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Diet specialization in a colonial seabird studied using three complementary dietary techniques: effects of intrinsic and extrinsic factors

Maëlle Connan[1,2] · Bo T. Bonnevie[1] · Christina Hagen[3,4] · Carl D. van der Lingen[5] · Christopher McQuaid[1]

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Abstract The breeding period has a critical influence on the trophic ecology of seabirds because of the energetic costs of egg production for females, the need to return regularly to the nest to provision chicks, the combined energetic demands of adults and chicks, and potential intraspecific competition if resources around the colony are scarce. The present study combined three dietary methods to investigate if and how these intrinsic and extrinsic factors influenced diet specialization in a colonially breeding seabird, the Cape gannet *Morus capensis*. The diet of this species was studied from November 2009 to October 2010 at the species' largest colony at Bird Island, Algoa Bay (33°50′S, 026°17′E; South Africa). Potential prey species were sampled concurrently and dietary tracers (stable isotopes and fatty acids) were analysed. Stomach content and carbon and nitrogen stable isotope analyses indicated that adults relied heavily all year round on small pelagic fish (anchovy and sardine), with prey species composition and individual prey size changing with season, probably reflecting prey biology. Dietary tracers did not show any differences between adult and chick diets. Subtle differences were found between stable isotope values of adult males and females but these were not supported by a Bayesian mixing model. In contrast, differences between the sexes were highlighted in blood fatty acids. The combined results suggest that these were probably related to the cost of egg production rather than to inter-sex differences in diet. Individual diet specialization was observed using stable isotopes in adults. Altogether this dataset indicates the importance of combining complementary methods to understand multiple facets of seabirds' trophic ecology, and highlights interactions with fisheries that require future monitoring.

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✉ Maëlle Connan
maelle.connan@gmail.com

[1] Department of Zoology and Entomology, Rhodes University, PO Box 94, Grahamstown 6140, South Africa

[2] Present Address: Department of Zoology, Nelson Mandela University, PO Box 77000, Port Elizabeth 6031, South Africa

[3] Percy FitzPatrick Institute of African Ornithology, DST-NRF Centre of Excellence, University of Cape Town, Cape Town, South Africa

[4] Seabird Conservation Programme, BirdLife South Africa, Cape Town, South Africa

[5] Department of Agriculture, Forestry and Fisheries, Cape Town, South Africa; and Marine Research Institute and Department of Biological Sciences, University of Cape Town, Cape Town, South Africa

Introduction

Seabirds breed on land but forage at sea and this shapes their life histories dramatically. During the breeding period, seabirds must commute between their nesting site and foraging areas to sustain themselves and raise their chicks (Nelson 1980). Trophic resources in the waters surrounding breeding sites come under pressure during the breeding season, especially as most seabird species breed colonially. If resources become depleted as the season progresses, then following the Ashmole's halo concept (Ashmole 1963), intraspecific partitioning may be exhibited, leading to foraging and/or dietary segregation

between adults. Horizontal as well as vertical foraging segregations between male and female breeders have been highlighted in both dimorphic (e.g. Weimerskirch et al. 1997; Clarke et al. 1998) and monomorphic seabird species (e.g. Lewis et al. 2002; Welcker et al. 2009). During chick rearing, males and females have also been shown to target different prey species or sizes (Clarke et al. 1998; Bearhop et al. 2006; Awkerman et al. 2007). A number of factors have been proposed to explain these differences including differences in parental care (Elliott et al. 2010; Burke et al. 2015), and different physiological needs, particularly with the additional cost to females of producing eggs (Lewis et al. 2002; Welcker et al. 2009; Hayward and Gillooly 2011). Outside the breeding season, such intraspecific tensions may be relaxed as the birds are free to travel much longer distances to obtain their food and do not need to support their chicks. Sexual differences in non-breeding foraging areas and diet have, however, been observed in a number of species including albatrosses (Phillips et al. 2009), shags (Bearhop et al. 2006) and penguins (Thiebot et al. 2014; Whitehead et al. 2017), but not in Northern gannet, *Morus bassanus* (Stauss et al. 2012).

The Cape gannet *Morus capensis* is a typical colonial breeder with one of the highest nesting site densities of all seabirds (Klages 1994) and, like all of these, it acts as a central place forager when breeding. It is endemic to southern Africa, where it breeds on six islands: five on the west coast in the Benguela upwelling system and one on Bird Island, Algoa Bay, on the south-east coast of South Africa in the temperate shelf system over the Agulhas Bank (BirdLife International 2017a). This easternmost colony on Bird Island supports >70% of the world's population (average 2000–2016: ~85,000 breeding pairs; Department of Environmental Affairs, unpubl data). In contrast to the colonies situated in Namibia and on the west coast of South Africa, the colony on the south-east coast has increased in size considerably in the last twenty years (Crawford et al. 2009; Distiller et al. 2012), concomitant with an eastward shift of small pelagic fish (sardine *Sardinops sagax* and anchovy *Engraulis encrasicolus*; Roy et al. 2007; Coetzee et al. 2008; van der Lingen et al. 2011; Department of Agriculture, Forestry and Fisheries 2014). Recently, continuous fine-scale time-activity budget data obtained using very high frequency devices attached to birds from the Bird Island colony have shown that trip duration and chick provisioning during breeding are clearly sex specific (Rishworth et al. 2014b). Females tend to make fewer, longer foraging trips and spend less time at the nest than males, with differences in behaviour between the sexes becoming increasingly apparent as the chicks develop (Rishworth et al. 2014b). Longer foraging trips for females had also been shown for the west coast colonies with male and female parents bringing back similar prey to their chicks (Mullers and Navarro 2010).

The diet of Cape gannets has been extensively studied using traditional stomach content analysis (e.g. Klages et al. 1992; Berruti et al. 1993; Pichegru et al. 2007; Green et al. 2015b). Although the analysis of stomach contents provides invaluable information (e.g. identification, quantification and measurement of prey remains), it has well-known limitations. For example, only the last few meals of the birds will be reflected (mean retention time in Cape gannet is 12 h; Laugksch and Duffy 1986) and during the breeding season, only the chick diet will be assessed, not the adult diet (Barrett et al. 2007). To overcome these drawbacks, stomach content analysis is now often combined with indirect tracers such as stable isotopes and/or fatty acids (FAs) to provide better insight into the diets of these marine top predators (Barrett et al. 2007; Karnovsky et al. 2008, 2012; Connan et al. 2014b). The ratios of the stable isotopes of carbon ($^{13}C/^{12}C$; $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$; $\delta^{15}N$) in proteins provide insight into the source production of the food web and the trophic level of the consumers, respectively (Michener and Kaufman 2007). Lipids, specifically FAs, have also been used to decipher the diet of seabirds (e.g. Raclot et al. 1998; Iverson et al. 2007; Williams and Buck 2010; Wold et al. 2011; Owen et al. 2013). Depending on the tissue analysed (e.g. plasma, red blood cells, whole blood, adipose tissue, feathers), this provides dietary information reflecting different time scales from the last few days up to several months (Dalerum and Angerbjörn 2005; Wang et al. 2010; Williams and Buck 2010).

The Cape gannet is categorized as Vulnerable on the IUCN red list (BirdLife International 2017a). Further discussion may result in its conservation status being soon uplisted to Endangered (BirdLife International 2017b). Effective management plans for its conservation require an understanding of when and where it is most vulnerable. Vulnerability can take many forms, but constraints on feeding are clearly important. In the North Sea, the use of FAs (Käkelä et al. 2007) and stable isotopes (Stauss et al. 2012) combined with stomach content analysis has highlighted the importance of interactions between the Northern gannet and fisheries. Such interactions have been observed for Cape gannets during chick rearing on the west coast of South Africa and contact with fisheries during this particularly vulnerable phase poses a potential threat to the species (Pichegru et al. 2007; Moseley et al. 2012; Tew Kai et al. 2013).

The high nesting densities and important population number in Algoa Bay make birds from that colony ideal candidates to investigate further the effects of various factors on diet specialization in colonial seabirds. Furthermore, knowledge of diet of adult Cape gannets over the entire annual cycle is lacking, particularly for regions away from nesting grounds. In the present study, we therefore use three complementary techniques (stomach content analysis, stable isotope analysis of blood and feathers, and FA

analysis of blood) to investigate (1) whether season has an effect on Cape gannet diets at their colony and also away from their nesting grounds, (2) whether adult male and female Cape gannets exhibit the same diet over time, (3) whether chicks are fed the same prey as adults use themselves, (4) whether individual diet specialization exists in adults, and (5) whether Cape gannets from Bird Island rely heavily on fisheries discards (which may have important consequences for conservation). We hypothesized that season would have an effect on Cape gannet diet because of changes in the availability of prey species at different times of year. Due to differing physiological needs, we hypothesized that diet would differ according to age as well as adult sex. We expected to detect individual specialization in Cape gannets as has been shown in Northern gannets (Votier et al. 2010). Finally, interactions with fisheries were believed to be likely detected (Tremblay et al. 2014).

Materials and methods

Study site and seabird sampling

Fieldwork was conducted on Bird Island in Algoa Bay (33°50′S, 026°17′E) which hosts the largest gannetry in the world (Crawford et al. 2007). Cape gannets are summer breeders, with a breeding season that extends from October to March, but individuals are found in the colony all year around (Pistorius et al. 2015).

Adult Cape gannets were sampled four times: in November 2009 during incubation, in February 2010 during chick rearing, in July 2010 after the breeding season, and in October 2010 before the next breeding season. These four periods were used as categories of the explanatory variable "sampling session" in our statistical models. In addition, fully feathered chicks were sampled in late February 2010. Adult gannets were caught at the nest by passing a hook attached to a 3 m long pole around their necks. Nests within 3 m of the border of the colony were targeted to avoid unnecessary disturbance in the colony (Carney and Sydeman 1999). Only adults seen coming back from a foraging trip were caught to ensure they had not been fasting in the days preceding the capture while incubating or chick guarding. Outside of the breeding season, adults were caught by slowly approaching them and passing the hook around the neck before they flew off. Birds were ringed to prevent repeated sampling of the same individuals.

Standard measurements [culmen length (±0.1 mm), tarsus length (±0.1 mm), flattened wing cord (± 1 mm) and body mass (± 25 g)] were collected for each sampled bird. A body condition index was then estimated by dividing the body mass over the wing cord to the power of three to account for isometric scaling (Green 2000; Bonnevie 2014). Morphometric data for adult male and female gannets were then compared using parametric or nonparametric tests depending on the outcomes of the tests for normality and homoscedasticity.

Stomach contents were collected by inducing birds to regurgitate by gently massaging the stomach. The regurgitated contents were then stored at −20 °C until further processing. For stable isotope and FA analyses, a maximum of 2 ml of blood was drawn from the tarsal vein using a 25 G needle and a syringe rinsed with heparin. Half a millilitre of blood was added to 70% ethanol for stable isotope analyses and DNA sexing, and the remaining blood was added to a solution of chloroform + 0.01% butylatedhydroxytoluene in a glass tube closed under nitrogen for FA analysis. Up to six body feathers were plucked for stable isotope analyses. Blood and feathers were then stored at −20 °C until laboratory analyses.

Genetic sexing

With the exception of the length of the gular stripe, the Cape gannet is sexually monomorphic (Rishworth et al. 2014a). Males and females were thus identified genetically by adapting a protocol developed by Fridolfsson and Ellegren (1999). Total genomic DNA was extracted from whole blood using a DNeasy Blood and Tissue extraction Kit (Qiagen) following the manufacturer's instructions. The sex linked chromo-helicase-DNA-binding gene (CHD) was then amplified using a polymerase chain reaction amplification and the two 2550F and 2718R primers (Fridolfsson and Ellegren 1999). Amplification reactions were performed in 20 µl final volumes, including 3 mM $MgCl_2$, 2 µl 10 × NH_4 Buffer, 160 µM each deoxyribonucleotide triphosphate (dNTP), 0.4 µM each primer, 0.5 U Bioline BioTAQ™ DNA polymerase, and 5 µl of template. Thermocycling conditions for amplification included an initial denaturation step of 2 min at 94 °C, followed by a touch-down cycle lowering the annealing temperature in 1 °C decrements, from 50 to 42 °C. Thirty additional cycles were then run at 42 °C. Cycles comprised a denaturation step at 94 °C for 30 s, 30 s at annealing temperature, and extension at 72 °C for 1 min. A final extensive phase at 72 °C lasted 5 min. PCR products were bound with SYBR® Green I and checked via electrophoresis on a 1.8% agarose gel with TBE Buffer and subsequent visualization under UV radiation. The CHD-Z gene is present in both sexes, whilst the CHD-W gene is present in females only.

Fish sampling

To reconstruct the gannet's diet from indirect markers using Bayesian mixing models (Parnell et al. 2013; Galloway et al.

2015), predators and their potential prey were collected concurrently. The potential prey species were identified from earlier stomach content studies (Batchelor and Ross 1984; Klages et al. 1992): these included anchovy, sardine, redeye round herring *Etrumeus whiteheadi*, hake *Merluccius capensis* and chokka squid *Loligo reynaudii*. The identification of hake in gannet stomach contents is indirect evidence of gannet-fisheries interactions (Adams and Walter 1993). Carbon and nitrogen stable isotope ratios and FA compositions were determined for each of these species. Anchovy and redeye round herring were collected by the Department of Agriculture, Forestry and Fisheries during a 2009 pelagic spawner biomass survey in the Algoa Bay region. Additional sardine were collected south of Cape Agulhas, the southernmost tip of Africa, approximately 500 km west of Algoa Bay. The hake and squid specimens were sourced from local fisheries operating as close as possible to the Cape gannet colony. Fish and squid were frozen at −20 °C as soon as possible after capture and were stored frozen until subsequent analyses.

Analysis of Cape gannet stomach contents

In the laboratory, all stomach contents were defrosted, weighed and individual prey or prey remains were identified to the lowest taxon possible using published keys (Clarke 1986; Smith and Heemstra 1986; Smale et al. 1995). Each taxon was then weighed separately to estimate the stomach content composition by wet mass. Considering the high variability in the number of stomach content samples collected between sampling sessions, no further data analyses were conducted on dietary composition.

Because a high proportion of prey remains consisted of fish tails or heads, the standard lengths of fish were estimated from the diameter of otoliths using the equations detailed in Smale et al. (1995). Anchovy was a common prey item in all sampling sessions so seasonal variation in anchovy standard length was then investigated using a Kruskal–Wallis *H* test (KS *H*) followed by Mann–Whitney *U* tests (MW *U*) to infer whether the birds were targeting different size classes depending on sampling sessions.

Stable isotopes

Stable isotope analysis of Cape gannet and potential prey samples

Gannet blood samples were dried at 50 °C for up to 48 h before being ground to a homogeneous powder. Whole blood does not usually require any pre-treatment before stable isotope analysis (Bearhop et al. 2000; Cherel et al. 2005b). Individual feathers were washed in a chloroform:methanol (2:1) solution, then rinsed in successive baths of methanol and distilled water, before being dried at 50 °C for 24 h. Feathers were then homogenized by finely cutting the barb.

Most freeze-dried fish flesh and squid mantle were ground to a fine powder before lipids were removed using cyclohexane (Jaquemet et al. 2008). Once delipidated, the powdered fish and squid were dried at 50 °C for 24 h. Small quantities (0.4–0.5 mg) of homogenized feather, dried blood and dried delipidated fish and squid were weighed and placed in tin capsules for analysis. Relative isotope abundances of carbon and nitrogen were determined by combusting samples in a Flash 2000 organic elemental analyser and the gases being passed to a Delta V Plus isotope ratio mass spectrometer via a Conflo IV gas control unit (Thermo Scientific, Bremen, Germany; Stable Light Isotope Unit, University of Cape Town, South Africa). Carbon and nitrogen results are presented in the usual δ notation relative to Vienna Pee Dee Belemnite and atmospheric N_2 standards, respectively:

$$\delta^{13}\mathrm{C} \text{ or } \delta^{15}\mathrm{N}\ (‰) = \left[(R_{\mathrm{sample}}/R_{\mathrm{standard}}) - 1\right] \times 10^3$$

where R_{sample} and R_{standard} are the ratios of $^{13}C/^{12}C$ (for $\delta^{13}C$) or $^{15}N/^{14}N$ (for $\delta^{15}N$) for the samples and the references (Vienna Pee Dee Belemnite, atmospheric N_2), respectively. Replicate measurements of internal laboratory standards (Merck gel, seal bone, valine) indicated measurement error <0.1‰ for both carbon and nitrogen stable isotope measurements.

Analysis of stable isotope data

When carbon and nitrogen stable isotope data did not follow multivariate normality (Mardia tests), they were compared using one or two-way permutational analyses of variance (PERMANOVA; Anderson 2001). Prey species, Cape gannet blood and feather data were analysed separately, and comparisons were made between sexes and sampling sessions, as well as a comparison of data for adults and chicks that were sampled concurrently in February 2010. Isotopic richness, the total convex hull area filled by all organisms of each group, was estimated using a bootstrapping approach when the number of samples differed between groups in the comparisons (Cucherousset and Villéger 2015).

Diet investigation using stable isotopes and Bayesian mixing models

The stable isotope database comprised five species of fish and one species of squid. In addition to the specimens

analysed in the present study (four fish species, one squid species), data obtained from Moseley et al. (2012) were added to the database; in particular, specimens of saury *Scomberesox saurus* collected from gannet stomach contents. The additional data were either collected from fresh individuals in Cape gannet stomach contents (sampled in November 2009) or from samples collected within 50 km of Bird Island during the 2009 small pelagic fish survey.

A crucial factor when using mixing models is to ensure accurate discrimination factors (trophic enrichment of stable isotopes from one trophic level to the next) for the tissue and species of interest (Bond and Diamond 2011; Phillips et al. 2014). To date, no discrimination factor determined in captivity has been published for Cape gannet or its congeneric counterparts. We, therefore, followed the approach of Stauss et al. (2012) by calculating averaged discrimination factors for blood and feathers considering only studies on captive adult or subadult seabirds (Hobson and Clark 1992b; Mizutani et al. 1992; Bearhop et al. 1999, 2002; Cherel et al. 2005b; Becker et al. 2007; Sears et al. 2009; Polito et al. 2011; Barquete et al. 2013; Ciancio et al. 2016). The averaged discrimination factors for the blood were 0.1 ± 0.7‰ ($\delta^{13}C$) and 2.7 ± 0.5‰ ($\delta^{15}N$), and for the feathers 1.4 ± 1.0‰ ($\delta^{13}C$) and 4.1 ± 0.7‰ ($\delta^{15}N$). The adequacy of prey database and discrimination factors was checked using a simulated mixing polygon (Smith et al. 2013) before running the mixing models. Probability distributions for the proportional contribution of each of the five potential prey species in the diet of Cape gannets were then estimated using the Bayesian stable isotope mixing model MixSIAR GUI v3.0 (Parnell et al. 2013; Stock and Semmens 2015). This Bayesian approach accounted for the uncertainty in sources (Moore and Semmens 2008; Ward et al. 2010), and allowed the inclusion of categorical covariates (sex and age class) into the models where appropriate (Semmens et al. 2009; Parnell et al. 2013). Markov Chain Monte Carlo parameters for blood and feathers were set as follows: chain length = 100,000, burn in = 50,000, thin = 50, number of chains = 3, except for juveniles were parameters were chain length = 300,000, burn in = 200,000, thin = 100, number of chains = 3. Models were assessed for convergence using Gelman-Rubin and Geweke metrics (Stock and Semmens 2015). Models were run once including all Cape gannets per age class/sex/sampling session to examine the proportions of potential prey species for the overall population.

Fatty acids

Fatty acid analysis of Cape gannet and fish samples

Total lipids were extracted from the Cape gannet whole blood, fish flesh and squid mantle samples using a modified Folch et al. (1957) method (Allan et al. 2010). From the total lipid extracts, fatty acid methyl esters (FAMEs) were prepared by heating the samples suspended in dichloromethane with sulphuric acid at 100 °C for 1 h, and then cleaning them via three successive rinses with hexane and milliQ-distilled water (Budge et al. 2006; Allan et al. 2010). The FAME composition of individual samples was determined using an Agilent Technologies 7890A gas chromatographic System with a ZB-Waxplus 320 column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness; Zebron Corporation), and helium as carrier gas, at the National Research Foundation facility at Rhodes University. The injection temperature was 250 °C, the flame ionization detector was set at 260 °C, and the oven was initially set at 150 °C. After 5 min, the oven temperature was raised to 225 °C at a rate of 2.5 °C min^{-1} and held there for 9 min. Peaks were integrated using GC ChemStation software (Agilent Technologies, version B.04.02), identified by comparison with retention times of external standards (37 component fatty acid methyl ester mix Supelco, marine PUFA no. 1 Supelco, bacterial acid methyl esters mix Supelco), as well as by mass spectrometry analysis (Agilent Technologies 7000 GC/MS Triple Quad; Agilent MassHunter Qualitative Analysis, version B.03.01).

Each FA was measured as an averaged proportion of the total fatty acid composition (TFA) in the shorthand form *X*: $a(n-6)$, where '*X*' is the number of carbon atoms in the acyl chain, '*a*' is the total number of double bonds in the chain, and '*6*' is the position of the first double bond from the methyl end of the molecule.

Analyses of fatty acid data

FA compositions of potential prey species and Cape gannet blood were arcsine transformed before data analysis. Overall FA profiles were compared among the five potential prey species, among the various groups of adult Cape gannets (sampling session, sex, and their interaction), and between adult and chick samples collected in February 2010 (age class, sex, and their interaction) using PERMANOVA. Subsequent similarity percentage (SIMPER) analyses identified the FAs primarily responsible for the observed differences between groups (Clarke 1993). Three-dimensional visualization was conducted using non-metric multidimensional scaling (NMDS) with a maximum stress of 0.15 (Bray–Curtis similarity index; Clarke 1993).

Diet investigation using fatty acid profiles and multivariate analyses

As with stable isotope data, FA profiles can now be used in Bayesian mixing models such as MixSIAR (Stock and Semmens 2015) and FASTAR (Galloway et al. 2015). However,

one of the prerequisites is that the database of potential prey species contains all the possible prey. Unfortunately, saury were not caught during fish sampling and those collected in the Cape gannet stomach contents were not fresh enough to have their FA analysed. Lipids are prone to degrade quickly (Christie 1973) so only freshly caught immediately frozen prey species were used for FA analyses. In addition, quantitative analyses require FA-specific calibration coefficients to be applied to FA proportions. To our knowledge, very few captive studies have been published on seabirds (Grahl-Nielsen et al. 2005; Käkelä et al. 2009; Wang et al. 2010, 2014; Polito et al. 2012) and none of them deal with total blood but have used yolk, adipose tissue or plasma. A comparison of the calibration coefficients determined in those studies highlighted that they were highly species- and tissue specific and so using calibration estimates from another species/tissue can be misleading (Wang et al. 2014). Considering the limitations of missing calibration coefficients for the species and the tissue used, as well as the limited prey database, no quantitative analysis was conducted on the FA data. Instead, multivariate analyses were used to qualitatively investigate the prey exhibiting the prey with FA profiles most similar to those of the birds. Only FAs that could arise from dietary origin alone were considered (30 FAs, Table 3; Iverson et al. 2004; Wang et al. 2010). Data for these 30 FAs were extracted, renormalized and arcsine transformed. The number of FA variables exceeded the number of individuals in the smallest group of Cape gannets ($n = 8$); hence, FA variables were first reduced by conducting a preliminary principal component analysis with the first eight principal components considered. Linear discriminant analysis was then conducted with the potential prey data only (sardines, anchovy, redeye round herring, hake and squid). Cape gannet FA data were then included as supplementary individuals so as not to interfere with the discriminant functions defined previously.

Individual specialization

Individual specialization was assessed using stomach contents and stable isotope values of whole blood and feathers from the same individuals. Stomach contents give information on prey eaten during the last foraging trip (Laugksch and Duffy 1986), while whole blood would represent an integration of all prey consumed over the last few weeks (Hobson and Clark 1992a, 1993). Feathers inform on the trophic ecology of the gannets while they moult. Carbon and nitrogen stable isotope values of the contents of each stomach were estimated using the prey stable isotope values (see above) and the following equation (adapted from Provencher et al. 2013):

$$SI_{SC} = \Sigma\,(wm_i s_i)/\Sigma\, wm_i$$

where wm_i is the wet mass of prey species i found in the gannet stomach contents and s_i is the carbon (or nitrogen) stable isotope value. Individual specialization was then estimated by testing the correlation between SI_{SC} and stable isotope ratios of whole blood for the same individual. Five assumptions were made (Provencher et al. 2013): (1) no measurement errors in stable isotope values, (2) prey isotope ratios represent the isotope ratios of the prey actually consumed by gannets, (3) equal digestive turnover rates and equal routing of prey muscle protein into gannet whole blood protein content for all prey species, (4) the ratio of prey body mass to prey muscle mass is the same for all prey species, and (5) complete specialization, i.e. stomach contents remain constant over the timescale of whole blood turnover rates. The regression's slope and R^2 value should equal one and the regression intercept should approximate the seabird fractionation value. Any deviation from those values indicates relaxation of one or more of the assumptions. Individual specialization was further examined by correlating whole blood stable isotope values with feather stable isotope values.

All statistical analyses were conducted using R v3.2.5 (R Development Team 2015) and Past 3.06 (Hammer et al. 2001). The significance level was set at 0.05.

Results

Morphometrics

None of the Cape gannet measurements taken of culmen, tarsus or mass yielded statistically significant differences between adult males and females, among seasons or their interaction (Two-way ANOVAs, all $P > 0.086$; Table 1). Slight seasonal variation was highlighted in adult wing cord with birds caught during incubation exhibiting a smaller measurement than in the three following samplings (Two-way ANOVA $F_{3,68} = 2.79$, $P = 0.048$) but neither sex nor the interaction season × sex yielded significant differences (both $P > 0.101$). Body condition index was similar between sexes (Two-way ANOVA $F_{1,69} = 0.12$, $P = 0.730$) but adults caught during breeding had a significantly lower body condition than during the other three seasons ($F_{3,67} = 4.09$, $P = 0.010$) and this was particularly so for females ($2.18 \pm 0.07 \times 10^{-5}$ vs $2.59 \pm 0.21 \times 10^{-5}$ g mm^{-3}). The interaction of the two factors was not significant ($F_{3,63} = 1.30$, $P = 0.283$).

Stomach contents

Five-hundred and twenty-seven prey items belonging to seven species (six fish and one squid species) were

Table 1 Means of morphometric measurements collected for adult and chick, male and female Cape gannets

	Adults				Chicks			
	Males		Females		Males		Females	
Culmen (mm)	91.3 ± 2.6 86.3–96.2	(50)	90.0 ± 3.8 84.1–96.3	(22)	88.6 ± 5.6 82.0–97.1	(6)	88.5 ± 2.5 84.7–92.8	(11)
Tarsus (mm)	62.0 ± 2.2 56.8–66.8	(38)	62.1 ± 3.3 56.6–68.9	(14)	62.4 ± 2.1 59.2–64.4	(6)	62.6 ± 2.2 59.4–66.4	(11)
Wing (mm)	477.7 ± 10.2 455–511	(50)	481.8 ± 8.5 460–498	(22)	438.2 ± 20.7 409–463	(6)	446.3 ± 17.7 407–467	(11)
Mass (g)	2735 ± 227 2125–3250	(49)	2830 ± 260 2425–3375	(22)	2523 ± 423 2050–3150	(6)	2627 ± 388 2000–3250	(11)
Body condition (10^{-5} g mm^{-3})	2.51 ± 0.26 1.95–3.42	(49)	2.53 ± 0.24 2.10–2.94	(22)	2.99 ± 0.38 2.41–3.46	(6)	2.96 ± 0.37 2.18–3.52	(11)

Sample size in parenthesis. Means ± standard deviations, and ranges

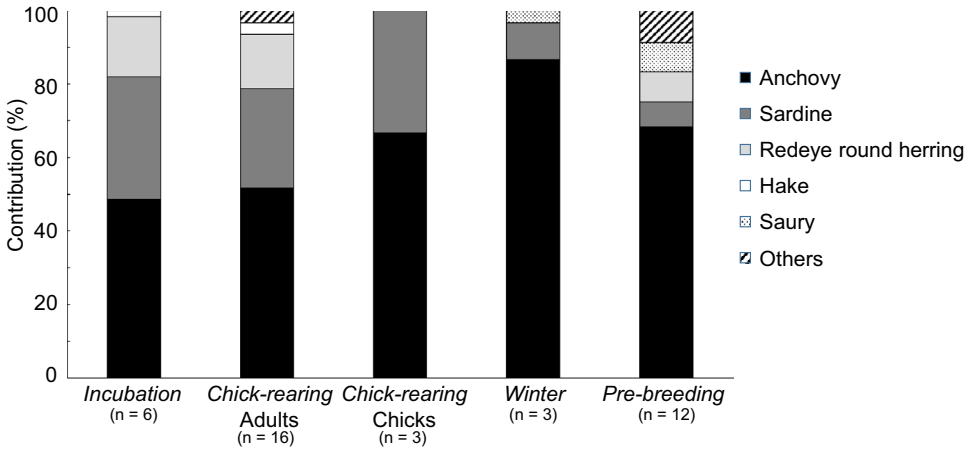

Fig. 1 Composition (by wet mass) of Cape gannet stomach contents collected from adults at four stages of their annual cycle as well as from chicks (others includes mullets and squid)

identified in Cape gannet stomach contents. Small pelagic fish dominated the stomach contents by wet mass (Fig. 1) and by number (Table 2). Anchovy represented between 49 and 87% of wet mass depending on the sampling session, followed by sardine (7–33%), redeye round herring (0–16%), others (squid or mullet *Liza richardsonii*; 0–9%), and saury (0–8%). In terms of numbers, anchovy represented between 80 and 97% of the prey remains and sardine between 2 and 15%, depending on sampling session. Hake comprised 0–4% in wet mass of stomach contents and its frequency of occurrence was 12.5% (found in five stomach contents out of 40).

Significant seasonal differences were observed in the standard length of anchovy ingested by adult gannets (KS $H_{3,266} = 61.66$, $P < 0.001$). Anchovy eaten prior to laying (October) were significantly smaller than those eaten in the previous sampling sessions (MW pairwise: all $P < 0.001$; Table 2). Anchovy brought back during chick rearing (February) were also slightly smaller than those brought back during incubation (November; MW pairwise: $P = 0.03$).

Stable isotopes

Potential prey

Stable isotope data from 68 individuals of the five fish and one squid species were gathered in the prey isotope database (Supplementary material Table S1). Saury exhibited the lowest $\delta^{13}C$ (−17.4‰) and hake the highest (−15.7‰). $\delta^{15}N$ ranged from 10.3‰ for the saury to 14.6‰ for the hake. The comparison of carbon and nitrogen stable isotope values among the fish and squid species showed that there were all significantly different from each other (PERMANOVA $F_{5,63} = 128.8$, $P < 0.001$; Supplementary material Table S1), with the exception of anchovy and redeye round herring, of saury and hake, and saury and squid (MW pairwise comparisons all $P < 0.036$ except anchovy-redeye round herring $P = 0.740$, saury-hake $P = 0.060$, and saury-squid $P = 0.057$). As the differences between saury and hake and saury and squid were only marginally non-significant and likely to be influenced by the number of saury

Table 2 Composition of the stomach contents by number (a; Nb and %) and standard length (b; mm; mean, standard deviations and ranges are given where appropriate)

		Incubation		Chick rearing				Winter		Pre-breeding	
				Adults		Chicks					
		Nb	%	Nb	%	Nb	%	Nb	%	Nb	%
(a) Composition by number											
Anchovy	*Engraulis encrasicolus*	37	80.4	245	91.4	18	85.7	62	96.9	112	87.5
Sardine	*Sardinops sagax*	7	15.2	15	5.6	3	14.3	1	1.6	6	4.7
Redeye round herring	*Etrumeus whiteheadi*							1	1.6	7	5.5
Hake	*Merluccius capensis*	1	2.2	3	1.1					2	1.6
Saury	*Scomberesox saurus*	1	2.2	4	1.5						
Mullet	*Liza richardsonii*			1	0.4						
Squid	*Loligo reynaudii*									1	0.8
Total		46		268		21		64		128	
(b) Standard length											
Anchovy	*Engraulis encrasicolus*	117.4 ± 9.4 (94.8–132.7)		108.6 ± 12.3 (67.2–129.7)		111.3 ± 5.1 (103.9–120.6)		113.9 ± 7.0 (99.3–125.2)		99.7 ± 8.5 (82.6–119.1)	
Sardine	*Sardinops sagax*			167.3 ± 27.1 (103.9–190.8)				200.8		116.7 ± 34.1 (96.6–185.7)	
Redeye round herring	*Etrumeus whiteheadi*									167.0 ± 12.0 (153.7–177.2)	
Hake	*Merluccius capensis*			275.1						252.6	
Saury	*Scomberesox saurus*			207.3							
Mullet	*Liza richardsonii*			210.2							

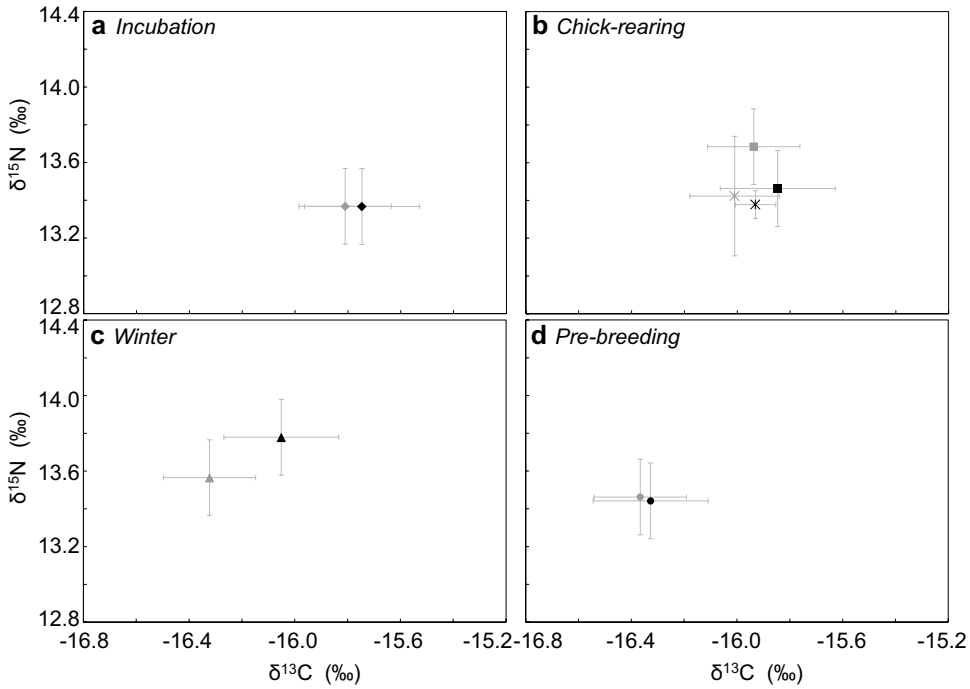

Fig. 2 Carbon and nitrogen stable isotope values measured in the blood of adult (incubation, end of chick rearing, wintering, pre-breeding) and chick (end of chick rearing; *stars*) Cape gannets sampled in 2009–2010. *Black* females, *grey* males. *Error bars* indicate standard deviations

samples ($n = 3$), five groups were consequently considered for further mixing models using stable isotope data: anchovy/redeye round herring, sardine, saury, hake and squid.

Cape gannets

Eighty-five blood samples from known-sex Cape gannets were collected for carbon and nitrogen stable isotope analysis. $\delta^{13}C$ values ranged from −16.4‰ (±0.2‰; males before breeding) to −15.7‰ (±0.2‰; incubating females), and $\delta^{15}N$ from 13.4‰ (± 0.2‰; incubating males and females) to 13.8‰ (±0.4‰; females in winter) (Fig. 2, Supplementary material Table S2). Sampling session had a statistically significant effect on adult blood $\delta^{13}C$ and $\delta^{15}N$ values (Two-way PERMANOVA $F_{3,68} = 11.38$, $P < 0.001$) but neither sex (Two-way PERMANOVA $F_{1,70} = 1.37$, $P = 0.163$) nor the interaction season × sex had a significant effect (Two-way PERMANOVA $F_{3,64} = -4.14$, $P = 0.461$). Blood collected in winter and before the breeding season exhibited a significantly lower $\delta^{13}C$ compared to the two preceding sampling sessions (incubation and chick rearing; all $P < 0.001$; Fig. 2). $\delta^{13}C$ values recorded during the two breeding sampling sessions (incubation, chick rearing) did not exhibit a statistical differences ($P = 0.316$) whereas $\delta^{15}N$ values recorded in the incubation period were significantly lower than those recorded during chick rearing ($P = 0.005$) or in winter ($P = 0.037$; Fig. 2). Overall, age class had a strongly significant influence on stable isotope values (PERMANOVA $F_{1,86} = 6.50$, $P < 0.002$) when comparing adult and chick samples collected at the end of chick rearing. Considered separately, adults and chicks had similar $\delta^{13}C$ values (MW $U = 103$, $P = 0.355$), but chicks exhibited significantly lower $\delta^{15}N$ values than adults (MW $U = 45$, $P < 0.002$; Fig. 2). Overall, average $\delta^{13}C$ for females were consistently higher than for males from the same sampling session (Fig. 2), but this was never significant. Isotopic richness was higher for samples collected outside the breeding season (>0.15‰^{2}) than for samples collected during incubation (0.07‰^{2}) or chick rearing (chicks: 0.08‰^{2}, adults: 0.13‰^{2}; adult value strongly affected by one sample with very different carbon and nitrogen isotope values; isotopic richness: 0.08‰^{2} without this outlier).

Feather samples were collected from 89 known-sex Cape gannets during 2009–2010. Considering only the adult samples, sampling session and sex had significant effects on the stable isotope values (PERMANOVA $F_{3,69} = 2.67$, $P = 0.006$; $F_{1,71} = 3.15$, $P = 0.023$; Supplementary material Table S2) but the interaction season × sex did not (PERMANOVA $F_{3,66} = -2.45$, $P = 0.264$; Fig. 3). Only $\delta^{13}C$ was found to vary significantly between sampling sessions (KS $H_{3,69} = 8.51$, $P = 0.037$) and sex (MW $U = 381.5$, $P = 0.028$). Adult and chick feathers differed significantly in their stable isotope ratios (MANOVA Wilk's lambda $F_{1,30} = 28.68$, $P < 0.001$). No statistical differences were observed for $\delta^{13}C$ values (t test $t = 0.86$, $P = 0.399$), but chicks exhibited significantly lower $\delta^{15}N$ values than adults (MW $U = 0$, $P < 0.001$) (Fig. 3). As expected, isotopic richness was lower in feathered chicks

(0.05‰^{2}) than adults (from 0.12‰^{2} for breeding adults to 0.41‰^{2} for incubating birds). Considering only adult samples, isotopic richness was higher in feathers than in blood (average: 0.23 vs 0.13‰^{2}).

Determination of diet using Bayesian mixing models

When corrected with tissue-specific trophic enrichment factors, all Cape gannet isotope values with one exception fell within the simulated mixing polygons calculated with the five potential prey groups (Supplementary material Fig. S1), allowing further diet determination using mixing models. The exception was the feather of one incubating female which displayed high $\delta^{13}C$ (−13.4‰) and low $\delta^{15}N$ (13.0‰) compared to other individuals from the same sampling session ($\delta^{13}C$: −14.5 ± 0.5‰, $\delta^{15}N$: 14.3 ± 0.4‰); this data point was removed from the dataset entered in the mixing models.

Overall, MixSIAR models run with blood data indicated that sardine made up the largest contribution to the diet of Cape gannet adults and chicks, followed by anchovy-redeye round herring combined (Fig. 4, Supplementary material Table S3). The importance of saury increased outside of and immediately before the breeding season (Fig. 4). Hake and squid were not found to be major contributors to the nutrients assimilated. Feather data confirmed the importance of small pelagic fish during moult (Fig. 5). The effects of season and sex observed to be statistically significant earlier were not revealed by the mixing model, possibly because MixSIAR is not sensitive to relatively small changes in diet. During breeding, adults fed their chicks similar prey species to those they fed on themselves (Figs. 4, 5).

Fatty acids

Potential prey

The FA composition of 46 individuals (four fish and one squid species) was determined (Supplementary material Table S1). Twenty-three FAs were identified as forming more than 0.5% of the TFA in at least one potential prey species. All five species were characterized by high amounts of PUFAs (>57% TFA), followed by SFAs (20–30% TFA), and MUFAs (9–20% TFA). Among species, the most common FAs in decreasing order of importance were docosahexaenoic acid [22:6(n-3), DHA; 37–43% TFA], palmitic acid (16:0; 14–24% TFA), and eicosapentaenoic acid [20:5(n-3), EPA; 10–16% TFA]. Overall analysis showed that the five species were distinguished on the basis of their FA composition (PERMANOVA $F_{4,41} = 19.52$, $P < 0.001$; Fig. 6a, b); however, pairwise comparisons highlighted that sardine and anchovy could not be distinguished on the basis of their FA profiles only ($P = 1$), but all the other species could ($P < 0.003$). SIMPER identified, in decreasing order of importance, 22:1(n-11), DHA, 18:1(n-9), 16:0, 20:1(n-9), 16:1(n-7), 20:5(n-3), and 16:4(n-1) as the main FAs for the separation of the five potential prey species (cumulative contribution 74%). Redeye round herring was statistically richer in 22:1(n-11) than the other four species (KS $H_{4,41} = 38.75$, $P < 0.001$, all pairwise comparisons

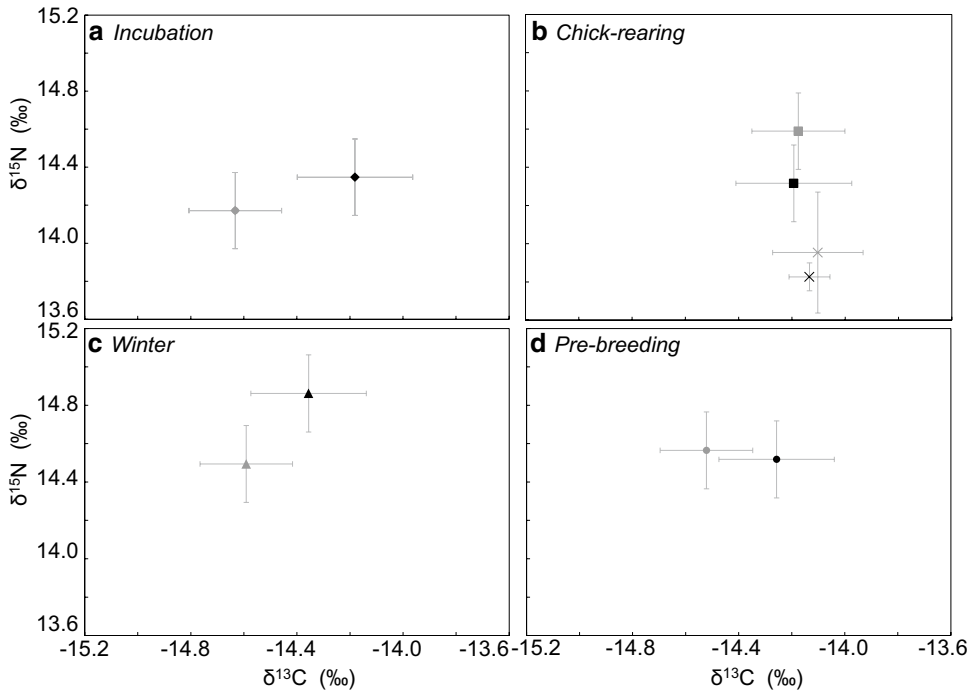

Fig. 3 Carbon and nitrogen stable isotope values measured in the feathers of adult (incubation, end of chick rearing, wintering, pre-breeding) and chick (end of chick rearing; *stars*) Cape gannets sampled in 2009–2010. *Black* females, *grey* males. *Error bars* indicate standard deviations

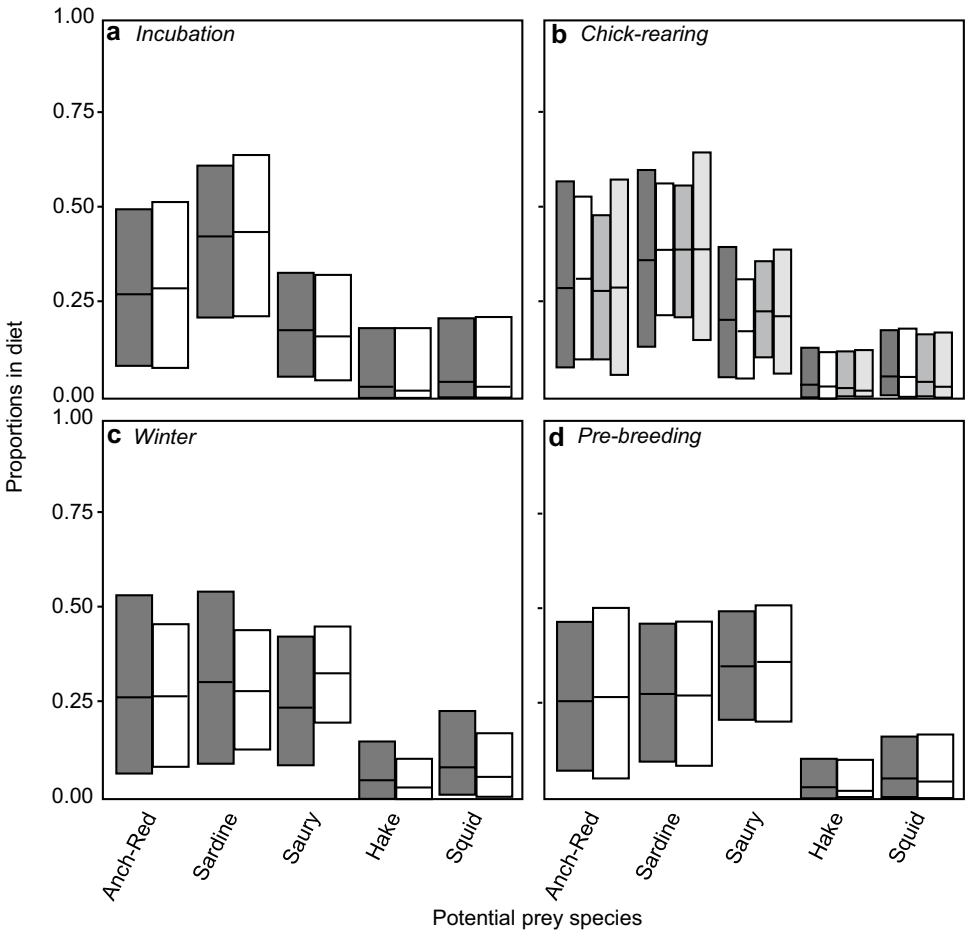

Fig. 4 Stable isotope mixing model (MixSIAR) results with predicted diet proportions (median values and 5th to 95th percentiles) of each five prey group compared to $\delta^{13}C$ and $\delta^{15}N$ values of Cape gannet blood. *Dark grey* adult females, *white* adult males, *light grey* chick females, *very light grey* chick males. Anch-Red: anchovy and redeye round herring combined

$P < 0.007$). Chokka squid exhibited the highest proportions of palmitic acid (16:0 KS $H_{4,41} = 24.02$, $P < 0.001$, all pairwise comparisons $P < 0.001$), and chokka squid and redeye round herring exhibited the highest amounts of 20:1(n-9) (KS $H_{4,41} = 37.81$, $P < 0.001$, all pairwise comparisons $P < 0.040$). Hake was particularly rich in 18:1(n-9) (KS $H_{4,41} = 33.15$, $P < 0.001$, all pairwise comparisons $P < 0.001$).

Cape gannets

Sixteen FAs were identified as forming more than 0.5% of TFA in the whole blood samples from Cape gannets (Supplementary material Table S2). Overall, SFAs were dominant in all groups (37–47% of TFA), followed by PUFAs (36–43% of TFA) and MUFAs (16–19% of TFA) except in blood collected from females before egg laying where the order of MUFAs and PUFAs was reversed (33 and 30%, respectively). The dominant FAs in decreasing order of importance were palmitic acid (>21% of TFA), stearic acid (>12% of TFA), EPA (>7% of TFA), DHA (>7% of TFA), and arachidonic acid [20:4(n-6), AA; >6% of TFA].

Both sex and sampling sessions were found to have an effect on the FA composition of whole blood of adult Cape gannet samples (Two-way PERMANOVA $F_{1,61} = 8.97$, $P < 0.001$, $F_{3,59} = 10.73$, $P < 0.001$) as well as their interaction (Two-way PERMANOVA $F_{3,55} = 0.74$, $P < 0.001$; Fig. 6c, d). The SIMPER analysis indicated that oleic acid was the most informative FAs to distinguish among groups followed by EPA, AA, DHA, and palmitic acid (77% of the dissimilarity was explained by these five FAs). The most striking dissimilarity among all the samples (sex and sampling session combined) was from six out of the eight samples collected from females before egg laying. These samples had three times the amount of oleic acid than all the other samples (33.5 ± 3.0 vs 11.3 ± 2.0% of TFA) and half of the amount of AA (5.6 ± 2.0 vs 11.2 ± 3.4% of TFA).

Comparison of the FA composition of adult and chick blood collected at the end of chick rearing indicated that the interaction age class × sex had a highly significant effect on FA compositions (Two-way PERMANOVA $F_{1,24} = 2.48$, $P = 0.001$). Taken individually, however, these two factors either were only marginally significant (age class $F_{1,26} = 2.01$, $P = 0.038$) or not significant

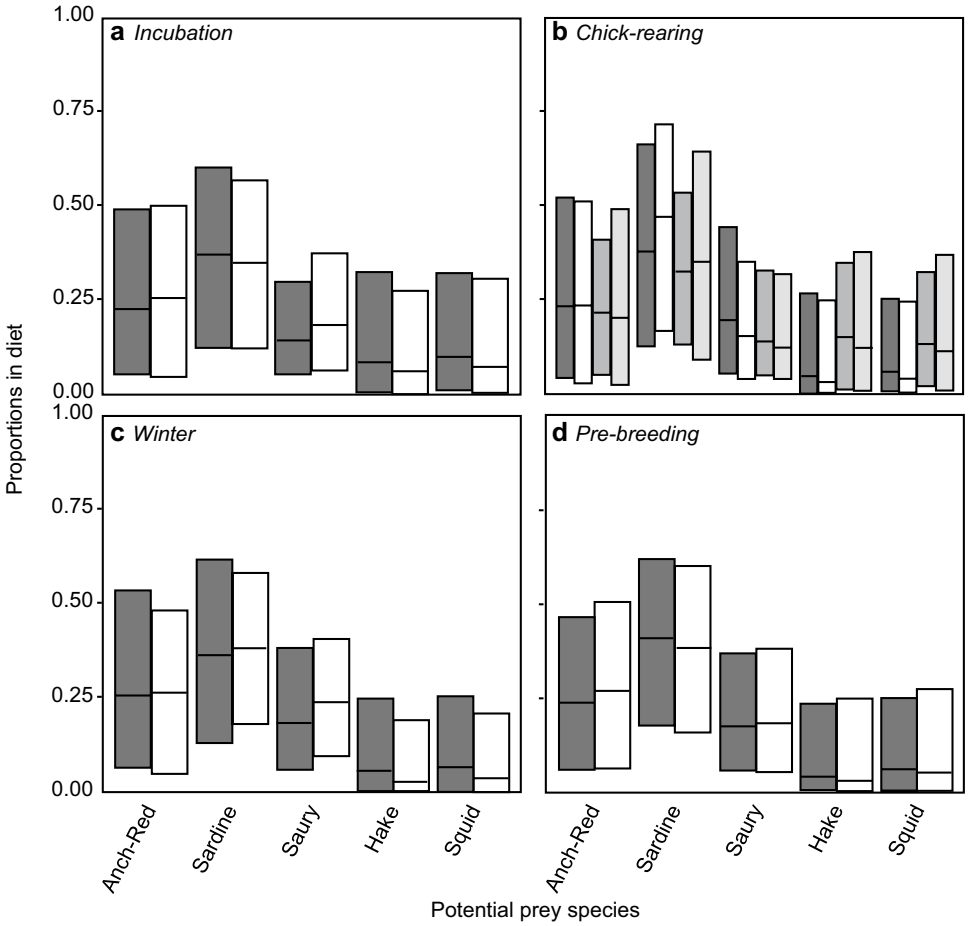

Fig. 5 Stable isotope mixing model (MixSIAR) results with predicted diet proportions (median values and 5th to 95th percentiles) of each five prey group compared to $\delta^{13}C$ and $\delta^{15}N$ values of Cape gannet feathers. *Dark grey* adult females, *white* adult males, light grey: chick females, *very light grey* chick males. Anch-Red: anchovy and redeye round herring combined

(sex $F_{1,26} = 1.41$, $P = 0.111$). The significant sex × age class interaction was mainly driven by the FA composition of adult female blood compared to the three other groups (adult males, chick females and males; Fig. 6e, f). Adult female blood samples were richer in myristic acid (14:0; 4.8 ± 2.5 vs 1.5 ± 0.5% of TFA) and EPA (14.9 ± 6.7 vs 8.9 ± 2.6% of TFA) but poorer in DHA (8.2 ± 2.1 vs 13.1 ± 2.1% of TFA). These comparisons were never significant, however, probably because of the small sample size for adult females. Considering more specifically the eight FAs identified that distinguished the potential prey species, none of the comparisons among the four groups of Cape gannets (female and male adults, female and male chicks) showed significant differences (all $P > 0.05$).

Investigation of diet using fatty acid composition of total blood samples

Principal component analysis yielded eight principal components with eigenvalues >1 which together represented 98.5% of the total variance. The subsequent discriminant analysis identified four discriminant functions to separate all five prey groups (Supplementary material Fig. S2). As seen earlier (Fig. 6a, b), anchovy and sardine exhibited a close FA composition. When gannet samples were included as supplementary individuals, all but one were classified with the hake group. The last sample was classified with the sardine group.

Individual specialization

Stomach content $\delta^{13}C$ values (SI_{SC}) correlated with whole blood $\delta^{13}C$ values for the same individuals across all sampling sessions (Pearson $r = 0.390$ $P = 0.040$; intercept = −9.579, slope = 0.399, $R^2 = 0.152$). However, the addition of R^2 and the slope markedly differed from 1 indicating relaxation of one or more of the assumptions. Overall, stomach content $\delta^{15}N$ values did not correlate with whole blood $\delta^{15}N$ values (Pearson $r = 0.023$ $P = 0.982$).

Both $\delta^{13}C$ and $\delta^{15}N$ values of whole blood correlated to feathers values of the same individuals ($\delta^{13}C$ Pearson $r = 0.473$ $P = 0.011$; intercept = −4.448, slope = 0.613, $R^2 = 0.223$; $\delta^{15}N$ Pearson $r = 0.477$ $P = 0.010$; intercept = 2.057, slope = 0.916, $R^2 = 0.227$). This indicates

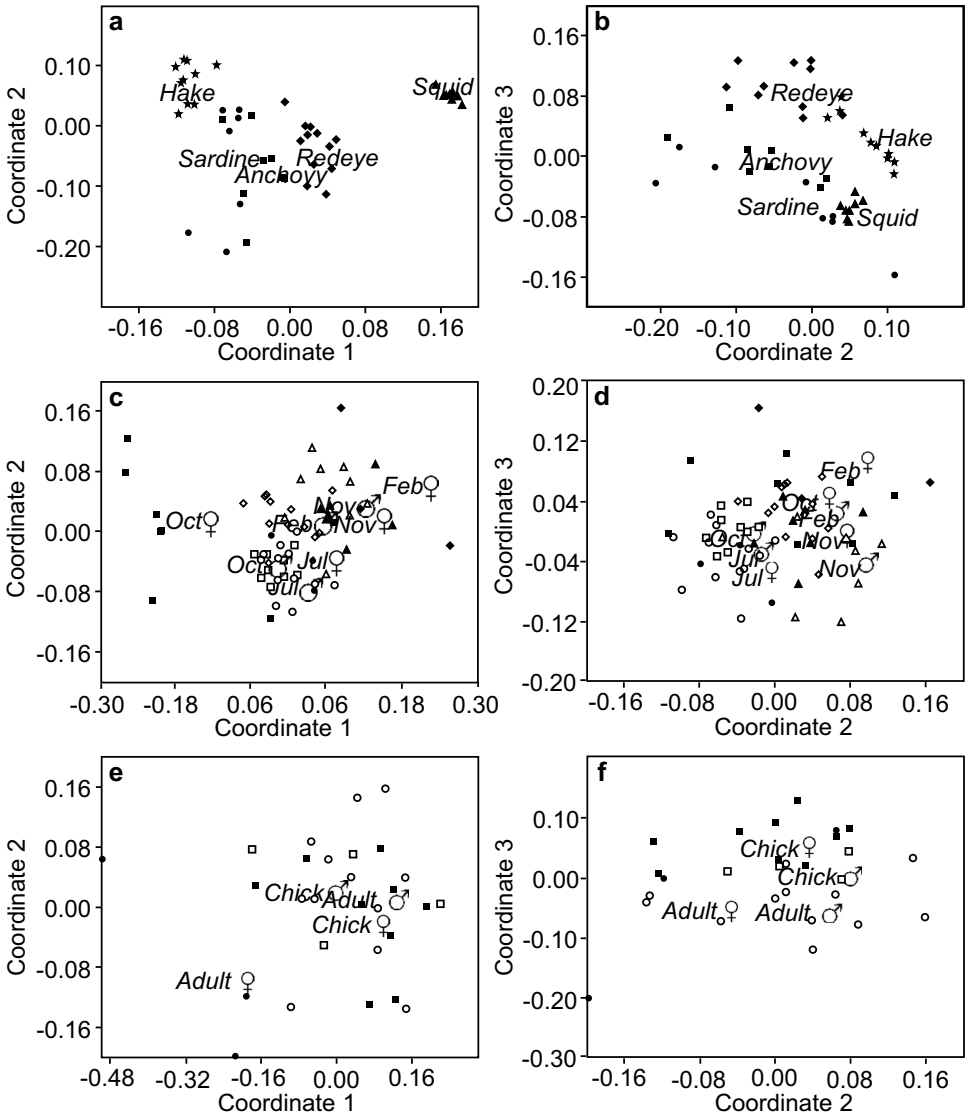

Fig. 6 Ordination (non-metric multidimensional scaling plots) of fatty acid profiles of prey species (*upper panels* **a**, **b**; 3D stress = 0.10; *circle* sardines, *squares* anchovy, *diamonds* redeye round herring, *stars* hake, *triangles* squid), adult Cape gannet blood samples (*middle panels* **c**, **d**; 3D stress = 0.11; females: *closed symbols*, males: *open symbols*), and adult and chick blood samples collected in February 2010 (*lower panels* **e**, **f**; 3D stress = 0.12; females: *closed symbols*, males: *open symbols*)

that individual diet was similar during both the few weeks before sampling, and during moult at an individual level.

Discussion

The breeding period has a critical influence on the trophic ecology of seabirds because of the energetic costs of egg production for females in terms of macro- and micro-nutrients (Williams 2005), the need to return regularly to the nest, the combined energetic demands of adults and chicks on resources, and potential intraspecific competition if resources around the colony are scarce. The present study combined three techniques to investigate how these factors influence the diet specialization of the colonially breeding Cape gannet. Variation in the diet of Cape gannets was linked to seasonality, but neither age nor sex. The combination of stomach content data with dietary tracers and repeated sampling over the course of a year indicated that adults were targeting similar prey species whether close to or far from their nesting sites. The combination of techniques further allowed us to detect diet specialization at the individual level. Finally, interactions with fisheries were revealed.

Seasonal variation of Cape gannet diets

Despite the unbalanced sampling of stomach contents in 2009–2010, their analysis confirmed the seasonal trend found in previous studies (e.g. Batchelor and Ross 1984; Klages et al. 1992; Green et al. 2015b; Table 3) with small pelagic fish dominating, and the proportion of saury increasing in winter (July) and before the breeding season (October). Smaller anchovy were caught in winter as seen for Cape fur seals *Arctocephalus pusillus pusillus* studied in

Table 3 Summary of all studies on Cape gannet diet conducted on Bird Island, Algoa Bay

Technique	Years of study	References
Stomach contents	1936	Courtenay-Latimer (1954)
	Jan 1978–Mar 1981	Batchelor and Ross (1984)
	May 1979–Mar 1991	Klages et al. (1992)
	Nov 1994–Dec 1995	Adams and Klages (1999)
	Sum 2004–Sum 2009	Moseley et al. (2012)
	Nov 2009–Oct 2010	This study
	May 1979–Mar 2013	Green et al. (2015b)
Stable isotopes (blood, feathers)	Feb–Mar 2006 (Adu and Chi)	Jaquemet and McQuaid (2008)
	Nov 2009 (Adu)	Moseley et al. (2012)
	Nov 2009–Oct 2010 (Adu and Chi)	This study
Fatty acids (blood)	Nov 2009	Moseley et al. (2012)
	Nov 2009–Oct 2010	This study

Adu adults, *Chi* chicks, *sum* summer

the same bay (Connan et al. 2014a), reflecting the life history and abundance of that fish species in the area (Barange et al. 1999). This highlights again the role of marine top predators as useful indicators of the marine ecosystem (Boyd et al. 2006).

Bayesian mixing models of carbon and nitrogen stable isotope ratios measured in Cape gannet blood and five potential prey groups also identified small pelagics as the main prey in all four sampling sessions. As with stomach contents, higher proportions of saury were indicated during winter (July) and autumn (October). Considering the longer integration time of the dietary signal in blood (Hobson and Clark 1993) compared to the few hours of integration in stomach contents (Laugksch and Duffy 1986), stable isotopes thus suggest that when they are not bound to the colony during breeding, adult Cape gannets target similar prey species at both long and short time scales. The seasonal decrease in $\delta^{13}C$ (from ~−15.7‰ during incubation to ~−16.4‰ before breeding) suggests that outside the breeding season, gannets use foraging areas that are farther offshore, where background stable isotope levels are more depleted (Hill et al. 2006). While breeding, Cape gannets can forage at least 250 km away from the colony at Bird Island (Moseley et al. 2012; Green et al. 2015a). Outside of the breeding season, they may disperse even more widely; individuals of unknown origin have been observed from northern Mozambique on the east coast of Africa to Nigeria on the west coast, more than 4000 km from the closest breeding colony (South African Bird Ringing Unit 2017). In addition, a large number of Cape gannets are associated with the KwaZulu-Natal sardine run in winter (O'Donoghue et al. 2010). The ages and breeding stages of these birds were, however, unknown, and it is possible that immature birds (and failed breeders) may disperse further than successful breeders (Votier et al. 2011).

Feather stable isotope values also highlighted the importance of small pelagic fish during moulting. The timing and duration of moult in the Cape gannet are not known, but in the Northern gannet, breeding parents exhibit moult-breeding overlap (Nelson 1965). The feathers analysed during our study are, therefore, likely to represent data from two years: feathers newly grown in winter and pre-breeding, and old feathers from the incubation period of the previous year. Feathers collected previously in 2006 exhibited similar values, strengthening the idea that Cape gannets from Bird Island (Algoa Bay) generally rely mainly on small pelagic fish during moulting (Jaquemet and McQuaid 2008).

In contrast to the findings of stomach content and stable isotope analyses, the outputs of the multivariate analyses of FA data suggested that hake was the dominant prey item for all but one of the Cape gannets sampled. A captive study conducted on the African penguin, *Spheniscus demersus*, has shown that a dietary signal can be detected in whole blood when those birds are fed hake rather than sardine, and that integration time between the two methods (stable isotopes and FAs) seems similar (Connan unpubl. data). In our analysis, we focused on dietary FAs rather than the whole array of FAs detected (Iverson et al. 2004; Wang et al. 2010), but there may still have been elongation/shortening of FAs during digestion. A study on captive gannets would allow the calculation of species-specific calibration FAs (Wang et al. 2010), which could then be used in more quantitative analyses (i.e. mixing models) to confirm or refute the importance of hake.

Influence of adult sex on dietary tracers

Blood biochemical markers can be influenced by the condition of the birds (e.g. Cherel et al. 2005a; Käkelä et al. 2009). Body condition indices showed that adult females

at the end of chick rearing exhibit poorer body condition than both males, and females at other stages of the annual cycle. Females make longer trips to sea as their chicks get older (Rishworth et al. 2014b). Female Northern gannets have also been found to undertake longer foraging trips and to dive deeper than males (Lewis et al. 2002). This can be interpreted in two ways. It has been suggested that increased foraging effort is an attempt to replenish their reserves following breeding (Rishworth et al. 2014b). Alternatively, competitive exclusion of females by males as the chick-rearing season advances and resources become scarce around the colony may oblige females to forage further afield so that greater foraging effort drives poor condition, rather than being a response to poor condition. Male Northern gannets have been described as more aggressive than females at the breeding colony (Nelson 1965) and this may well also be the case for Cape gannet. The hypothesis that males would be more efficient than females at capturing prey has already been suggested by Lewis et al. (2002). Unfortunately, no at-sea data are currently available but this will change with the increased use of miniaturized video cameras that may provide this kind of data in the next few years (e.g. Thiebault et al. 2014). The slightly lower body condition and higher foraging effort of females during breeding suggest that females may be especially vulnerable to changes in prey availability. A captive study with an extreme fasting animal, the king penguin *Aptenodytes patagonicus*, has revealed that fasting increased $\delta^{15}N$ (Cherel et al. 2005a). The lower body condition of female gannets was not reflected in their stable isotope ratios; chick-rearing females had slightly lower $\delta^{15}N$ values than chick-rearing males, but the difference was not significant, nor was this subtle difference detected by the Bayesian mixing models. This indicates that males and females relied on the same resources at that time of the year. The biggest difference between male and female blood samples was found in winter when females exhibited a higher $\delta^{13}C$ and lower $\delta^{15}N$ values than males. This may indicate that females showed a stronger preference than males for small pelagics rather than saury. Sexual segregation in isotopic data has been found in Northern (Stauss et al. 2012) and Australasian *Morus serrator* (Angel et al. 2016) gannets. In the latter, however, the difference between males and females reflected differences in the origins rather than the species of prey eaten.

The differences between sexes and sampling sessions in FA whole blood composition were mainly driven by six of the eight females sampled prior to egg laying (in October 2010). The blood of these six females was three times richer in oleic acid and contained half the arachidonic acid of the other samples. These females were not yet incubating when caught, so these changes in FA composition were probably related to egg production. Oleic and arachidonic acids have been found in high quantities in Northern gannet eggs (Surai et al. 2001). No such pattern was found in the other samples suggesting that metabolism and diet were similar in all birds. A difference in lipid mobilization between the sexes cannot, however, be ruled out (Jacobs et al. 2013).

Diet of adults vs chicks

Differences between adult and chick diets could only be investigated through dietary tracers as prey brought back to the colony during breeding reflect chick rather than adult diet (Barrett et al. 2007). No important differences were obvious in the stable isotopes or FAs of adult and chick blood, which suggests that adults mainly feed their chicks on prey that they themselves feed on. Chicks were sampled at the end of the chick-rearing period to reduce the impact of intense growth on biochemical markers (Sears et al. 2009). During feather growth, however, stable isotope data suggested that either chicks were fed prey of slightly lower trophic levels than moulting adults or we witnessed some physiological effect of differences in the metabolism of adults and chicks. For example, growth results in lower $\delta^{15}N$ values in rhinoceros auklet *Cerorhinca monocerata* chicks (Sears et al. 2009). Because the whole feather was homogenized, gannet chick feathers may carry both the dietary signal during intense growth and the growth plateau before fledging (20–80 days; Cooper 1978; Mullers et al. 2009). However, the difference between adult and chick feathers was also found in 2006 for Cape gannets from Bird Island and the west coast Malgas Island though not for gannets from Ichaboe Island in Namibia (Jaquemet and McQuaid 2008). This suggests that a difference in diet rather than physiology may be the most plausible explanation. The blood of male and female chicks did not exhibit any differences in their stable isotope or FA compositions, indicating that they were likely fed similar prey species. Differential allocation between male and female offspring has been found in dimorphic (Weimerskirch et al. 2000) but also monomorphic birds (Cameron-MacMillan et al. 2007). The similarity in dietary tracers in our study does not provide any information on the amount of food provided by the parents. Further work combining dietary tracers with continuous weighing of sexed chicks over the whole chick rearing would provide that information and may have important implications as post-fledging survival is lower in lighter fledgling Cape gannets (Jarvis 1974).

Diet specialization at the individual level

The combination of stomach contents with stable isotopes of blood and feathers provided information on the diet of

individuals at three different time scales: diet over the last 12 h, diet over the last few weeks, and diet during moult. When stomach contents and whole blood stable isotopes were compared, the R^2 values and slopes suggested that one or more assumptions were violated giving little power to the outcomes of the correlation. This may be due to the way stomach contents were collected, i.e. spontaneous regurgitation, implying that the entire stomach contents were not collected; very likely only a portion. Despite this, the highest $\delta^{15}N$ value observed in the blood samples (~+1‰ compared to the mean of all the other samples) corresponded to the stomach content of an adult male which comprised primarily hake remains. For whole blood and feather stable isotope values, high correlations were detected for both $\delta^{13}C$ and $\delta^{15}N$, indicating individual diet specialization at longer time scales. Together, this suggests that individuals may specialize on particular prey species or on fisheries discards. Individual specialization indicated by stable isotopes, combined with an observed increase in abundance in the Eastern Cape (Crawford et al. 2015), suggests increasing food availability. Indeed, following the optimal foraging theory, one would expect a higher level of specialization with an increase in prey availability (Pyke 1984). Such individual specialization may have important ecological implications for population dynamics (Ceia and Ramos 2015).

Interactions with fisheries

The consumption of fisheries discards, such as hake, by Cape gannets has been highlighted in numerous studies on the west coast of South Africa (e.g. Pichegru et al. 2007; Mullers and Navarro 2010; Tew Kai et al. 2013). Rarely found above 50 m deep (Smale 1984), hake would be unavailable to gannets other than as fishery discards (Adams and Walter 1993). Fishery discards have been identified from time to time in the stomach contents of Cape gannets on the south-east coast (Green et al. 2015b), and were also found in 5 stomach contents (out of 40) collected in our study. Hake were reported in the diet of Cape gannets from this colony in the early 1980s (Batchelor and Ross 1984), but whether the link between gannets and fisheries has increased over the years cannot be confirmed due to the relatively low number of stomach contents collected. Direct evidence of gannet-fishery interactions on the south-east coast has been produced using miniaturized cameras, with equipped gannets originating from the study colony (Tremblay et al. 2014). Foraging on hake discards by Cape gannets is a concern not only in terms of energetic needs (fishery discards are of lower calorific values than natural prey; Pichegru et al. 2007), but also because of accidental mortality during interactions with fishing gear, either through plunge diving into trawl nets or collisions with the warp cables (Watkins et al. 2008). While mitigation measures such as bird scaring lines have reduced incidences of albatross mortality, they have not had the same success in reducing gannet mortality (Maree et al. 2014). Furthermore, attractiveness is often sex-biased with, for example males Northern gannets exhibiting stronger association with fishing vessels than females (Votier et al. 2013), and males procellariiforms being over-represented in fishery by-catch (Ryan and Boix-Hinzen 1999). Further research will be necessary to assess whether fisheries interaction is also sex biased in Cape gannets.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest and that consent was obtained from all parties.

Ethical approval All work on Cape gannets was performed under permits issued by the Ethic committee from Rhodes University and South African National Parks (CONM746).

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