Pelagic or benthic prey? Combining trophic analyses to infer the diet of a breeding South American seabird, the Red-legged Cormorant, *Phalacrocorax gaimardi*

Annick Morgenthaler[A,C], *Ana Millones*[A], *Patricia Gandini*[A,B] *and Esteban Frere*[A,B]

[A]Centro de Investigaciones de Puerto Deseado, Universidad Nacional de la Patagonia Austral,
 Av. Prefectura s/n, cc 238 (9050), Puerto Deseado, Santa Cruz, Argentina.
[B]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Prefectura s/n,
 cc 238 (9050), Puerto Deseado, Santa Cruz, Argentina.
[C]Corresponding author. Email: annick.morgenthaler@gmail.com

Abstract. The available information about the feeding habits and preferences of the Red-legged Cormorant (*Phalacrocorax gaimardi*) generally suggests that this near-threatened South American cormorant is a sedentary benthic forager that also incorporates pelagic prey in its diet. In order to describe the dietary composition and assess the importance of certain prey types (pelagic vs benthic), we studied the diet of this cormorant on the Argentine Atlantic coast during four breeding seasons, using a combined technique of conventional diet assessment (pellets) and stable isotope analysis. Our results show that the Red-legged Cormorant forages mainly on pelagic and demersal–pelagic prey. Results of both techniques showed the main prey to be the Patagonian Sprat, a small high-energy-content pelagic forage fish, and the Patagonian Squid, a low-energy-content demersal–pelagic invertebrate. We also found an overall low prey diversity and important interannual variation for the main prey types, as well as variation between the different breeding stages. This study therefore contributes new and unambiguous information about the Red-legged Cormorant's use of trophic resources and suggests that the combination of a conventional technique with stable isotope analysis provides a solid framework for this seabird diet assessment.

Additional keywords: Argentina, mixing model, Patagonia, shag, stable isotope analysis, trophic ecology.

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Introduction

Seabirds are good indicators of changes in the marine environment and may be indicative of fluctuations in fish stocks and oceanographic conditions (Furness and Camphuysen 1997). The abundance and distribution of prey can affect the reproductive success and the population size of seabirds through 'bottom-up' processes (Suryan *et al*. 2006). For example, changes in prey availability due to overfishing together with oceanographic changes have negatively affected the populations of cormorants and pelicans in Peru and South Africa (Schreiber and Clapp 1987). It is therefore important to rely on good basic dietary and trophic ecology knowledge of seabirds in order to understand the possible changes that can affect their population trend.

To assess the diet of seabirds, the analysis of pellet casts (regurgitated indigestible prey remains) has been widely used, particularly on cormorants and shags, as sampling is simple and non-invasive (Barrett *et al*. 2007). Nevertheless, this conventional approach can present some biases, caused by the temporal snapshot represented by the sampled pellets, the differential erosion rate of the prey remains that can over- or under-represent certain prey types, and, finally, also because some secondary prey remnants can be retained (Casaux *et al*. 1995; Barrett *et al*. 2007). Stable isotope analysis (SIA) of carbon and nitrogen in consumer tissues does not suffer from these biases and is a powerful dietary assessment technique, especially when used in combination with conventional techniques (Inger and Bearhop 2008). The stable isotope ratios in tissues of a consumer reflect assimilated diet, and under appropriate conditions allow quantification of the relative importance of main dietary items through the use of stable isotope mixing models (Phillips and Gregg 2001; Parnell *et al*. 2010).

The Red-legged Cormorant (*Phalacrocorax gaimardi*) is found along the Pacific coast from northern Peru to southern Chile, and along the Atlantic coast, where its range is restricted to the Santa Cruz Province of Argentina (Zavalaga *et al*. 2002; Frere *et al*. 2005). This species is classified by the IUCN as Near Threatened because its population is showing a moderately rapid decline owing to mortality in fishing operations and unsustainable exploitation (BirdLife International 2012). In Argentina the species is considered 'vulnerable' (López-Lanús *et al*. 2008) and its small population (945 breeding pairs) showed a slight decline between 1990 and 2009 (Millones *et al*. 2015).

Coastal ocean productivity has been identified as a key factor influencing the usage patterns of the breeding habitat of the Red-legged Cormorant, and it could also be an important factor affecting temporal variations in the Argentine population (Millones and Frere 2012; Millones *et al.* 2015).

Little is known about the diet and foraging behaviour of the Red-legged Cormorant throughout its global range. Frere *et al.* (2002) and Gandini *et al.* (2005) reported that the Red-legged Cormorants from the breeding colony of Isla Elena, at the Ría Deseado estuary (Santa Cruz, Argentina) (Fig. 1) feed close to the colony (<3 km), in inshore shallow waters (<15 m) and mostly near the seabed. Millones *et al.* (2005) found that the diet from the Isla del Rey colony, Ría Deseado (Fig. 1), based on pellet analysis only, is composed of a wide variety of prey, most of benthic habits, particularly *Patagonotothen* spp. Nevertheless, some pelagic prey (*Sprattus fuegensis* and *Odonthestes* sp.) has been seen to be important too, as evidenced during one of the two breeding seasons studied by Millones *et al.* (2005). The Red-legged Cormorant usually feeds individually, but it sometimes feeds in mixed flocks (multispecific seabird assemblage), preying on schools of pelagic fishes such as sprats or silversides (Frere *et al.* 2002; Nasca *et al.* 2004). Preliminary unpublished conventional dietary analysis from samples collected at Isla Elena (Fig. 1), Ría Deseado (Morgenthaler *et al.*, pers. obs.) suggested that, during breeding period, pelagic prey might play an important role. We therefore investigated the diet of breeding Red-legged Cormorants using a combined technique of conventional diet assessment (pellet analysis) and stable isotope analysis of carbon and nitrogen. By adding the dimension of stable isotope analysis we hoped to resolve some of the uncertainties concerning the effectively assimilated prey types and to assess the importance of pelagic vs benthic prey in their diet. Our objectives were: (1) to describe, compare and discuss the diet of the Red-legged Cormorant in detail using two techniques, (2) to assess the differences in diet composition between breeding stages and years, and (3) to compare and contrast the diet of this cormorant at two colonies using SIA.

Materials and methods

Study area

Our study area is a ria, a long (>40 km), narrow inlet formed by the partial submergence of a river valley, located near the town of Puerto Deseado, and belongs to Ría Deseado Provincial Nature Reserve (Fig. 1). This area hosts a population of 263 breeding pairs of Red-legged Cormorant, breeding in six colonies (Millones *et al.* 2015). The main fieldwork was carried at Isla Elena (47°45′S, 65°56′W), the second-largest colony for the species in the Atlantic coast, holding 112 breeding pairs. The secondary study site was the colony of Isla del Rey (44 breeding pairs, 47°46′S, 66°03′W), 10 km from Isla Elena.

Conventional diet sampling

Pellets, which can also include small remains of undigested food, were collected at Isla Elena during four breeding seasons (2009, 2011, 2012 and 2013) by fixing two collecting bags (2 × 0.7 × 0.5 m) on the cliffs, underneath two groups of 3–5 nests each. The bags containing the pellets were emptied every 1–2 weeks between November and February. The pellets were collected during three breeding stages: incubation (approximately early November to mid-December), early chick-rearing (chicks up to four weeks old; mid-December to mid-January), and late chick-rearing (chicks between approximately four weeks old and fledglings; mid-January to end February). In studies of Great Cormorants (*P. carbo*) and European Shags (*P. aristotelis*), chicks <7 weeks old did not produce pellets, apparently digesting all calcareous materials (Derby and Lovvorn (1997)). So, chick-rearing samples of true pellets should be attributed to adults only. Nevertheless, in our case, due to the heterogeneity of the pellet unit (including undigested food rests), it is not possible
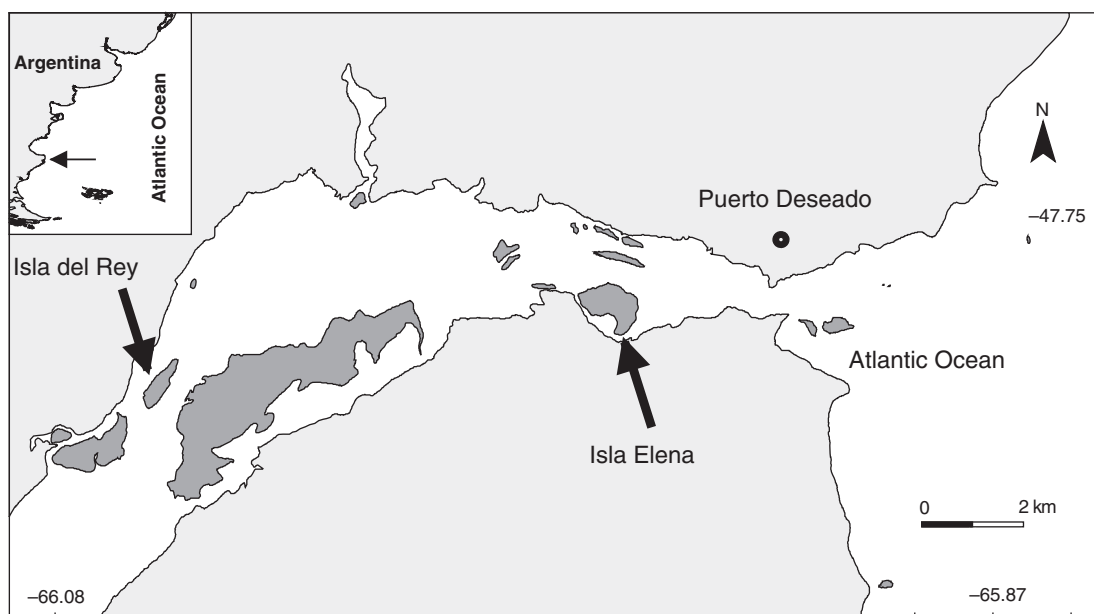

Fig. 1. Location of the two studied colonies of Red-legged Cormorant at Ría Deseado, Argentina.

to attribute chick-rearing samples to adults only, and these samples might partially include chick's diet.

The pellets were then disaggregated under a binocular microscope and all hard prey remains (fish otoliths and bones, cephalopod beaks, crustacean exoskeletons and polychaete mandibles) were used to quantify and identify the prey at the lowest taxonomic level possible. Identification was made by using our own collections and available literature and catalogues (Cousseau and Gru 1982; Boschi *et al*. 1992; Gosztonyi and Kuba 1996; Pineda *et al*. 1996; Volpedo and Echeverría 2000). The frequency of occurrence (%FO) and the number of occurrence (%N) were calculated for all prey items, and expressed as percentages. Allometric regressions were used to estimate the average total length (TL) and wet weight (W) of the different prey types (Cousseau and Gru 1982; Pineda *et al*. 1996; Hansen 1999). When no regression from the study area was available, data from our own collection were used. Fishes' otoliths (OL) and cephalopods' beak lengths from the 2009 season pellets were used for TL and W calculations. The %W was calculated for the same prey used for SIA mixing models (see below) and the unidentified fishes, in order to compare the results of both techniques. The unitary weight of unidentified fish (W_{un}) was estimated on the basis of the ratio of %N of main identified fishes (*Sprattus*: 94.3%; *Odontesthes*: 5.7%; $n = 333$):

$$W_{un} = (W_{sprattus} \times 94.3 + W_{odontesthes} \times 5.7) \times 10^{-2}.$$

In order to express the prey diversity, Shannon–Weaver's diversity index was calculated for (1) all data together (all years and breeding stages), (2) each year separately and (3) each breeding stage separately (mean value of all four years) (Tramer 1969).

Stable isotope analysis sample collection and processing

Whole blood was chosen for SIA as it is a frequently used tissue in non-destructive SIA seabird studies, providing one- to five-week time-integrated information before the sampling date (Cherel *et al*. 2005). Whole blood samples of adults and chicks of Red-legged Cormorants were collected during three consecutive breeding seasons (2011–13) at Isla Elena (overall $n = 53$) for SIA. Some of the SIA sampled birds came from the nests that were used for conventional diet sampling, but most came from neighbouring nests. Adults were captured from their nest with a pole with a noosed cable during late incubation stage (around mid-December), corresponding to the period when they were less prone to fly away (Frere *et al*. 2002). Each manipulation lasted less than 5 min and on release birds flew directly to the water and returned to their nest shortly afterwards. The 2–5-week-old chicks were captured from their nests and returned promptly after manipulation (early to mid-January). Blood (0.5 mL) was extracted from the brachial vein of adults and chicks and preserved in 70% ethanol before processing in the laboratory (Hobson *et al*. 1997). No mathematical correction for the effect of ethanol was applied to our datasets as a precautionary measure given that most authors found no effect of ethanol on the $\delta^{13}C$ values of blood in different bird species (Hobson *et al*. 1997; Therrien *et al*. 2011; but see Bugoni *et al*. 2008). In addition, some whole blood samples of chicks were collected at the Isla del Rey colony during the 2012 and 2013 breeding seasons

(overall $n = 9$), following the same procedure. This small sample size was due to the secondary importance of this complementary study site and also due to the position of nests in the cliffs that made captures more difficult. In spite of the small sample size this site was included because a conventional diet study was conducted some years ago at Isla del Rey (Millones *et al*. 2005) and the comparison of the SIA values of these two sites (one at the entrance and the other further inside the inlet) would provide a more complete overview and a clearer view of the diet and prey use in the entire estuary (Fig. 1).

Samples of the main prey sources for SIA were collected from 2011–12 to 2013–14 spring–summer seasons. Four potential prey sources were chosen according to published data from the same area (Millones *et al*. 2005) and preliminary conventional diet data from this study. These were: two small pelagic fishes, the Patagonian Sprat (*Sprattus fuegensis*) and the Silverside (*Odontesthes nigricans*), a demersal–benthic fish, the notothenid (*Patagonotothen cornucola*), and a demersal–pelagic squid, the Patagonian Squid (*Loligo gahi*). These samples were collected under intertidal rocks (notothenids), found from regurgitates in the collecting bags or around nests (sprats and squid), or captured by local fishermen (silversides), and identified by using the available literature and catalogues (Menni *et al*. 1984; Bovcon *et al*. 2007). The collected prey samples were frozen until processed in the laboratory.

The samples were dried at 60°C for >24 h for whole blood and for >48 h for fish muscle and squid mantle samples, and then they were ground to a fine, homogenised powder. Sprats are considered fatty fishes and high lipid content could affect their $\delta^{13}C$ values (Post *et al*. 2007). Therefore, a subset of sprat muscle samples was analysed before and after chemical lipid extraction, in order to develop a mathematical lipid model between $C:N_{bulk}$ and $\delta^{13}C_{lipid-free}$–$\delta^{13}C_{bulk}$, to be applied to the remaining $\delta^{13}C_{bulk}$ sprats data (Logan *et al*. 2008; Bond and Jones 2009). The lipids were extracted using chloroform–methanol (2:1) (Logan *et al*. 2008). The linear fitting model obtained and subsequently applied to bulk data was:

$$\delta^{13}C_{lipid-free} = -4.45 + 1.41 \times C:N_{bulk} + \delta^{13}C_{bulk}$$

$$(n = 8, \ R^2 = 0.834).$$

The shift between $\delta^{13}C_{bulk}$ and $\delta^{13}C_{lipid-free}$ ranged from 0.2 to 1.3‰ (mean = 0.6, $n = 8$). The sprats' isotopic values ($\delta^{13}C$ and $\delta^{15}N$ used in all subsequent analyses refer to the $\delta^{13}C_{lipid-free}$ values (obtained either through chemical extraction or mathematical correction) and the $\delta^{15}N_{bulk}$ values. The cormorant's blood and other prey samples were not lipid extracted due to their low lipid content, verified by their values of $C:N$ mass ratio <4 (Bond and Jones 2009). The yolk could affect the isotopic values of young chicks and therefore lead to misinterpretation of the diet. Yolk has been shown to have a turnover of 7–28 days after hatching in captive auklets (Sears *et al*. 2009). The effect of yolk on chicks' $\delta^{13}C$ and $\delta^{15}N$ values seems to be negligible in our datasets as no differences for these values were found between chicks <20 days and chicks >20 days within each year's datasets.

Carbon and nitrogen isotope ratios were measured in the Center for Stable Isotopes at the University of New Mexico,

USA, by Elemental Analyser Continuous Flow Isotope Ratio Mass Spectrometry using a Costech ECS 4010 Elemental Analyser coupled to a Thermo Fisher Scientific Delta V Advantage mass spectrometer via a CONFLO IV interface. Isotope ratios are reported using the standard delta (δ) notation relative to AIR and Vienna Pee Dee Belemnite (V-PDB), respectively, and expressed in units per thousand (‰) as follows:

$$\delta = (R_{sample} : R_{standard} - 1),$$

where R_{sample} and $R_{standard}$ are the molar ratios of the heavy to light isotopes ($^{13}C : {}^{12}C$ or $^{15}N : {}^{14}N$) of the sample and standard, respectively. Average analytic precision based on routine analysis of a laboratory protein standard was <0.1‰ (1σ). The laboratory standard was calibrated against IAEA-N-1, IAEA-N-2, USGS 42 and USGS 43 for nitrogen and NBS 21, NBS 22 and USGS 24, USGS 42 and USGS 43 for carbon.

Stable isotope analysis mixing models

The relative contribution of the potential prey to the cormorants' diet was estimated using the Bayesian stable isotope mixing model SIAR 4.2 (Parnell *et al.* 2010) implemented in R software (R Core Team 2014). The SIAR model is fit via the Markov Chain–Monte Carlo method to produce simulations of plausible values of dietary proportions of sources consistent with the data, using a Dirichlet prior distribution (Parnell *et al.* 2010). Due to interannual differences of isotopic data for cormorant and prey, one model was run for each year, with adults and chicks from the different colonies considered as different groups. The prey sources data used for each model (year) were from the same year as the consumers' data, except for the squid. Because no squid samples were obtained in 2011 and only one in 2013, the 2012–13 dataset (2012: $n = 5$; 2013: $n = 1$) was used for the three years. The Diet Tissue Discrimination Factor (DTDF) is a key parameter for stable isotope mixing models (Bond and Diamond 2011). No DTDF is known for the Red-legged Cormorant, and only one study determined experimentally whole blood DTDF for a cormorant species: the Double-crested Cormorant (*Phalacrocorax auritus*), which was fed with whole farm-raised Channel Catfish (*Ictalurus punctatus*) (Craig *et al.* 2015). Instead, the whole blood DTDF of Rockhopper Penguins (*Eudyptes chrysocome*) was used (Cherel *et al.* 2005). Although this DTDF was obtained from a taxonomically different bird family, the feeding trials were made with capelin muscle (*Mallotus villosus*), an oceanic forage fish, ecologically and isotopically more similar to sprats (one of the Red-legged Cormorant's prey) than the whole freshwater farm-raised catfish fed to the Double-crested Cormorant. Furthermore, Cherel *et al.* (2005) recommend the use of data from the same prey tissue in the mixing models as the tissue used in the feeding trials from which the DTDF come (fish muscle in our study case and the Rockhopper Penguins feeding trials, instead of whole fish in the Double-crested Cormorant feeding trials). The used values of the discrimination factor were therefore +1.86‰ for $\Delta^{15}N$ and +0.46‰ for $\Delta^{13}C$ (Cherel *et al.* 2005); a standard deviation of ±1‰ was added to account for uncertainty due to potential differences in discrimination factors between penguins and cormorants (Votier *et al.* 2010).

For each model (year) we applied the simulation method of Smith *et al.* (2013) to ensure that the consumer data were situated within 95% of the source isotopic mixing polygon.

We ran the models for 500 000 iterations, with 50 000 initial discards, without data on concentration dependence and without prior information. The means and 95% credible intervals of the estimates of source contribution to diet were reported for all groups and models. Furthermore, comparisons of the proportion estimates for a prey type between groups (i.e. adults vs chicks' sprat proportions) were realised and approximated by the proportion of samples that were higher in one group than another and expressed as the probability that the proportion of a prey type in the diet of one group is higher than that of the other group.

Statistical analyses

Similarities in dietary composition of the main prey between years and between breeding stages, in terms of biomass, were determined with a multivariate analysis of similarities (ANOSIM) using the R-package 'vegan' (Oksanen *et al.* 2016). A Bray–Curtis similarity matrix was generated to assess similarities in prey-group composition among samples. This procedure uses this matrix to compute a statistical test R that takes a value of 1 when all samples within a group are more similar to each other than to any sample from other groups, and is ~0 when there are no differences between groups. A randomisation process is used to find the probability of gaining particular values of R by chance (Clarke and Warwick 2001).

In order to compare isotopic values between years and between adults and chicks, the isotopic niche positions were examined using nested linear models and residual permutation procedures (Turner *et al.* 2010). The isotopic niche locations of two groups are considered to be different if the Euclidean Distance (ED) between the centroid locations is significantly greater than zero. The mean distance of each individual to the niche centroid position (MDC) was examined for each year's cormorant dataset, as an indicator of niche breadth (reflecting the prey diversity). All calculations were based on Turner *et al.* (2010).

Results

Conventional diet

From 209 analysed pellets, 13 different prey items were identified, belonging to five different taxa: teleost fishes, cephalopods, crustaceans, polychaetes, and algae (Table 1). Overall prey diversity was low (Shannon–Weaver Index: 0.65) and showed variability between years (2009: 0.82; 2011: 0.28; 2012: 0.46; 2013: 0.34) and between breeding stages (mean value of all four years: incubation: 0.61; early rearing: 0.34; late rearing: 0.42). The dietary composition, considering the biomass estimates of the main prey types (squid, sprats and silversides), showed significant differences between years ($r = 0.055$, $P = 0.013$) and between breeding stages ($r = 0.057$, $P = 0.003$) despite a high level of overlap.

Squid showed the highest biomass estimates during the incubation periods of all four years (Fig. 2) During chick-rearing stages the squid proportions varied more according to the year, and were present in high proportions during the early and late

Table 1. Percentages of prey in the diet of breeding Red-legged Cormorants at Isla Elena

Number of individual prey (*n*), percentage number (%N), percentage frequency of occurrence (%FO) and percentage estimated wet weight (%W) of prey, obtained from 209 pellets, are shown. The ecological group of each prey is shown in parentheses (P, pelagic; B, benthic; DP, demersal pelagic; DB, demersal benthic). The most abundant prey types are shown in bold

Prey	*n*	%N	%FO	%W
Teleost fishes	1037	89.3	79.9	39
Sprattus fuegensis (P)	**934**	**80.4**	**68.1**	**27.0**
Odontesthes sp. (DP)	**24**	**2.1**	**7.8**	**4.7**
Ramnogaster arcuata (P)	4	0.3	1.5	
Patagonotothen sp. (DB)	3	0.3	1.5	0.6
Pinguipes brasilianus (DB)	1	0.1	0.5	
Parona signata (DP)	1	0.1	0.5	
Iluocoetes sp. (DB)	1	0.1	0.5	
Unidentified fishes	**69**	**5.9**	**14.2**	**6.7**
Cephalopods	95	8.2	26.5	61.0
Loligo gahi (DP)	**95**	**8.2**	**26.5**	**61.0**
Crustaceans	8	0.7	4.4	
Eurypodius latreilli (B)	3	0.3	1.5	
Nauticaria magellanica (DB)	1	0.1	0.5	
Unidentified crustaceans	6	0.5	2.9	
Polychaetes	21	1.8	4.9	
Polynoidae (B)	6	0.5	0.5	
Nereididae (B)	15	1.3	3.4	
Algae			26.0	

chick-rearing stages of 2012 and the early-rearing stage of 2013, but completely absent in 2011 (Fig. 2). In contrast, the sprats were consistently more abundant during the chick-rearing stages than the incubation stage (Fig. 2). Sprats showed a high interannual variability too (Fig. 2). In 2011, 100% of the estimated biomass comprising the diet during the two chick-rearing stages was attributed to sprats. Silversides were present in low proportions and only in 2009 (all stages) and the late-rearing stage of 2013 (Fig. 2). Apart from the three main prey types, the benthic notothenid fishes were also present but appeared only in very low proportions during the late-rearing stage of 2009 (Fig. 2).

Stable isotope analysis and diet estimated by the mixing models from Isla Elena

Stable isotope values of Red-legged Cormorant whole blood ranged between –17.89 and –14.54‰ for $\delta^{13}C$, and between +14.73 and +17.03‰ for $\delta^{15}N$ (Table 2). The mean isotopic niche position of the cormorants (adults and chicks together) differed significantly between all pairs of years (ED = 1–1.8‰, all $P < 0.001$) (Fig. 3). The mean distances to centroid position, for adults and chicks together, were low for all three years, showing a low data dispersion (all MDC values <0.9). The mean isotopic niche position of adults and chicks differed significantly within each year (ED = 0.6, 1.6 and 0.6 ‰ respectively, all $P < 0.001$). The $\delta^{13}C$ was the value that varied most between years, with lower values in 2011, higher in 2012 and intermediate in 2013 (Fig. 3). A similar pattern of shifts was observed for sprats, with mean isotopic niche position differing significantly between all pairs of years too (ED = 1.6–2.6‰,
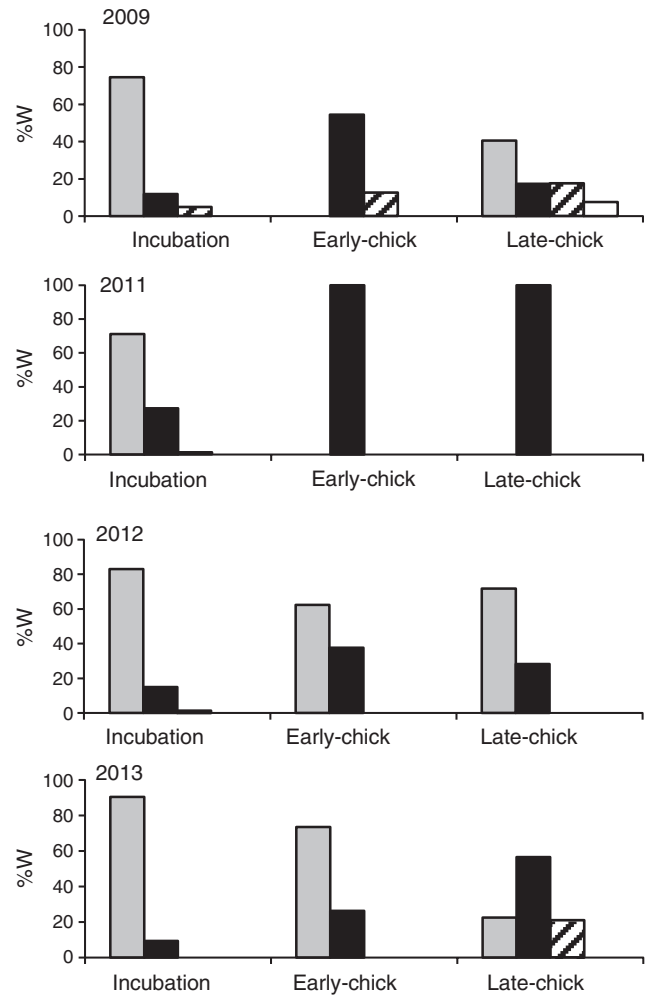
Fig. 2. Percentage estimated wet weight (%W) of the main prey for each year and breeding stage, in the diet of the Red-legged Cormorant. The prey shown in the figure are: *Loligo gahi* (Patagonian squid) in grey, *Sprattus fuegensis* (Patagonian sprat) in black, *Odontesthes nigricans* (silverside) with black-white diagonal lines, and *Patagonotothen* sp. (notothenid fish) in white. Values are shown for each of the four years and their respective breeding stages: incubation, early-chick-rearing and late-chick-rearing from Isla Elena colony (no. of pellets analysed: $N_{2009} = 105$, $N_{2011} = 42$, $N_{2012} = 41$, $N_{2013} = 21$).

all $P < 0.005$) (Fig. 3). Notothenid fishes have high $\delta^{15}N$ values (on average 1.5‰ higher than cormorants) (Table 2, Fig. 3), suggesting that they may not belong to the same trophic web as the consumer, and are therefore not expected to contribute in an important way to cormorant diet. Nevertheless, as they appeared in important numbers in the diet obtained from Millones *et al.* (2005) they were included as a possible source in the mixing models.

The simulation method for inspection of mixing polygons confirmed that the consumer data were situated within the 95% mixing regions for each model (probability ranges for each model: 2011: 0.36–0.61; 2012: 0.12–0.33; 2013: 0.14–0.62), although on visual inspection some consumers seemed to partially fall out of it (Fig. 2). The results for all three models

Table 2. Stable isotope values for adults and chicks of the Red-legged Cormorant from Isla Elena, and of potential prey
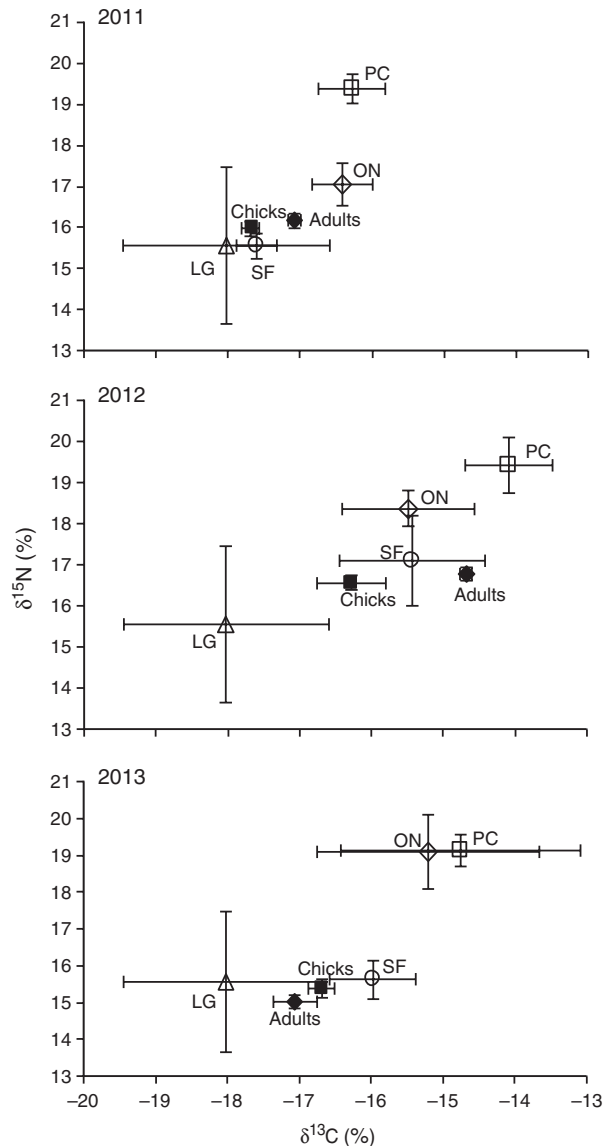
All data correspond to the year shown, aside from *Loligo gahi* ($N_{2012} = 5$, $N_{2013} = 1$). Data are presented as means with standard deviation (s.d., in parentheses)

	N	$\delta^{13}C$ (s.d.)	$\delta^{15}N$ (s.d.)
Red–legged Cormorants			
2011			
Adults	10	−17.08 (0.09)	+16.17 (0.18)
Chicks	10	−17.69 (0.12)	+15.97 (0.18)
2012			
Adults	6	−14.68 (0.08)	+16.77 (0.15)
Chicks	10	−16.28 (0.47)	+16.56 (0.17)
2013			
Adults	7	−17.06 (0.31)	+15.02 (0.17)
Chicks	9	−16.69 (0.18)	+15.38 (0.24)
Prey			
2011			
Sprattus fuegensis	4	−18.07 (0.28)	+13.95 (0.80)
Odontesthes nigricans	5	−16.87 (0.41)	+15.19 (0.52)
Patagonotothen cornucola	3	−16.74 (0.47)	+17.52 (0.36)
2012			
Sprattus fuegensis	4	−15.39 (0.57)	+15.24 (1.10)
Odontesthes nigricans	5	−15.95 (0.92)	+16.50 (0.43)
Patagonotothen cornucola	5	−14.54 (0.61)	+17.56 (0.67)
2013			
Sprattus fuegensis	5	−16.44 (0.60)	+13.75 (0.52)
Odontesthes nigricans	4	−15.66 (1.55)	+17.24 (1.01)
Patagonotothen cornucola	5	−15.22 (1.67)	+17.26 (0.43)
Years combined			
Loligo gahi	6	−18.48 (1.43)	+13.69 (1.91)

showed that the sprats appeared to be the main prey type in adults and chicks' diet, followed by squid (Table 3). Only for 2012's chicks was the proportion of sprats slightly smaller than that of squid. Overall, more than 40% of the assimilated diet was composed of sprats. The proportion estimates of sprats were slightly larger for the chicks than for the adults in 2011 and 2013 (probabilities of proportion of sprats from chicks being higher than from adults: $P2011 = 0.66$; $P2013 = 0.67$). The squid were the second-most-important prey type after sprats for chicks of all years and for 2013 adults. The silversides were second in importance for 2011 adults, and the notothenid fishes were the second most important prey type for 2012 adults. Nevertheless the notothenid and silverside proportion estimates of all years and ages (except silversides proportion estimates of 2011 adults) presented uncertainties that included some probabilities that these prey type were actually not part of the diet (lower 95% CI = 0) (Table 3).

Stable isotope analysis: comparative results of the two colonies

The mean isotopic niche position of chicks from Isla Elena and Isla del Rey colonies differed significantly in 2012 and 2013 (ED = 0.6 and 1.0‰ respectively, both $P < 0.001$). The chick dietary proportions obtained for 2012 and 2013 at Isla del Rey are in general comparable to those for Isla Elena. Both colonies showed important and similar interannual differences, with

Fig. 3. Mixing diagram of adults and chicks of Red-legged Cormorants (solid diamond) from Isla Elena and their potential prey (open triangles), presented by year. All data correspond to the year shown, aside from *Loligo gahi* ($N_{2012} = 5$, $N_{2013} = 1$). Values are mean ± s.d. Stable isotope values of the prey were corrected by adding the Diet Tissue Discrimination Factor (see Methods). Prey items: LG, *Loligo gahi* (Patagonian squid); SF, *Sprattus fuegensis* (Patagonian sprat); ON, *Odontesthes nigricans* (silverside); PC, *Patagonotothen cornucola* (notothenid fish).

comparatively lower sprat proportions, and with higher proportions of all other prey items in 2012 than in 2013 (Table 4). Nevertheless, Isla del Rey presented, for both years, slightly higher proportions of silversides and notothenid fishes compared with Isla Elena (probabilities for the proportions of a prey type from Isla del Rey being higher than from Isla Elena: $P2012$silverside: 0.56; $P2013$silverside: 0.78; $P2012$notothenids: 0.72; $P2013$notothenids: 0.80), and slightly lower proportions of sprats and squid compared with Isla Elena (probabilities for the proportions of a prey type from Isla del Rey being lower

Table 3. Diet composition proportions for the Red-legged Cormorant from Isla Elena obtained from stable isotope mixing models

The proportions were estimated using SIAR and are presented as mean estimates with 95% confidence intervals (in parentheses)

	Squid	Sprat	Silverside	Notothenids
2011				
Adults	19.5 (3.8–34.6)	37.8 (19.6–56.6)	31.7 (11.7–50.7)	11.0 (0–22.4)
Chicks	26.3 (9.0–42.8)	43.4 (22.3–65.6)	22.5 (2.1–40.3)	7.8 (0–18.6)
2012				
Adults	17.8 (0.6–34.7)	42.5 (15.6–72.1)	18.8 (0–39.8)	20.9 (0–40.2)
Chicks	37.0 (25.0–49.4)	29.1 (6.7–49.1)	23.1 (1.4–41.7)	10.9 (0–25.4)
2013				
Adults	42.3 (21.8–63.4)	44.8 (17.6–71.2)	6.7 (0–18.4)	6.1 (0–16.8)
Chicks	34.1 (15.9–52.0)	52.6 (29.7–75.6)	6.9 (0–17.1)	6.4 (0–16.0)

Table 4. Diet composition proportions for Red-legged Cormorant chicks from Isla Elena and Isla del Rey colonies, obtained from stable isotope mixing models

Proportions were estimated using SIAR and are presented as mean estimates with 95% confidence intervals (in parentheses)

	Squid	Sprat	Silverside	Notothenids
2012				
Is. Elena	37.0 (25.0–49.4)	29.1 (6.7–49.1)	23.1 (1.4–41.7)	10.9 (0–25.4)
Is. del Rey	31.0 (14.0–48.0)	24.7 (0.7–44.8)	25.6 (1.0–46.8)	18.7 (0–36.5)
2013				
Is. Elena	34.1 (15.9–52.0)	52.6 (29.7–75.6)	6.9 (0–17.1)	6.4 (0–16.0)
Is. del Rey	26.1 (4.1–44.9)	41.7 (15.2–70.2)	16.0 (0–33.9)	16.2 (0–33.0)

than from Isla Elena: P2012sprat: 0.61; P2013sprat: 0.71; P2012squid: 0.72; P2013squid: 0.73) (see Table 4).

Discussion

The combined dietary approach of this study provided a detailed description of the diet composition of the Red-legged Cormorant during several breeding seasons. Our results showed a low prey diversity and important interannual variation for the main prey types, as well as variation between the different breeding stages. Furthermore, this study provides evidence for the importance of pelagic and demersal–pelagic prey in the diet of this cormorant. These latter results contrast with the previous general foraging ecology descriptions obtained from conventional methods and from spatial foraging studies at Ría Deseado, which suggested that the feeding behaviour of this cormorant is mainly benthic (Frere *et al*. 2002; Millones *et al*. 2005). Millones *et al*. (2005) have found pelagic and demersal–pelagic prey in the cormorant's diet, especially during the breeding season; nevertheless, the general diet was considered mainly benthic and the extent of effectively assimilated pelagic prey remained unclear. In the present study, thanks to the combined dietary approach, we were able to show that pelagic species (sprats and squid) play a dominant role in the diet of breeding Red-legged Cormorant. Furthermore, our results raise concerns about the vulnerability of this low-mobility predator that depends on high-energy-content migrating fish (sprat), whose availability can be variable within the breeding season and among years.

Frere *et al*. (2002) showed that most of the dives corresponded to bottom-feeding behaviour, which is not necessarily in contradiction with our results. For this pursuit-diver seabird, the time spent feeding on benthic prey on the seafloor is much less effective than preying upon large school of pelagic fishes. For this reason, benthic feeding time is actually not comparable in terms of prey catch rate to that of feeding on pelagic prey schools. Furthermore, these cormorants constantly look for algae to bring to the nest during the breeding period, increasing time spent on the bottom that is not used for feeding purposes.

Seabirds sometimes feed their chicks with different prey items than the adults eat for themselves (Davoren and Burger 1999). In the case of our study, caution has to be taken when comparing SIA values for adults and chicks, because they could be showing not only temporal differences in the diet due to sampling dates (incubation vs chick-rearing) but also differences between adult and chick diet in the case where the chick would have been fed differently from what the adults eat for themselves. The same bias applies with pellet analysis of incubation vs chick-rearing stages: the incubation stage pellets reflect the adult's diet, and the pellets from the chick-rearing stages probably reflect a mix of adult and chick's diet. Nevertheless, although we have no direct evidence showing that adults and chicks of the Red-legged Cormorant share the same diet, we can assume that this is the case. The low diversity of prey items found in the diet, along with the sedentary and opportunistic behaviour of this cormorant, lead us to think that adults and

chicks probably eat similarly, and even more when pelagic prey are abundant. In order to compare the two techniques we considered that pellets from the incubation period and SIA adult samples both reflect the incubation period diet, and the pellets from the chick-rearing period and SIA chick's samples both reflect the chick-rearing period.

The two dietary assessment techniques used in this study showed similar overall results, with Patagonian sprats and Patagonian squid as the two main prey. The two techniques showed differences in the proportions of these two prey, with SIAR mixing models predicting overall lower proportions of squid and higher proportions of sprats than the conventional technique. Pellet analysis might have overestimated the squid proportions, due to the slow erosion rate of the beaks (Barrett *et al.* 2007). Both techniques showed an increase of sprats in the chick-rearing period compared with incubation, although the difference was less marked with SIAR mixing models. SIAR mixing models might have failed to detect clear differences between the incubation and chick-rearing stages due to the short period (average 19.7 days) between the blood sampling of adults (corresponding to the late incubation period) and young chicks (2–5 weeks old), with a concomitant partial overlap of the integration period of blood samples (1–5 weeks: Cherel *et al.* 2005) of both groups, and therefore blurring the differences in diet between the two periods. Even so, the general pattern of our results, showing more squid during incubation and more sprats during the chick-rearing stage, is in accordance with the increased availability of migrating sprats (first-choice prey) close to the coast during late spring and early summer (see below).

Both techniques showed important interannual variability in their prey proportions, although the way they varied was not always correlated from one technique to the other. Interannual variability in prey availability is common in marine ecosystems and in the case of Patagonian squid and sprats it may be related to variability in the productivity of the Southern Patagonian Shelf Ecosystem, as none of these prey stocks are being targeted by industrial fisheries in this region.

Our results comparing the two colonies with SIA allowed a better comprehension of resource use at the level of the entire ria, which seems to be dominated by interannual variation rather than spatial variation (location of colonies). Despite the similarities of diet obtained for both colonies, we found that the diet from Isla del Rey, situated 15 km inside the estuary, differed slightly from that of Isla Elena, situated at its entrance, insofar as it incorporated more demersal–pelagic silversides and benthic notothenids, and fewer pelagic sprats and squid. Our SIA results for Isla del Rey are therefore in agreement with the diet obtained from pellet analysis at the deeper location of Isla del Rey by Millones *et al.* (2005). The spatial differences in the use of trophic resources are in agreement with the prey availability: pelagic species like sprats are not residents of the ria and periodically migrate inside it; these events are probably more frequent near the outflow, allowing the migrating fish to penetrate deep inside the inlet, only to a lesser degree. Furthermore, the different Red-legged Cormorant colonies, along with other important seabird and marine mammal populations, are probably contributing to the depletion of the schools of sprats as they move further inside the estuary. Therefore this prey must be less accessible to the inner colonies, considering that Red-legged

Cormorants are feeding close to their colonies (Frere *et al.* 2002; Gandini *et al.* 2005).

The Patagonian sprat is a high-quality prey, with higher energy content by comparison with any other available seabird prey from the Southern Patagonian Shelf Ecosystem (Ciancio *et al.* 2007). Sprats congregate and spawn in the south of this ecosystem, and when larvae develop, they drift north and west (Ciancio *et al.* 2015). Therefore, sprats are present in high number in our study area only during late spring and summer, and, within this period, they are more abundant during the chick-rearing stage of cormorants than during incubation. This explains the shift of diet between the breeding and the non-breeding season (Millones *et al.* 2005) and possibly the increased proportions of sprat during the chick-rearing period, as observed in our study with pellets. Sprats seem to be the first-choice prey, but when their availability is reduced, as in 2009 and 2012, the cormorant will feed upon lower-energy-content prey (squid, silversides, and notothenids) and on a broader prey spectrum. Therefore we can conclude that this sedentary central-place forager might depend on the migration timing and availability of energetically rich prey (sprat) close to its breeding grounds for optimal foraging.

We consider that the framework used, which includes combining different methods, together with an appropriate study design, provided a more accurate overview than using each method separately. Nevertheless, an optimal use of SIA mixing models has to take into account the need for prior information about the prey-to-consumer discrimination factor, and for best results a thorough sampling of the possible prey, preferably from the same study site and period as the consumers, has to be planned and undertaken. Indeed, our results show the importance of sampling alongside prey and consumers each year of the study period due to the possible interannual variability of SIA values. One of the main prey of our study, the sprat, and also our consumer, showed strong variability of $\delta^{13}C$ and $\delta^{15}N$ values between the three sampled years, at the same location (Fig. 3).

To our knowledge, no study has been undertaken on the use of trophic resources on the Pacific coast, where a large proportion of the total world population of Red-legged Cormorants is found. Considering that small forage fishes are an important diet component for this species in the Atlantic coast during the breeding season, and that numerous seabird species are feeding on small forage fish in the Humboldt Current, we can expect that the Red-legged Cormorant might feed on sprats and other small forage fish along the Pacific coast too. A decline in numbers of small pelagic fish has negatively affected the population trends of many seabirds worldwide, including in the Humboldt Current area. These temporary or permanent declines can be the result of overfishing or oceanic changes related to low-productivity years, such as the ENSO event. ENSO events are known to negatively affect ocean productivity mainly on the Pacific coast – and to a lesser degree on the Atlantic coast too (Duffy 1990). Considering the results of our study, we expect that possible changes in the availability of the Patagonian sprat in the Southern Patagonian Shelf Ecosystem, and, by extension, changes in the availability of other small and low trophic forage fishes throughout the distribution range of the Red-legged Cormorant, could be important factors affecting the breeding population of this central-place forager seabird.

Finally, our study results suggest that the combination of conventional technique with SIA provides a solid framework for this seabird diet assessment. Our results have important conservation implications for this locally endangered species as they contribute a detailed description of its dietary composition during several breeding seasons, enhancing the low prey diversity, the variability of the main prey proportions along breeding stages and an important interannual variation. Furthermore, this study provides evidence for the importance of pelagic prey in the diet of this under-studied cormorant during its breeding season. These results therefore contribute new and unambiguous information and a better understanding of the Red-legged Cormorant's use of trophic resources.

Acknowledgments

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