is unlikely to be the result of a major technical bias in our DNA metabarcoding system. Target sequences from all prey groups were aligned and checked for primer mismatches or any insertions/deletions that may have affected DNA amplification, none were detected. There is also no evidence from other DNA metabarcoding studies that cephalopod DNA degrades more during digestion than fish DNA, with equal detection of DNA from both prey groups in scats during feeding trials even when squid was a small proportion of the

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diet (Casper *et al.* 2007; Deagle *et al.* 2005). We tested the marker used in our study on both pure squid material and faecal DNA extracts from other albatross and penguin species to ensure that the PCR could detect cephalopod DNA. These tests revealed up to 50% occurrence of cephalopod DNA in scat samples of two other seabird species (unpublished data). Furthermore, to reduce the impact of technical biases, we analysed both the RRA and FOO across samples. The latter analyses will detect cephalopod ingestion even if there is a bias against amplification of their DNA relative to other prey groups. The overall conclusions of these analyses are similar. More broadly, the reason for these differences between our study using DNA and those using stomach contents is inconclusive. We cannot determine if this simply reflects technical biases introduced by different methods of diet determination or whether cephalopod predation rates were generally lower than previously reported. It would be good to test this observation in future studies with more samples collected in different years, simultaneous stomach content collections or an alternate DNA metabarcoding system which includes group specific markers.

DNA metabarcoding provides a useful new way to study the diet of seabirds. Our study demonstrates that it could enhance long-term ecological monitoring studies to enable all prey groups to be detected. This is particularly important where seabirds used as indicator species (Cairns 1987). For example, in the Southern Ocean an international program uses diet analyses of stomach contents from marine predators as biological indicators of ecosystem health (CEMP; SC-CCAMLR 1997). Two of the key predators studied are black-browed albatross and Adélie penguins (*Pygoscelis adeliae*), both of which have been now been shown using metabarcoding to consume substantial amounts of jellyfish. If there are shifts away from krill towards more gelatinous species in the Southern Ocean (Atkinson *et al.* 2004), the consequent impacts on predator diets are likely to be difficult to detect using current methods. If the biomass of jellyfish increases and/or their distribution shifts, it will be important to identify changes to the food-webs and monitor the short and long-term effects that an abundance of a low nutritional food may have on the body condition, breeding success and survival prospects of marine predators.

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Data accessibility statement:

Data deposited in the Australian Antarctic Data Centre (doi:10.4225/15/5955d989a1940).

Author contribution:

JM, SJ, RA and MAL conceived and designed the project; RA, RP, AS, DT, PC, HW, CS contributed samples, JM performed laboratory work; JM and BD performed bioinformatics; MG provided fishery catch data; JM and BR carried out statistical analysis and prepared figures; JM wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

Supporting Information

Appendix S1 – Summary of sample sizes, non-food DNA groups and amplification success.

Appendix S2 – Custom bioinformatics script used to analyse sequences data in R

Appendix S3 – Frequency of occurrence of prey groups across breeding stages

Appendix S4 – Generalised linear model results for dietary comparisons between colonies, years and breeding stages

Appendix S5 – Black-browed and Campbell albatross published hard-part dietary results.

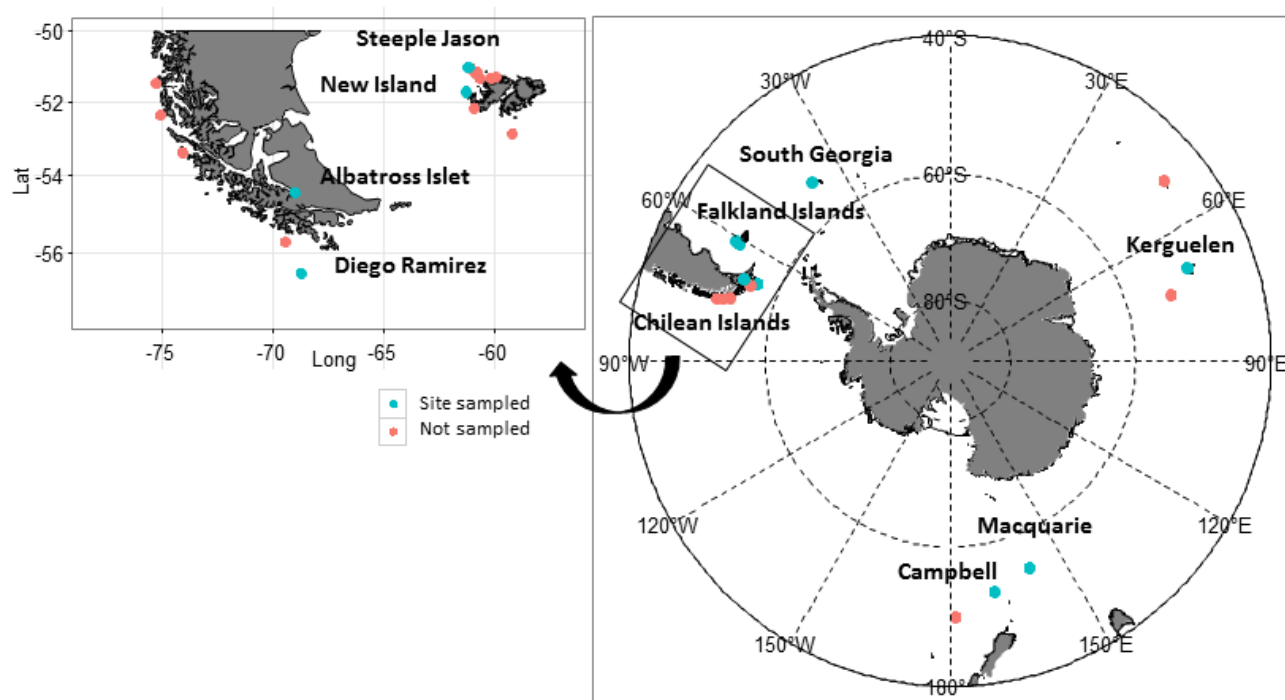


Figure 1: Breeding distribution of Black-browed and Campbell albatrosses. Blue dots represent the eight colonies where scat samples were collected, and the red dots the remaining colonies not sampled during the study. The inset shows the individual Chilean and Falkland Island colonies.

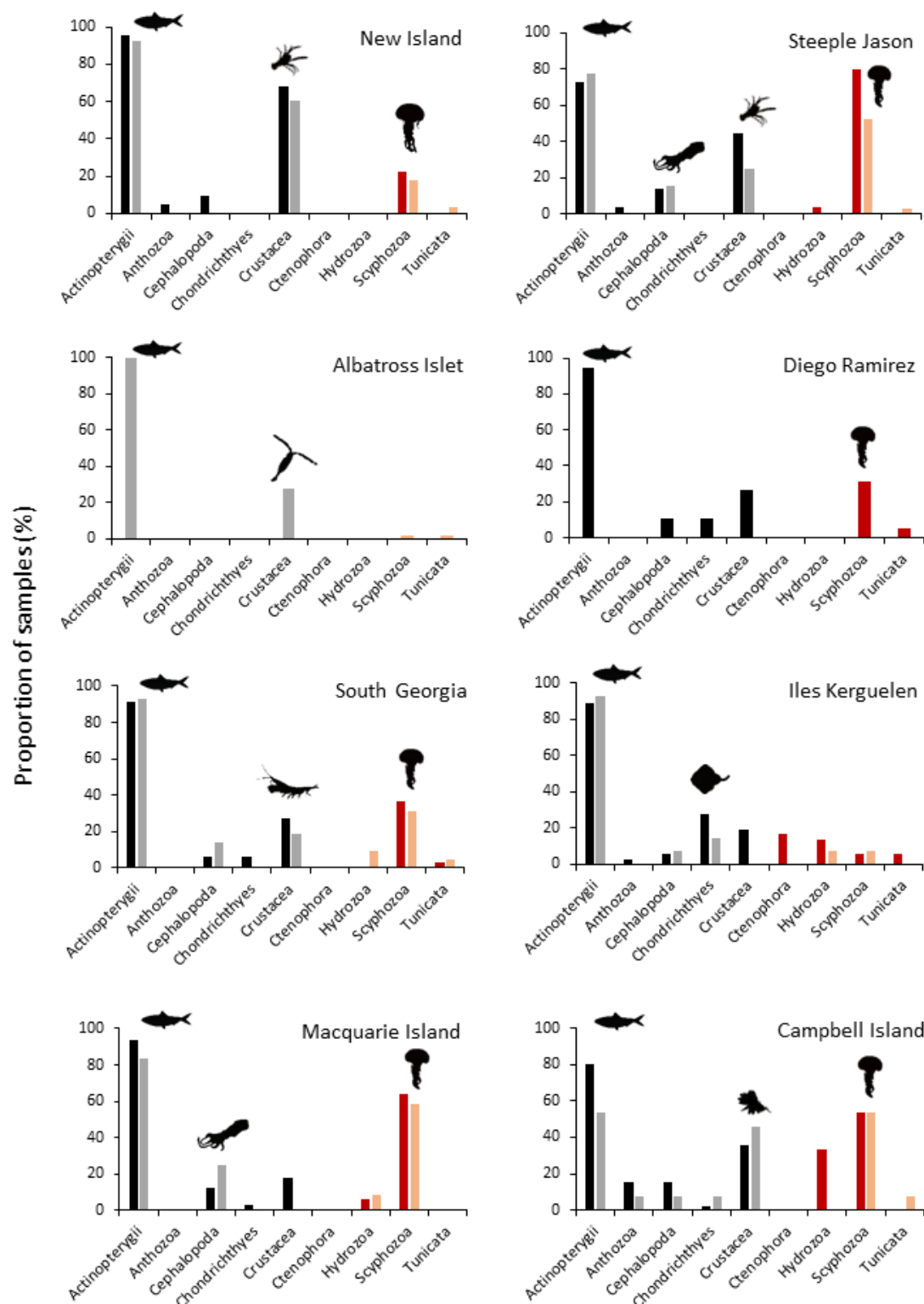


Figure 2: The frequency of occurrence of prey groups in the diet of black-browed and Campbell albatrosses from austral summer 2013/14-2015/16. Dark bars represent 2013/14 collections and the lighter bar 2014/15 (or in the case of Iles Kerguelen, 2015/16). The red and orange bars highlight the gelatinous prey items detected.

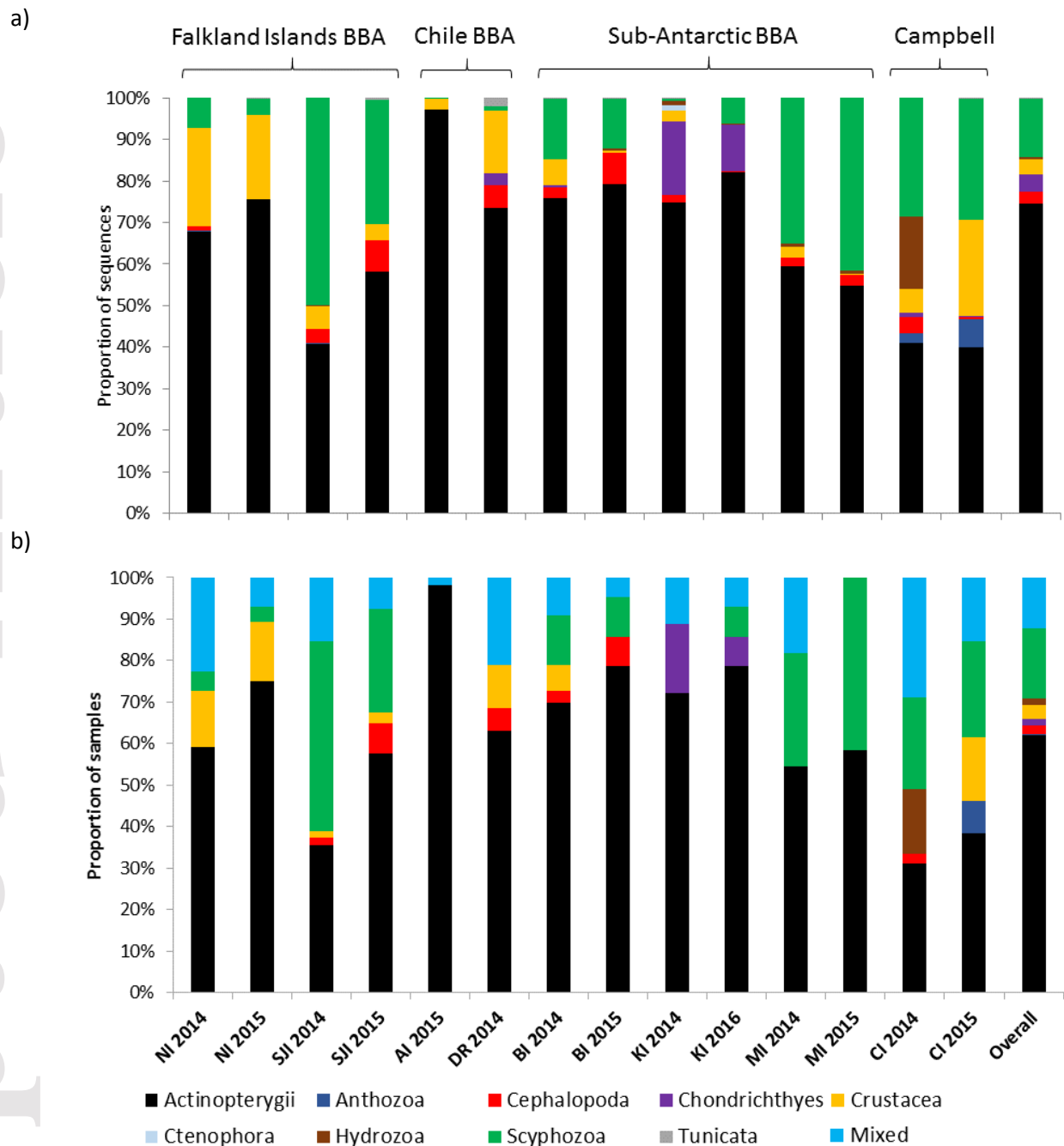


Figure 3: The relative read abundance and major prey groups consumed by black-browed and Campbell Island albatrosses from austral summer 2013/14-2015/16. Values represent (a) relative read abundance for each site and year and (b) the proportion of samples with >70% of sequences assigned to each prey group. Mixed samples have <70% of the sequences from any one group. Sites were: New Island (NI) and Steeple Jason Island (SJI), Falkland Islands; Diego Ramírez (DR) and Albatross Islet (AI), Chile; Bird Island, South Georgia (BI); Kerguelen Archipelago (KI), France; Macquarie Island, Australia (MI); and Campbell Island, NZ (CI).

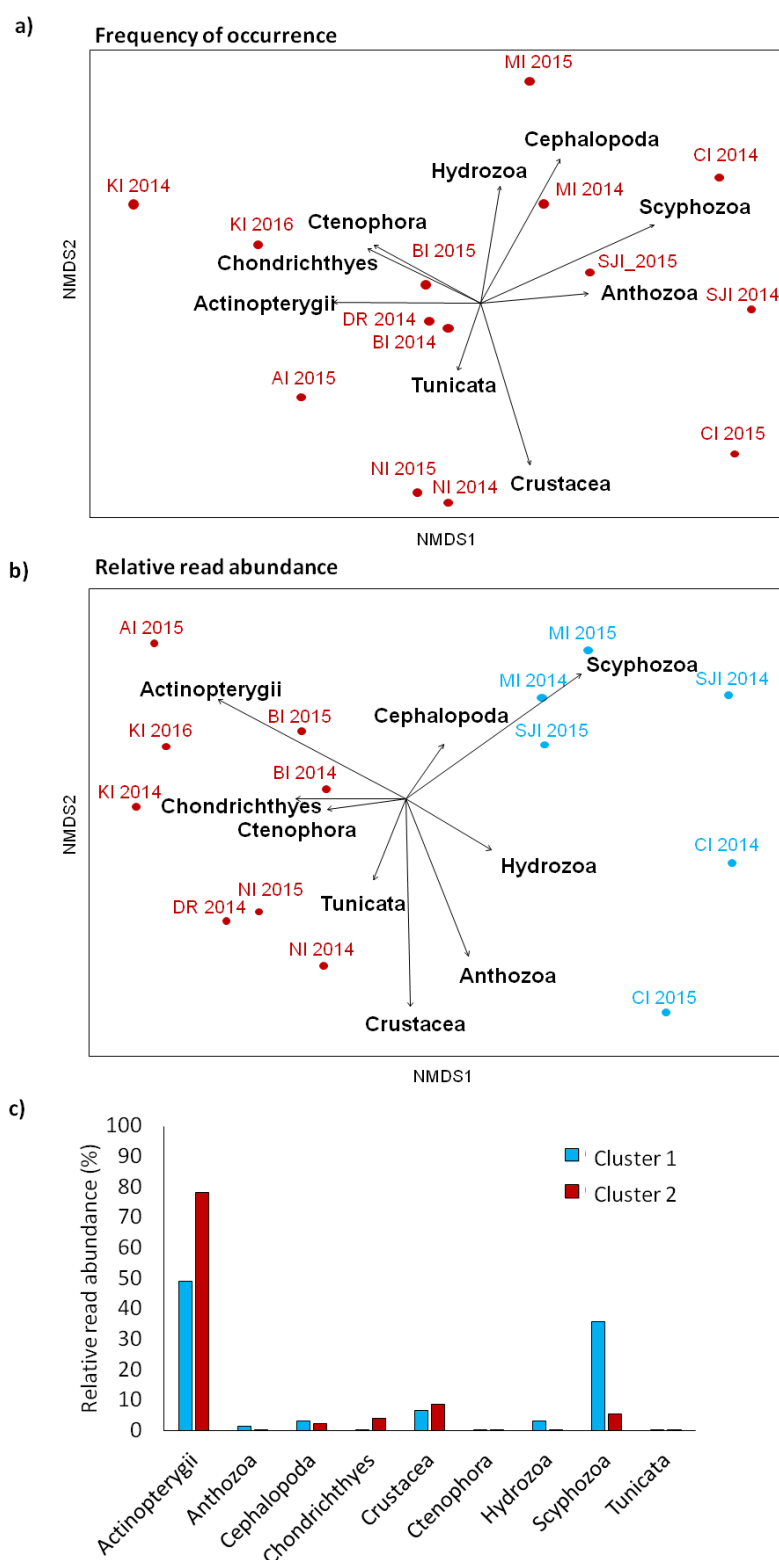


Figure 4: Correspondence of breeding sites with prevalence of major prey groups indicated by multi-dimensional scaling using: a) frequency of occurrence (FOO) and b) relative read abundance (RRA). Significantly different site clusters are shown in red and blue in figure b and the RRA for each group

in figure C. The mean RRA of prey sequences for each group are shown in the bar plot with the ratio of Actinopterygii (bony fish) to Scyphozoa (jellyfish) resulting in the major division. Clusters were assigned using dissimilarity indices calculated with the Manhattan method and hierarchical clustering was calculated using the average agglomeration method.

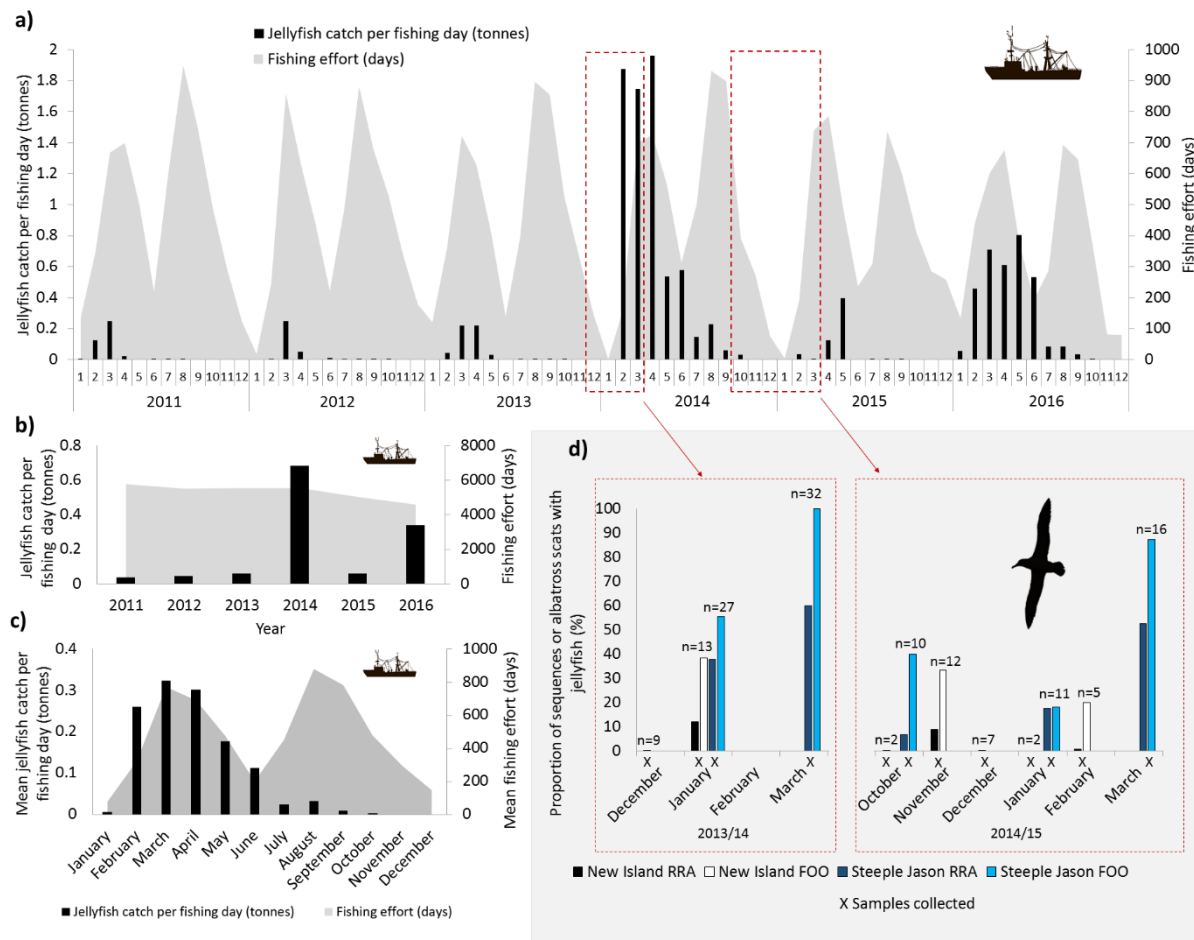


Figure 5: The amount of jellyfish caught in trawl fisheries off the Falkland Islands from 2011-2016 and amount of jellyfish in the diet of black-browed albatross during this study. Grey shading represents the trawl fishing effort in days and the black bars show the amount of jellyfish caught in tonnes per fishing day across: a) each month from 2011-2016, b) each year from 2011-2016 and c) average monthly totals. Figure d) shows the average monthly RRA and FOO of jellyfish DNA in albatross scat samples at New Island and Steeple Jason Island. The 'x' represents sampling periods for each site to distinguish between no jellyfish detection and no dietary sampling.

Table 1: Prey groups consumed by black-browed albatross at each site and Campbell albatross at Campbell Island in each year. Values represent the frequency of occurrence (FOO) with relative read abundance (RRA) in parenthesis. FOO calculations were calculated for any food item which comprised >1% of food sequences for that sample.

	NI 2014	NI 2015	SJI 2014	SJI 2015	AI 2015	DR 2014	BI 2014	BI 2015	KI 2014	KI 2016	MI 2014	MI 2015	CI 2014	CI 2015	Overall
Sample size	22	28	59	40	51	19	33	42	36	14	33	12	45	13	447
CHORDATA	95.5	92.9	72.9	77.5	100	100	90.9	92.9	100	100	93.9	83.3	80.0	53.8	88.1
	(67.9)	(75.8)	(40.8)	(58.5)	(97.2)	(78.6)	(76.6)	(79.5)	(92.6)	(93.4)	(59.4)	(54.9)	(42.1)	(40.1)	(68.4)
Actinopterygii	95.5	92.9	72.9	77.5	100	94.7	90.9	92.9	88.9	92.9	93.9	83.3	80	53.8	86.4
	(67.9)	(75.7)	(40.8)	(58.2)	(97.2)	(73.6)	(75.9)	(79.3)	(74.8)	(82.1)	(59.4)	(54.9)	(41.1)	(39.9)	(65.8)
Clupeocephala	95.5	92.9	72.9	77.5	100	94.7	90.9	92.9	88.9	92.9	93.9	83.3	80	53.8	86.4
	(67.9)	(75.7)	(40.7)	(58.2)	(97.2)	(73.6)	(75.9)	(79.3)	(74.8)	(82.1)	(59.4)	(54.9)	(41.1)	(39.9)	(65.8)
Chondrichthyes						10.5	6.1		27.8	14.3	3		2.2	7.7	5.1
						(3)	(0.5)		(17.6)	(11.3)	(<0.1)		(1)	(0.1)	(2.4)
Batoidea						10.5	6.1		27.8	14.3	3			7.7	5 (2.3)
						(3)	(0.5)		(17.6)	(11.3)	(<0.1)			(0.1)	
Selachimorpha													2.2		0.2 (0.1)
													(0.9)		
Tunicata		3.6		2.5	2	5.3	3 (0.1)	4.8	5.6					7.7	2.5
		(0.1)		(0.4)	(<0.1)	(2.1)		(0.2)	(0.1)					(0.1)	(0.2)
CNIDARIA	31.8	17.9	79.7	52.5	2	31.6	36.4	35.7	22.2	14.3	72.7	58.3	77.8	53.8	41.9
	(7.3)	(4)	(50.4)	(30.1)	(0.1)	(1.1)	(14.7)	(12.5)	(1.7)	(6.4)	(35.9)	(42.5)	(48.3)	(36.1)	(20.8)
Anthozoa	4.5		3.4						2.8				15.6	7.7	2.4
	(0.1)		(0.2)						(<0.1)				(2.3)	(6.9)	(0.7)
Actiniaria	4.5		3.4						2.8				15.6	7.7	2.4 (0.7)
	(0.1)		(0.2)						(<0.1)				(2.3)	(6.9)	
Hydrozoa	0		3.4					9.5	13.9	7.1	6.1	8.3	33.3		5.8
	(0.1)		(0.4)					(0.6)	(1)	(0.2)	(0.8)	(0.8)	(17.5)		(1.5)
Anthomedusae	0		3.4					9.5	13.9	7.1	6.1	8.3			3.5 (0.3)

	(0.1)		(0.4)					(0.6)	(1)	(0.2)	(0.8)	(0.8)			
Siphonophorae													31.1 (17.5)		2.2 (1.3)
Undetermined													2.2 (<0.1)		0.2 (<0.1)
Scyphozoa	22.7 (7.1)	17.9 (4)	79.7 (49.9)	52.5 (30.1)	2 (0.1)	31.6 (1)	36.4 (14.6)	31 (11.9)	5.6 (0.6)	7.1 (6.2)	63.6 (35.1)	58.3 (41.7)	53.3 (28.5)	53.8 (29.2)	36.8 (19.8)
Coronatae			1.7 (0.2)				24.2 (12.9)	7.1 (2.7)	5.6 (0.6)		63.6 (35.1)	50 (41.1)	44.4 (21.1)	23.1 (12.5)	15.7 (7.5)
Semaeostomeae	22.7 (7.1)	17.9 (4)	78 (49.7)	52.5 (30.1)	2 (0.1)	31.6 (1)	12.1 (1.7)	23.8 (9.1)		7.1 (6.2)		8.3 (0.6)	20 (7.3)	30.8 (16.7)	21.9 (12.3)
Undetermined							3 (<0.1)						2.2 (<0.1)		0.4 (<0.1)
CTENOPHORA															
Ctenophora									16.7 (1.3)						1.2 (0.1)
MOLLUSCA															
Cephalopoda	9.1 (1.1)		13.6 (3.4)	15 (7.6)			10.5 (5.3)	6.1 (2.6)	14.3 (7.5)	5.6 (1.8)	7.1 (0.2)	12.1 (2)	25 (2.6)	15.6 (3.9)	7.7 (0.6)
Octopoda			1.7 (0.1)							2.8 (0.6)					0.3 (0.1)
Teuthida	4.5 (0.2)		13.6 (3.3)	15 (7.6)			5.3 (5.2)	6.1 (2.6)	14.3 (7.5)	2.8 (1.2)	7.1 (0.2)	9.1 (1.9)	8.3 (0.3)	13.3 (3.8)	7.7 (0.6)
Unidentified	4.5 (0.9)						5.3 (0.1)					3 (0.1)	16.7 (2.3)	6.7 (0.2)	2.6 (0.3)
ANTHROPODA															
Crustacea	68.2 (23.6)	60.7 (20.1)	44.1 (5.4)	25 (3.7)	27.5 (2.7)	26.3 (15)	27.3 (6.1)	19 (0.5)	19.4 (2.6)		18.2 (2.6)		35.6 (5.7)	46.2 (23.2)	29.8 (7.9)
Calanoida	40.9 (4.5)	14.3 (3.6)	28.8 (2.4)	5 (0.2)	23.5 (2)	10.5 (3.2)	3 (0.1)	7.1 (0.2)	13.9 (1.2)		6.1 (0.2)		20 (3.7)	15.4 (0.7)	13.5 (1.6)

Decapoda										2.2 (0.1)		0.2 (<0.1)
Eumalacostraca	4.5 (0.8)	28.6 (5)	8.5 (0.3)	12.5 (2.9)		6.1 (0.2)		2.8 (0.1)	3 (0.1)		7.7 (0.3)	5.3 (0.7)
Euphausiacea	4.5 (0.1)	7.1 (0.4)		12.5 (0.5)		18.2 (5.7)	7.1 (0.3)				0 (0.1)	3.5 (0.5)
Isopoda			1.7 (1.6)	2.5 (0)				2.8 (1.1)				0.5 (0.2)
Munididae	27.3 (15.7)	21.4 (11)	5.1 (1)									3.8 (2)
Peracarida							2.4 (<0.1)		3 (0.1)	2.2 (0.1)	15.4 (0.2)	1.6 (<0.1)
Pleocyemata	9.1 (2.4)	3.6 (0.1)									7.7 (0.3)	1.5 (0.2)
Podoplea	4.5 (0.1)				9.8 (0.6)	21.1 (11.7)		2.8 (0.1)	6.1 (2.2)	13.3 (1.7)		4.1 (1.2)
Thoracica										4.4 (0.2)	30.8 (21.6)	2.5 (1.6)
Undetermined			1.7 (0.1)				3 (<0.1)					0.3 (<0.1)