# Adjustment of pre-moult foraging strategies in Macaroni Penguins *Eudyptes chrysolophus* according to locality, sex and breeding status

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The annual moult creates the highest physiological stress during a penguin's breeding-cycle and is preceded by a period of hyperphagia at sea. Although crucial to individual survival, foraging strategies before moult have been little investigated in keystone marine consumers in the Southern Ocean. The Macaroni Penguin *Eudyptes chrysolophus* demonstrates how individuals may adjust their foraging strategies during this period in line with constraints such as potential intraspecific competition between localities, foraging ability between dimorphic sexes and timing at sea between breeding and non-breeding population components. We recorded pre-moult behaviour at sea for 22 Macaroni Penguins from Crozet and Kerguelen Islands (southern Indian Ocean) during 2009 and 2011, using light-based geolocation and stable isotope analysis. Penguins were distributed in population-specific oceanic areas with similar surface temperatures (3.5 °C) south of the archipelagos, where they foraged at comparable trophic levels based on stable isotopes of their blood. Bayesian 'broken stick' modelling with concurrent analysis of seawater temperature records from the animal-borne devices showed that within each population, females remained 6 days longer than males in the colder waters before heading back towards their colonies. Finally, 17 other non-breeding individuals that moulted earlier had a higher mean blood $\delta^{15}$N value than did post-breeding birds, meaning that early moulters probably fed more on fish than did late moulters. Our findings of such adjustments in foraging strategies developed across locality, sex and breeding status help understanding of the species' contrasted pre-moult biology across its range and its ecology in the non-breeding period.

**Keywords:** Crested Penguins, light-based geolocation, marine habitat, marine resources, non-breeding, Southern Ocean, specialization, stable isotopes.

Moulting is generally associated with high energy expenditure in birds, among which penguins probably endure some of the most intense expressions of this stress. For penguins, moult takes place on land, requiring a complete and prolonged (2–5 weeks) fast, during which they renew their entire plumage (Stonehouse 1967, Brown 1986, Adams & Brown 1990). Unlike any other bird species, moulting penguins depend exclusively on their endogenous fat and protein reserves (Cherel *et al.* 1994), so this remarkable moult is preceded by the build-up of large nutrient reserves during a period of hyperphagia at sea (Cherel *et al.* 1993, Cherel 1995, Green *et al.* 2009a). Such an intensive feeding phase raises questions about the behavioural adjustments that penguins may make at that time, with respect to their use of the main ecological resources of space, time and food.

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For colonial breeders such as penguins, potential intraspecific competition at sea in such a context may be especially intense, particularly as several colonies may exist in close proximity to each other and each locality may contain several million pairs. In this context, the 'Hinterland' model proposed by Cairns (1989) predicts that breeding seabirds should develop specialized foraging strategies in areas where exploitation occurs, which may overlap between neighbouring colonies. Secondly, in diving seabirds, body mass is generally correlated with diving capacity (Schreer & Kovacs 1997), and larger penguin species may therefore feed more at greater depths (e.g. Wilson *et al.* 1991). Therefore, the existence of sexual size dimorphism within a given penguin species may result in different foraging abilities between sexes, thereby influencing their respective time needed to achieve sufficient body condition prior to moult. Thirdly, breeders and non-breeders generally differ in the timing of pre-moult foraging and moulting, the breeders being tied to the colony for longer in the season until the chicks fledge (Stahl *et al.* 1985, Williams 1995). It might therefore be possible that breeders and non-breeders rely on different marine resources and habitats to prepare for the moult fast.

In this study we focused on the pre-moult foraging trips of the Macaroni Penguin *Eudyptes chrysolophus*. This species breeds in large colonies that may contain hundreds of thousands of pairs, and is the most numerous penguin species, with more than 8 million breeding pairs estimated worldwide (Trathan *et al.* 2012, Crossin *et al.* 2013). Thus, it is considered to have the greatest prey biomass consumption of any seabird species, reaching about 9.2 million tonnes of marine resources each year (more than 13% of the total amount of food taken by all the world's seabirds; Brooke 2004). We therefore assume that potential competition levels might be particularly elevated in this predator between populations and might therefore lead to niche specialization at sea. Our first objective in this study was therefore to assess whether pre-moulting Macaroni Penguins from different populations actually segregate spatially at sea, following the 'Hinterland' model. Our second objective, considering the sexual size dimorphism consistently exhibited in eudyptid penguins (Warham 1975), was to test for an effect of sex on the time needed to complete hyperphagia at sea. Our third goal was to assess whether the shift in the

timing of moult between the breeders and non-breeders induces different at-sea trophic niche use prior to moult.

As inadequate foraging opportunities, insufficient storage of energy reserves or subsequent poor remobilization of stored reserves may reduce an individual's survival during moult (Brown 1985, Cherel *et al.* 1994, Green *et al.* 2004), this period of hyperphagia at sea appears critical in the penguins' life-cycle. However, studying the at-sea ecology of pre-moulting penguins poses severe methodological challenges. The relatively long time at sea (2–9 weeks; Warham 1975, Williams 1995) raises issues about the use of back-mounted satellite tracking units, notably because of the prolonged adverse drag effects the devices would induce on these highly streamlined animals (Bannasch *et al.* 1994, Wilson *et al.* 2004). Also, the use of miniaturized but less precise light-based geolocation loggers may seem ill-adapted because this at-sea trip spans the March equinox (Stahl *et al.* 1985), a period when light-derived estimates of latitude are usually unreliable (Wilson *et al.* 1992, Hill 1994). Consequently, at-sea movements of Macaroni Penguins before moult have been very little documented compared with other stages of its life-cycle (but see Waluda *et al.* 2010, and insights from Brown 1987 and Green *et al.* 2009b).

In this study we combined recent approaches in estimating both spatial (light-based geolocation improved by *in situ* water temperature recording, enabling the estimation of positional data even during equinoxes, e.g. Thiebot & Pinaud 2010) and temporal (Bayesian 'broken stick' modelling, e.g. Authier *et al.* 2012a) patterns of bird distribution, as well as in trophic ecology (stable isotope analysis). This combination allowed the characterization of at-sea foraging strategies in two Macaroni Penguin populations during the pre-moult hyperphagia trip. We studied Macaroni Penguins from Crozet and Kerguelen Islands, two of the main breeding sites for the species (Crossin *et al.* 2013) with potentially overlapping at-sea distributions in the southern Indian Ocean (Thiebot *et al.* 2011).

## METHODS

Our study was undertaken in the southern Indian Ocean, a well-structured oceanic region with sharp latitudinal separation of water masses

by oceanographic fronts (Park *et al.* 2009). From north to south, the Subantarctic and the Polar Fronts encompass the Polar Frontal Zone (PFZ), within which lie the Crozet (46.4°S, 51.8°E) and Kerguelen (49.1°S, 70.3°E) archipelagos, *c.* 1385 km apart. Further south is the colder Low Antarctic Zone (LAZ), limited by the southern boundary of the Antarctic Circumpolar Current.

## Surveying at-sea ecology before moult

At the Crozet archipelago, 10 Macaroni Penguins (six males and four females; sex inferred from bill morphometrics given by Williams & Croxall 1991) that raised chicks successfully were equipped with data-loggers at the Pointe Basse colony, Possession Island. The loggers were deployed on 11 February 2009, a few days before the expected departure date of the birds for their pre-moult foraging trip (Stahl *et al.* 1985). At the Kerguelen archipelago, 20 post-breeding Macaroni Penguins (12 males and eight females) were similarly equipped on 15–16 February 2011 at the Cape Cotter colony. To track penguins during their complete pre-moult foraging trips, we deployed BAS MK5 (Crozet) or MK15 (Kerguelen) miniaturized global location sensing (GLS) loggers (British Antarctic Survey, Cambridge, UK; weight *c.* 5 g), mounted on specially designed leg rings following Bost *et al.* (2009). Leg rings used were soft plastic bands finely adjusted to the diameter of the penguins' legs. To avoid causing injuries to the leg skin over deployment duration, the inner side of the rings was covered with marine mastic and both upper and lower edges were manually filed prior to deployment. GLSs measured light level every minute and recorded the maximum light level at the end of every 10-min period together with the time, enabling the determination of the location of the bird at that time (Wilson *et al.* 1992, Hill 1994). In addition, the GLSs recorded ambient sea temperature once during every 20-min period of continuous immersion with a resolution of 0.0625 °C and an accuracy of ± 0.5 °C. Penguins from Kerguelen were also weighed to the nearest 50 g on deployment and recapture. To assess whether carrying a logger would negatively affect the penguins' foraging ability, we weighed 20 control birds (15 males and five females) at the same average departure and arrival dates as the tracked birds and compared mass gain between the two groups.

## Analysis and comparison of at-sea ecology

Two daily location estimates (at noon and midnight) are commonly produced with the light-based geolocation approach, with an expected spatial error of *c.* 120–130 km for breath-holding diving animals (Staniland *et al.* 2012) and unreliable latitude estimates during the equinox periods when day length is equivalent everywhere on Earth. In our case, penguin movements were estimated from recovered GLSs with a maximum-likelihood approach using the package 'tripEstimation' in R 2.9.0 (R Development Core Team 2009, Sumner & Wotherspoon 2010). Following the computer software script given in Thiebot and Pinaud (2010), we combined additional data with the light-based geolocation procedure to calculate twice-daily realistic location estimates even during the equinox periods, hence avoiding the removal of any location estimates from the datasets. These additional data comprise the colony positions (which allowed us to set the penguins' start/end location), the assumed mean travel speed of penguins of 2 km/h (limiting the possible distance travelled between successive locations) and a geomatic land mask, preventing any location estimates on emerged areas. Another important component of this advanced approach described in Thiebot and Pinaud (2010) for the same species was the matching procedure of seawater temperature recorded *in situ* by the loggers throughout the trip, with sea-surface temperature satellite data. This procedure enabled us systematically to refine latitude estimates all through the trip, including the equinox period, as sea-surface temperature varies with latitude (cooler polewards). This approach is particularly valuable in the southern Indian Ocean, where surface water circulation produces a strong latitudinal stratification of the water masses (Park *et al.* 2009). Weekly satellite data were downloaded from the POET-PODAAC website (http://podaac-tools.jpl.nasa.gov/las), with a spatial resolution of 1° latitude and longitude, and *ecart* parameter in the script was set to 1 °C. To provide an estimate of the spatial error of our method, we generated a second batch of the trip estimations without constraining the departure point at the colony (*fixed release* = 'FALSE' in the R code). We then measured the distance between both first location estimates calculated through the two approaches for all individuals.

The start and end dates of the trips were inferred from the time of the first and last seawater temperature records from each GLS. At-sea movements of penguins were quantified using metrics such as the time spent at sea, linear distance travelled, maximum distance reached from the colony (hereafter called 'maximum range') and bearing from the colony to the point of maximum range. To infer the date on which penguins started to move back towards their respective colonies, we used a recently developed Bayesian approach ('broken stick' modelling technique, based on beta regression analysis with 'betareg' package in R; Cribari-Neto & Zeilis 2010). This 'homing decision date' of penguins at sea translates statistically as a change-point in the distribution of relative distance to the colony vs. time (Thiebot *et al.* 2014, Fig. 1 for an example). To estimate date of the change-point $T_i$, we used a profile likelihood approach following Authier *et al.* (2012a): the likelihood was computed for each location date spanning the
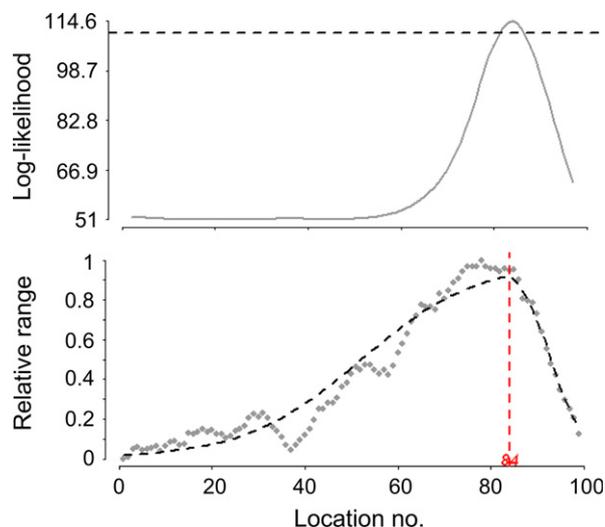


**Figure 1.** Example of a 'broken stick' determination process for homing decision date. The upper panel shows the profile of the model likelihood computed for the existence of a change-point at each location date spanning the at-sea period of the Penguins (grey curve), with the value of the approximate 95% confidence interval around the maximum likelihood (dotted line). The lower panel shows the relative distance reached from the colony by the individual, at each location estimate: grey dots show relative distance from the colony reached by the Penguin, dashed black curve shows the beta regression fitted, and vertical dashed line shows the inferred change-point. The example shown is a female from Crozet Islands; the change-point was determined for location no. 84, i.e. on 25 March 2009.

at-sea period of the penguins. The value of $T_i$ that maximized the likelihood was thus evaluated, and an approximate confidence interval for $T_i$ was computed with a Likelihood Ratio Test with 1 df. This method bypasses the need to inspect the data manually for identification of a turning point, which could only be uncertain in our case owing to the low spatial accuracy of individual GLS location estimates, especially during the equinox period (Wilson *et al.* 1992, Hill 1994). Depending on the results from a Shapiro–Wilk test for normality, we used parametric (Welch two-sample *t*-test) or non-parametric (Wilcoxon and Kruskal–Wallis rank sum tests) statistical tests to assess significant differences in metrics of the penguins' at-sea movements (see above) between populations and/or sexes. In all cases the threshold for statistical significance was set at $P = 0.05$. Bearings were categorized clockwise from 0 (North) to < 360 and were compared using circular analysis of variance (ANOVA) with a high concentration *F*-test. Values reported are mean $\pm$ 1 sd.

The marine home-range of the pre-moulting birds was measured as the 95% kernel density contours (Wood *et al.* 2000). The package 'adehabitat' (http://cran.rproject.org/web/packages/adehabitat/index.html) was used for the kernel calculations, with a constant smoothing parameter (search radius) $h = 2°$ relevant for these data (BirdLife International 2004). We used the function 'kernel-overlap' in this package, with the method 'Utilization Distribution Overlap Index' (UDOI), as recommended by Fieberg and Kochanny (2005), to quantify overlap between home-ranges. In this latter index, zero indicates two home-ranges that do not overlap and one indicates both home-ranges are uniformly distributed and have complete overlap.

## Stable isotopes

Following Cherel *et al.* (2007) the isotopic niche was used as a proxy of the trophic niche of Macaroni Penguins, with the ratios of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) reflecting their foraging habitats and diet, respectively (Newsome *et al.* 2007). The isotopic method has already been validated in the southern Indian Ocean, with seabird $\delta^{13}C$ values indicating their latitudinal foraging grounds (Cherel & Hobson 2007, Jaeger *et al.* 2010) and their $\delta^{15}N$ values increasing with prey trophic level (Cherel *et al.* 2007). Macaroni

Penguins were blood-sampled when they were fitted with a GLS and again on their return to the colony at the beginning of moult, except for one returning individual for which blood sampling was not possible in the field at Crozet, and another two at Kerguelen. As non-breeders and failed breeders moult earlier than successful breeders (Stahl *et al.* 1985), the isotopic niche of the former group was investigated by randomly sampling 20 early moulters (sex unknown; erroneous values omitted for three individuals) at Crozet in mid-March 2009, i.e. *c.* 3–4 weeks before the late moulters (GLS birds). Using allometric equations between body mass and carbon half-life in avian blood, half-life in Macaroni Penguins was estimated at *c.* 32 days (see Cherel *et al.* 2007). This means that two half-lives correspond to a *c.* 2-month duration period during which most (75%) blood carbon is renewed. A duration of 2 months is slightly longer than the pre-moult foraging period and thus includes the whole period of hyperphagia at sea. Noticeably, the effect of fasting is minimal on $\delta^{15}N$ values of penguin blood, thus precluding a nutritional bias for the trophic interpretation of blood $\delta^{15}N$ values (Cherel *et al.* 2005).

Blood was collected into a heparinized syringe by venipuncture of a Penguin flipper vein. Ethanol 70% was then added to whole blood because, in many cases, freezing was not possible in the field. The low lipid content of whole blood does not necessitate lipid extraction (Cherel *et al.* 2005), as verified here by its consistently low values of C/N mass ratio (Table 1). Before isotopic analysis, blood samples were dried in an oven at 60 °C. Relative abundance of $^{13}C$ and $^{15}N$ were determined with a continuous flow mass spectrometer (Thermo Scientific Delta V Advantage) coupled to an elemental analyser (Thermo Scientific Flash EA 1112). Results are presented in the $\delta$ notation relative to Vienna PeeDee Belemnite and atmospheric $N_2$ for $\delta^{13}C$ and $\delta^{15}N$, respectively. Replicate measurements of internal laboratory standards (acetanilide) indicate measurement errors < 0.15‰ for both $\delta^{13}C$ and $\delta^{15}N$ values.

## RESULTS

### Recapture statistics

Penguins were recaptured as soon as possible when they came back on land to moult. On Crozet, we retrieved nine loggers (of 10 deployed GLSs, five from males and four from females) between 5 and 18 April 2009. On Kerguelen, 14 loggers were retrieved between 24 March and 14 April 2011 and 13 were successfully downloaded (eight from males and five from females). Overall, 11 loggers were recovered < 3 days after the penguins landed at both localities, and 11 others 4–14 days after landing. On Kerguelen, mass gain of the penguins after their pre-moult foraging trip was similar in tracked birds that were weighed < 3 days after landing and control birds (2.17 ± 0.30 kg, $n$ = 6, vs. 2.09 ± 0.51 kg, $n$ = 15; $t_{15}$ = −0.41, $P$ = 0.69), indicating no adverse effect of GLS attachment. As fasting proceeds, normal mass loss precludes any further reliable comparison with control birds that were weighed later.

### Spatial distribution

The positional difference between the first location estimates calculated from constrained vs. unconstrained approaches averaged 118 ± 78 km, with a mean difference of 1.09° in longitude and 0.78° in latitude. The penguins from Crozet performed directional trips towards oceanic areas south of the Polar Front (Fig. 2). No penguin headed north, west or east from Crozet. On average, the tracked penguins travelled more than 2700 km and moved

**Table 1.** Stable isotopic signature of whole blood from Macaroni Penguins sampled on their departure from and/or return to the breeding colonies for moulting (see text for sample sizes). Values are mean ± 1 sd.

| Locality | Sampling time | $n$ | Blood $\delta^{13}C$ (‰) | Blood $\delta^{15}N$ (‰) | C/N |
|---|---|---|---|---|---|
| Crozet | Departure (GLS birds) | 9 | −22.5 ± 0.2 | 8.1 ± 0.5 | 3.39 ± 0.09 |
| | Return (GLS birds, late moulters) | 8 | −23.3 ± 0.1 | 8.2 ± 0.4 | 3.44 ± 0.16 |
| | Return (non- and failed-breeders, early moulters) | 17 | −23.5 ± 0.2 | 9.0 ± 0.2 | 3.52 ± 0.10 |
| Kerguelen | Departure (GLS birds) | 20 | −20.8 ± 0.5 | 8.8 ± 0.5 | 3.26 ± 0.07 |
| | Return (GLS birds, late moulters) | 12 | −22.1 ± 0.5 | 8.3 ± 0.3 | 3.29 ± 0.05 |

more than 824 km (and up to 1612 km) away from the colony, ranging south to 60.2°S (Table 2). Globally, 58% of all location estimates for the Crozet birds were within the PFZ and 42% in the LAZ.

Birds from Kerguelen headed southeastwards, generally following the 2000-m isobath around the Kerguelen Plateau, in a very coherent anticlockwise movement south of the Polar Front. Thus, all of these birds' locations were in the LAZ. The southernmost latitude estimate was 56.3°S for Kerguelen birds.

Penguins from Crozet and Kerguelen had similar average foraging ranges, travelled distances and bearings. Their respective home-range showed

very close values: 1.77 vs. 1.75 million km² for Crozet compared with Kerguelen, and were entirely separate (UDOI = 0). Within each population, significant differences between sexes were found only in the average bearing for Kerguelen birds (more southwards heading in females, circular ANOVA, $F_{1,20} = 13.10$, $P = 0.009$).

## Time-based analyses

Penguins from Crozet entered the water synchronously on *c.* 14 February and landed on *c.* 6 April, remaining at sea for about 50 days. Birds from Kerguelen also left the colony synchronously but later than on Crozet, on *c.* 17 February. They returned
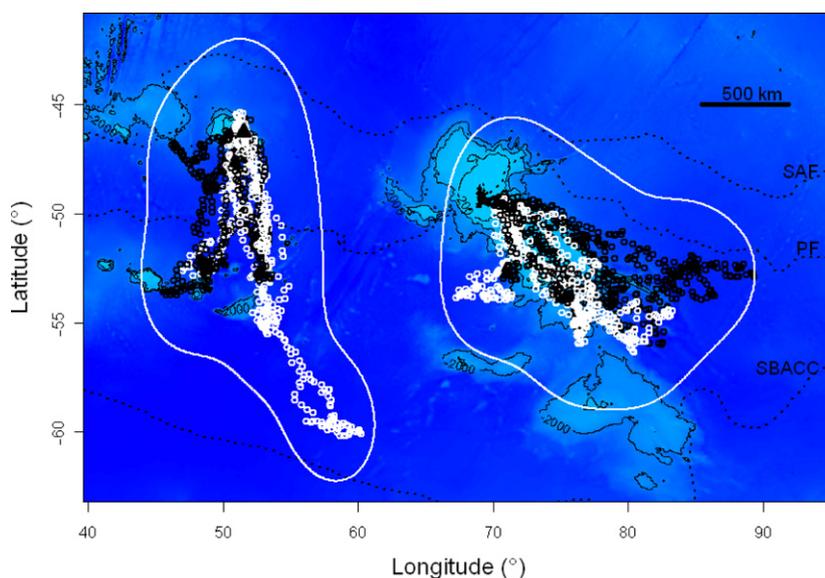


**Figure 2.** Geolocation estimates of Macaroni Penguins (in black: male, and in white: female individuals) surveyed during their pre-moult foraging trip from Crozet (black triangle, *n* = 9 birds, in 2009) and Kerguelen (*n* = 13 birds, in 2011), southern Indian Ocean. The white lines show the 95% kernel density contour computed for each population. The average position of the oceanographic fronts in the area is indicated with dotted lines, from north to south: the Subantarctic Front (SAF), the Polar Front (PF) and the southern boundary of the Antarctic Circumpolar Current (SBACC). The isobaths for 200, 1000 and 2000 m are also shown.

**Table 2.** Derived parameters for the at-sea movements of Macaroni Penguins tracked from Crozet (in 2009) and Kerguelen (in 2011) during their pre-moult foraging trip. Values are mean ± 1 sd.

| Locality | Individuals | Departure date | Return date | Time spent at sea (days) | Max. range (km) | Distance travelled (km) | Bearing to furthest point (°) |
|---|---|---|---|---|---|---|---|
| Crozet | All (*n* = 9) | 14 February ± 4 | 6 April ± 5 | 50.5 ± 7.3 | 824 ± 343 | 2708 ± 698 | 195 ± 39 |
| | Males (*n* = 5) | 15 February ± 5 | 4 April ± 2 | 46.0 ± 4.7 | 684 ± 181 | 2352 ± 401 | 209 ± 41 |
| | Females (*n* = 4) | 12 February ± 1 | 9 April ± 6 | 56.2 ± 6.1 | 998 ± 444 | 3153 ± 782 | 178 ± 31 |
| Kerguelen | All (*n* = 13) | 17 February ± 1 | 30 March ± 4 | 41.8 ± 4.0 | 941 ± 271 | 2713 ± 429 | 125 ± 29 |
| | Males (*n* = 8) | 17 February ± 1 | 29 March ± 4 | 40.7 ± 3.9 | 1009 ± 249 | 2786 ± 471 | 114 ± 13 |
| | Females (*n* = 5) | 17 February ± 2 | 1 April ± 3 | 43.5 ± 4.0 | 832 ± 225 | 2596 ± 369 | 142 ± 41 |

earlier, on *c.* 30 March, remaining for 9 days less at sea than the Crozet birds ($t_{17.028} = 11.31$, $P < 0.001$). Broken stick modelling allowed us to infer their homing decision dates at sea for all individual penguins with 95% confidence intervals of 3 days on average (Table 3). Within each population, significant differences between sexes were found in the time at sea for Crozet (greater in females, $t_{5.6} = 2.73$, $P = 0.037$) and homing decision dates (6 days earlier in males at both sites: $t_{5.029} = 2.85$, $P = 0.036$ and $t_{6.937} = 2.40$, $P = 0.048$ for Crozet and Kerguelen, respectively). Water temperature recorded by the loggers over the trip duration confirmed that the penguins from both colonies moved southwards into cooler waters (Fig. 3a). While birds from Crozet and Kerguelen departed from waters with significantly different temperatures (7.3 vs. 5.4 °C, respectively), both populations exploited waters of *c.* 3.5 °C during 60–70% of their trip duration, before diverging again when returning to their respective colonies. Within each population, significant differences were found in the water temperature used between sexes (Fig. 3b,c). Particularly during the second half of the foraging trip, females consistently exploited colder waters, on average, than males.

## Trophic niche

At Crozet, blood $\delta^{13}C$ values of returning Macaroni Penguins were lower than on departure ($t_{13.7} = 9.72$, $P < 0.001$), with no change in blood $\delta^{15}N$ values ($t_{14.6} = 0.60$, $P = 0.56$, Table 1). At Kerguelen, both blood $\delta^{13}C$ and $\delta^{15}N$ values were lower in returning birds ($t_{24.7} = 6.80$, $P < 0.001$, and $t_{29.9} = 3.01$, $P = 0.005$, respectively).

**Table 3.** Summary of homing decision dates inferred from broken stick modelling. Values are mean $\pm$ 1 sd.

| Locality | Individuals | Homing decision date (days in March) | 95% confidence interval (days) |
|---|---|---|---|
| Crozet | All (*n* = 9) | 21 $\pm$ 4.4 | 3.9 $\pm$ 2.2 |
| | Males (*n* = 5) | 18 $\pm$ 2.5 | 3.5 $\pm$ 1.1 |
| | Females (*n* = 4) | 24 $\pm$ 3.7 | 4.4 $\pm$ 3.1 |
| Kerguelen | All (*n* = 13) | 13 $\pm$ 4.8 | 2.8 $\pm$ 1.1 |
| | Males (*n* = 8) | 11 $\pm$ 3.6 | 2.6 $\pm$ 0.7 |
| | Females (*n* = 5) | 17 $\pm$ 4.7 | 3.1 $\pm$ 1.6 |

Moulting Macaroni Penguins from Crozet compared with Kerguelen birds had lower $\delta^{13}C$ values ($t_{13.3} = 8.08$, $P < 0.001$), but similar $\delta^{15}N$ values ($t_{12.0} = 0.61$, $P = 0.55$, Table 1). No sex-related differences were found in either blood $\delta^{13}C$ values (Crozet: $t_{5.3} = 0.51$, $P = 0.63$; Kerguelen: $t_{7.0} = 1.72$, $P = 0.13$) or $\delta^{15}N$ values (Crozet: W = 1, N = 8, $P = 0.07$; Kerguelen: $t_{10.0} = 1.37$, $P = 0.20$) of moulting birds. At Crozet Islands, early moulters showed similar blood $\delta^{13}C$ values to late moulters (W = 40, N = 25, $P = 0.11$), but early moulters had significantly higher $\delta^{15}N$ values (W = 132, N = 25, $P < 0.001$).

## DISCUSSION

We studied the at-sea ecology of Macaroni Penguins over the crucial hyperphagia period prior to moult during which penguins gained > 2 kg body mass (i.e. 60% of their initial mass) in 1–2 months. We revealed distinctive foraging strategies between pre-moulting penguins from the southern Indian Ocean at three biological levels. First, at the population level, the foraging areas used by parapatric penguins were spatially distinct, although overlap was theoretically possible and the parapatric penguins foraged at similar trophic level in waters of the same temperature. Secondly, females remained on average around 6 days longer than males in these cool southerly waters. Thirdly, between the non-breeding and post-breeding birds, the former group (early moulters) had significantly higher trophic level but similar trophic habitat to the latter (late moulters). In our study we compared data collected at two localities in two different years, and thus could not separate the effects of locality vs. year. However, environmental conditions were not conspicuously different in both years, as observed from satellite imagery (monthly-merged Aqua MODIS data from http://coastwatch.pfel. noaa.gov/coastwatch/CWBrowserWW360.jsp): indeed, the 3.5 °C isotherm was situated about 680 vs. 697 km south from Crozet and 585 vs. 576 km from Kerguelen in 2009 vs. 2011, respectively, supporting the validity of the comparison made in our study. Moreover, previous studies showed that at-sea distribution of the Macaroni Penguin was very similar across years, during both summer and winter periods (Trathan *et al.* 2006, Thiebot *et al.* 2011), minimizing the effect of year in our comparison.
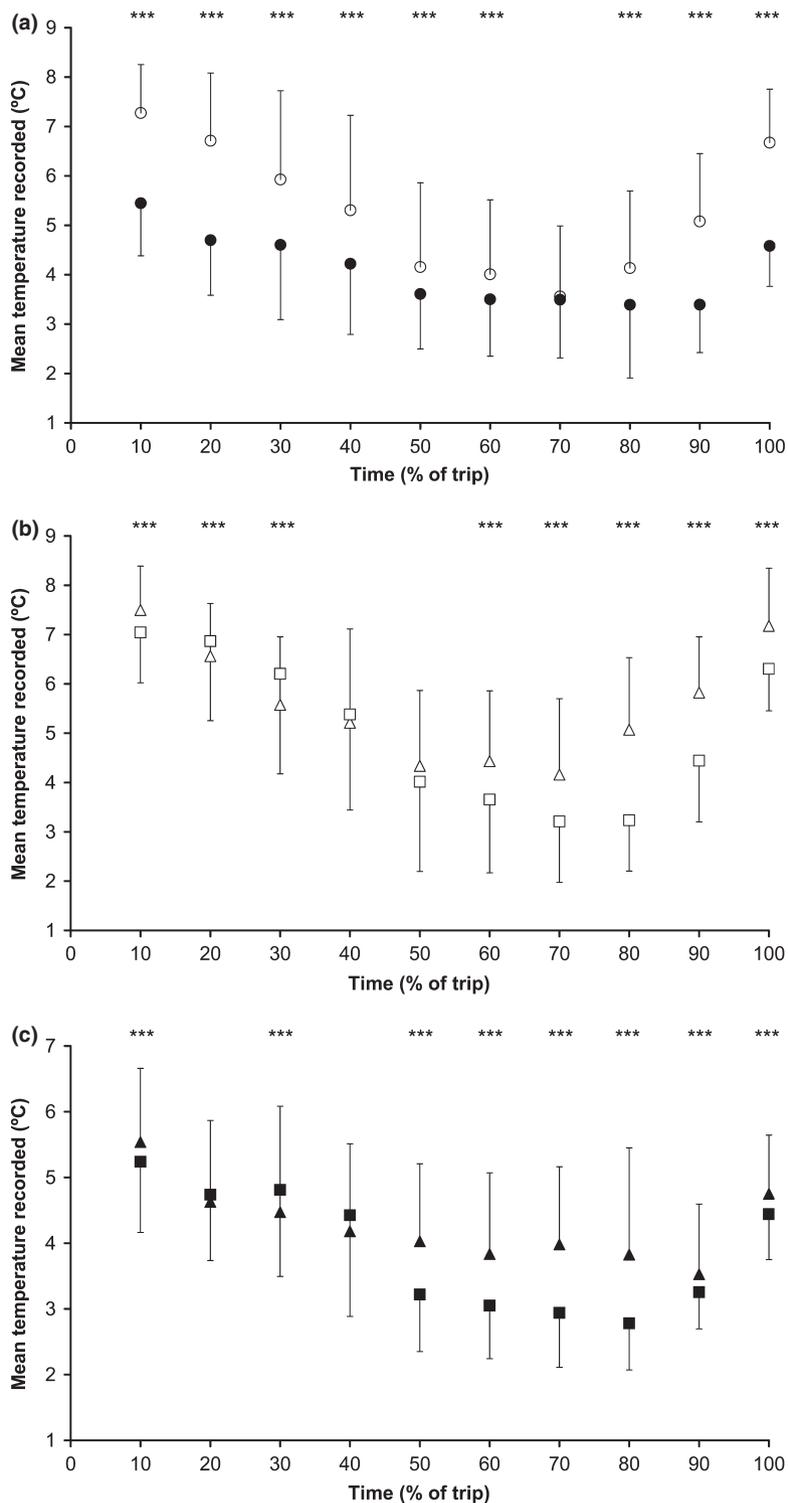
**Figure 3.** Seawater temperatures recorded by GLS loggers carried by Macaroni Penguins from Crozet in 2009 and Kerguelen in 2011 during their pre-moult foraging trip. (a) Penguins from Crozet ($n = 9$, open symbols) and Kerguelen ($n = 13$, filled symbols). (b) Males ($n = 5$, triangle symbols) and females ($n = 4$, square symbols) from Crozet. (c) Males ($n = 8$, triangle symbols) and females ($n = 5$, square symbols) from Kerguelen. Time is represented as the percentage of the total trip duration. Values are means over each 10% duration class with sd. Asterisks indicate significant differences between groups (*** $P < 0.001$).

## Marine habitat of pre-moulting Macaroni Penguins

Penguins from Crozet and Kerguelen showed similar at-sea ecology, reaching a specific sea-surface isotherm of 3.5 °C south of the Polar Front in oceanic waters. Importantly, these results from two of the major breeding localities of the species support previous distribution estimates by Brown (1987) and Green *et al.* (2009b) in showing a relatively large-scale offshore distribution (*c.* 900-km range) of Macaroni Penguins during this stage, without any return to land at their colony or elsewhere (see also Waluda *et al.* 2010). For individuals we tracked from Crozet, this pre-moult foraging trip had the longest average duration (> 50 days) ever reported for the species to our knowledge (reviewed in Williams 1995). As Crozet is the northernmost breeding locality of the species, this result supports the suggestion that latitude affects the duration of the pre-moult provisioning trip in eudyptid penguins (Williams 1995). The fact that both Crozet and Kerguelen birds moved towards the same 3.5 °C surface isotherm may allow estimations of this trip to be refined in the Macaroni Penguin, as it seems that pre-moult trip duration may be positively correlated with the distance of each colony to this 3.5 °C isotherm in March (Supporting Information Fig. S1), as suggested by durations of *c.* 13 days at South Georgia (54°00′S, 38°02′W, Williams & Croxall 1991) and *c.* 45 days at Marion Island (46°52′S, 37°51′E, Brown 1987). Contrasted site situations regarding this potentially common characteristic environment may hence explain why penguins from different sites take more or less time commuting.

In these cold oceanic waters, penguins from Crozet and Kerguelen fed at a similar trophic level. Blood $\delta^{13}$C values are in agreement with foraging in the LAZ, being intermediate between the higher $\delta^{13}$C values of King Penguins *Aptenodytes patagonicus* that forage in the vicinity of the Polar Front and the lower values of high-Antarctic penguins confined with the southernmost waters adjacent to the Antarctic continent (Cherel & Hobson 2007). Blood $\delta^{15}$N values of Macaroni Penguins were relatively low when compared with other seabirds and fur seals and with potential prey in the area (Cherel *et al.* 2007, 2010), probably indicating a crustacean-based diet with a low proportion of fish during the pre-moulting trip (Table S1). We therefore assume that the birds were feeding primarily upon the few species of swarming hyperiids (e.g. *Themisto gaudichaudii*, *Primno macropa*) and euphausiids (e.g. *Euphausia vallentini*, *Thysanoessa* spp.) and upon the schooling myctophid *Krefftichthys anderssoni*, which were identified in food samples of individuals from the same populations during the breeding season (Ridoux 1994, Cherel *et al.* 2007, 2010). Interestingly, only one female reached high latitudes at which Antarctic Krill *Euphausia superba* begins to occur in the southwestern Indian Ocean (Pakhomov 2000), thus suggesting that the species is not nutritionally important for Crozet and Kerguelen Macaroni Penguins during their pre-moult trip.

## Three levels of foraging specialization

Under the reasonable assumption of similar at-sea distribution of Macaroni Penguin populations between years (see earlier; Trathan *et al.* 2006, Thiebot *et al.* 2011), we demonstrated a specialization for the marine areas exploited at the population scale. This suggests that large-scale segregation of the foraging areas is an effective means for parapatrics to minimize intraspecific competition and achieve intense foraging with similar diets, activity schedules, thermal niches and potentially overlapping ranges (Thiebot *et al.* 2011, Wakefield *et al.* 2013). Such specialization would be especially clear-cut in the context of non-uniformly distributed food resource, which is probably the case here (Kozlov *et al.* 1991).

The second level of foraging specialization showed in our study was that females consistently remained in cold waters for longer than did males, whereas diet and large-scale geographical distribution were alike. Male Macaroni Penguins are larger and can dive deeper than the females (Green *et al.* 2005) and this may confer on them a greater foraging ability. Such a differential foraging ability is supported by the fact that, at Crozet, females made significantly longer trips than males. Females would thus take longer to acquire sufficient body reserves before coming back on land for moulting. On Kerguelen, a similar trend in sex-biased trip duration was observed, although not statistically significant: such lesser effects may be linked to the clearer difference in bearings measured between sexes in this population, suggesting more sex-specific feeding grounds in that case. This subtle difference in thermal niche between sexes could not be demonstrated by the carbon signature of

the individuals alone, which is more relevant for revealing contrasts at the larger scale of water masses (Cherel & Hobson 2007, Jaeger *et al.* 2010, Authier *et al.* 2012b). Possible sex-specific needs at sea related to different energetic investment during breeding (Green *et al.* 2009a) remain to be investigated in more detail.

Furthermore, our study showed that at the intrapopulation scale, pre-moult foraging strategies differed between early and late moulters along the trophic level dimension. Blood $\delta^{13}$C values indicated that both groups foraged within the same isotopic habitat (this does not preclude spatially distinct feeding grounds). But more importantly, early moulters had higher blood $\delta^{15}$N values than late moulters, indicating a greater proportion of higher trophic level prey, most probably the myctophid *K. anderssoni*, in the pre-moult diet (see Cherel *et al.* 2010 for the $\delta^{15}$N values of potential prey). The trophic differences may reflect a change over time in prey available to the birds in the exploited habitat, with increasingly lower proportions of myctophid fish as austral autumn progresses (Kozlov *et al.* 1991). Interestingly, this degradation of environmental conditions during autumn may be the reason why the non-breeders would benefit in moulting earlier than the breeders, the latter being supposedly unable to do so until the chicks fledge. In doing so, early moulters also have a longer time to forage at sea until the next breeding season, which may increase their chances to breed early and therefore with success at that time (Crawford *et al.* 2006), although it was not feasible to verify this in our study.

Apart from food limitation, the other hypothesis explaining why ecological niches between organisms may be segregated is the reinforcement of reproductive barriers ('Wallace effect', Rohde 2005). In our case, this hypothesis may provide evidence for this first level of specialization (i.e. between parapatrics). However, it does not explain other levels of foraging specialization documented here (between sexes and breeding status). Rather, previous studies stressed the high cost of moulting for land-breeding diving animals worldwide (penguins: Brown 1985, Cherel *et al.* 1994, Green *et al.* 2004, pinnipeds: Worthy *et al.* 1992, Boyd *et al.* 1993), emphasizing the importance of food availability prior to moult in the foraging ambit of these central-place foraging animals.

## At-sea ecology of the Macaroni Penguin during the complete inter-nesting period

A comparison between the pre-moult (this study) and winter periods at sea (from Bost *et al.* 2009, Thiebot *et al.* 2011) allows characterization of the foraging strategies of Macaroni Penguins during the complete inter-nesting period. In winter, the birds' estimated home-range was systematically larger than in pre-moult, both at Crozet and at Kerguelen. However, even during the longer winter period, these two populations showed non-overlapping at-sea distributions, suggesting that they are in total isolation throughout their life cycle (although juvenile dispersal and immaturity period have not yet been investigated). Moreover, Macaroni Penguins used a broader habitat encompassing more water masses during winter (Table S2). Importantly, they were chiefly distributed in the PFZ in winter, whereas during pre-moult this water mass was less used than the LAZ, or even, in the case of the Kerguelen birds, not used at all. However, Macaroni Penguins from both localities exploited waters of similar temperature in the middle of the pre-moult (*c.* 3.5 °C) and winter (3.0–3.5 °C) periods. This comparison suggests that (1) Macaroni Penguins specifically target this water temperature during the inter-nesting period, whatever the breeding locality, and (2) water masses may be mobile across seasons, possibly leading marine predators to adapt their bearing and movement while at sea. That Macaroni Penguins actually track a specific thermal niche at sea would potentially explain their southward movements during pre-moult in the late austral summer vs. their more longitudinal movements observed during winter. This is consistent with the northward movements during pre-moult trips surveyed in the same species from a more southerly locality (Green *et al.* 2009b, Waluda *et al.* 2010, Fig. S1). Finally, the present study, combined with previous knowledge for other periods (Cherel *et al.* 2007), suggests that in the southern Indian Ocean, Macaroni Penguins seem independent of Antarctic Krill year-round. Here again, information about at-sea ecology of this key consumer during the juvenile and immature stages is needed to clarify this point.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Situation of the Macaroni Penguin breeding locations (black dots) where pre-moulting trip has been documented, with respect to the average location of the 3.5 °C surface isotherm in March (black line).

**Table S1.** Stable isotopic signature of whole blood from Macaroni Penguins sampled on their departure from the colonies for the post-moulting winter exodus, thus partly reflecting trophic niche prior to moult.

**Table S2.** Average proportion of time spent in the different water masses by penguins from Crozet and Kerguelen during the pre-moult vs. post-moult (winter) stages.