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The diet of Olrog's Gull (*Larus atlanticus*) reveals an association with fisheries during the non-breeding season

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Abstract. In this study, we used stable-isotope analysis to determine the importance of different food resources in the diet of an endemic gull species of the Atlantic coast of southern South America during the non-breeding period. We compared the isotopes ¹⁵N and ¹³C in the blood of Olrog's Gulls (*Larus atlanticus*) of different ages with those in potential prey of the Gulls. We also determined the abundance of Olrog's Gull in their wintering areas and attending coastal fisheries operations in a small region of Argentina. An isotope-mixing model showed differences in the isotopic signatures of adult, subadult and juvenile Gulls. Although the isotope-mixing model showed crabs as the main prey, it also showed that demersal and pelagic fish may be important in the diet. We speculate that the demersal and pelagic fish in the diet, shown by their isotopic signatures in the Gulls, may be taken in association with coastal fisheries, particularly bottom otter-paired trawling. At-sea observations showed that Olrog's Gulls were one of the most-common birds attending fishing operations of the Argentine coastal fishing fleet, taking advantage of fish mostly made available through hauling operations.

Additional keywords: crabs, dietary habits, foraging, stable-isotopes.

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Introduction

Knowledge of predator–prey interactions is essential for understanding the animal's behaviour and the dynamics of populations, as well as the direct and indirect effects that humans have on ecosystems. In the marine environment, seabirds are important, top-level predators, consuming an estimated 7% of total marine productivity (Brooke 2004). However, their life-history traits (e.g. long-lived, low-fecundity, delayed maturity) make them among the most threatened marine species, comprising 25% of extinctions of all marine organisms (Dulvy *et al.* 2003).

Of the range of methods used to determine the diet of seabirds the analysis of regurgitated pellets has been used widely because it provides valuable information with little field effort by researchers and little disturbance to the species studied during the non-breeding season (Barrett *et al.* 2007). More recently, measurements of the levels of naturally occurring stable isotopes (¹⁵N and ¹³C) in the body tissues of prey consumers has become an established method of investigating predator–prey relationships, particularly in seabirds (Hodum and Hobson 2000; Forero *et al.* 2004; Cherel *et al.* 2005). The analysis of stable-isotope ratios in consumer tissues and potential prey is now widely used to study individual feeding ecology (Rubenstein and Hobson 2004; Inger and Bearhop 2008). For example, the analysis of stable isotopes of carbon and nitrogen and the use of mixing models has recently been successfully used in the study of the feeding

ecology of several species of seabird (Forero et al. 2004; Navarro et al. 2009; Ramos et al. 2009; Moreno et al. 2010) and in studies of marine birds congregating along the south-eastern coast of Argentina during their non-breeding seasons (Mariano-Jelicich et al. 2008). Stable isotopes provide advantages over more traditional methods of estimating the diet of seabirds because they represent assimilated, rather than ingested, food, indicate averages over an extended period of tissue formation, and sampling has little effect on the animals sampled (Tieszen et al. 1983; Hobson and Welch 1992). In combination with long-term monitoring programs, for the study of trophic relationships the analysis of stable isotopes may overcome the shortcomings of other methods of dietary analysis and constitute more effective tools for determining the importance of different food resources, the consumption of which may have implications for the conservation status of a species (Navarro et al. 2009).

Olrog's Gull (*Larus atlanticus*) is endemic to the Atlantic coast of southern South America and one of the few globally threatened species of *Larus*, being listed as vulnerable (BirdLife International 2012). Recently studies revealed that the size of the total breeding population is fairly small, with less than 8000 pairs (Yorio *et al.* 2012). Although most gulls are generalist foragers, Olrog's Gull is considered to be one of few gulls with specialised feeding habits (Burger and Gochfeld 1996). Using conventional methods (i.e. analysis of regurgitations), most studies of the diet

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of Olrog's Gulls during the non-breeding season have shown that they feed primarily on varunid crabs (Copello and Favero 2001; Berón and Favero 2010). However, Olrog's Gulls may have recently broadened their diet by associating with sport-fishing and land-based and offshore commercial fishing operations (Martínez *et al.* 2000; Berón and Favero 2009; Seco Pon *et al.* 2012; Seco Pon and Favero, in press). Earlier studies in Buenos Aires Province found that juvenile Olrog's Gulls are socially subordinate to adults in selection of foraging sites, are less skilled in capturing and handling crabs and compensate for this lack of skill by using alternative food resources, such as anthropogenic food sources (Berón *et al.* 2007, 2011).

A problem inherent in studying the interaction of individual marine predators and fisheries is the inability to study at-sea foraging behaviour in detail, such as simultaneously recording diet, feeding behaviour and activity of fishing vessels (Duffy 1983; Crawford 2007; Votier et al. 2010). However, for Olrog's Gulls, a combination of existing fine-scale data on the exploitation of coastal areas and attendance at small-scale fishing vessels with analysis of stable-isotope ratios in Gull tissues should make it possible to understand the interaction of Olrog's Gulls with Argentine fisheries operations. Counts from land and at sea have provided high-resolution data on the spatial and temporal distribution of Gulls in coastal areas and around fishing vessels (Hudson and Furness 1989; Garthe and Huppop 1994; Santora et al. 2009; Wickliffe and Jodice 2010; Seco Pon et al. 2012). In this study, we undertook an analysis of stable-isotope ratios in Olrog's Gulls and their potential prev to assess the relative contribution of coastal fisheries to the diet of individual Olrog's Gulls of different ages in the main wintering areas in Argentina. Analysis of stable isotopes should complement the results of traditional methods of dietary analysis used in studies of Olrog's Gulls (direct observations, analysis of regurgitations), which can overestimate the importance of prey with hard body parts.

Materials and methods

Study area

This study was conducted at two sites in south-eastern Buenos Aires Province, Argentina: dietary analysis was done at Mar Chiquita Coastal Lagoon (37°40′S, 57°22′W), and observations of associations with fishing vessels were made in Mar del Plata Harbour (38°03'S, 57°32'W). The sites are 30 km apart and widely used by Olrog's Gulls in winter, between April and September. Mar Chiquita Coastal Lagoon is a UNESCO Man and the Biosphere (MAB) Programme world biosphere reserve and a provincial reserve; it is ~46 km² in area, brackish with muddy sediments and mudflats, low tidal amplitude and a large surrounding marsh dominated by cordgrass (Spartina densiflora) (Botto and Iribarne 2000). Three crab species dominate the macro-invertebrate fauna of the estuary, two semi-terrestrial burrowing species, the Burrowing Crab (Neohelice granulata) and the Fiddler Crab (Uca uruguayensis), which both occur in the upper littoral, and the Mud Crab (Cyrtograpsus angulatus), which is mainly found in the lower littoral and on rocky seashores. Mar del Plata is the most important commercial harbour in Argentina, with ~70% of the coastal fishing fleet based there and ~80% of the national coastal fishery catch is processed through the Harbour (Lasta et al. 2001). Owing to the highly variable operability and utilisation of a diverse array of fishing gear, the fishery has been technically described as a non-selective, multispecies coastal fishery, locally known as 'variado costero' (Errazti and Bertolotti 1998; Consejo Federal Pesquero 2006).

Analysis of stable isotopes

Blood samples for isotopic analysis were collected between April and September 2007 from 19 Gulls (6 juveniles, 9 subadults and 4 adults) captured with bal-chatri traps (Blom 1987) in Mar Chiquita Coastal Lagoon. Individuals were released immediately after blood sampling and banding. Approximately 0.3 mL of blood was obtained from the braquial vein, transferred to a vial with 1.5 mL of absolute ethanol and stored at room temperature until analysis. Ethanol was used as a preserver as it has been shown to have negligible effect on the stable-isotope values for carbon (13C) and nitrogen (15N) in a variety of tissues (Hobson et al. 1997; Kelly et al. 2006; Bugoni et al. 2008). Whole blood was used because it integrates dietary information over 3-4 weeks before sampling (Hobson and Clark 1992; Bearhop et al. 2002; Cherel et al. 2007). Blood samples were dried in an oven at 60°C and ground using a hand mortar (Hobson et al. 1997; Cherel et al. 2007).

Stable-isotope signatures of $\delta^{13}C$ and $\delta^{15}N$ of each individual Gull were determined by mass spectrometry at the Centro de Aplicaciones de Tecnología Nuclear en Agricultura Sostenible (CATNAS, Montevideo, Uruguay). Results are presented in the usual δ notation, relative to Vienna Pee Dee Belemnite for $\delta^{13}C$ and to atmospheric N^2 (air) for $\delta^{15}N$.

To assess quantitatively the importance of different food sources, we used a Bayesian stable-isotope mixing model (Jackson et al. 2009). This model is very useful in providing quantitative indices of the contributions of various food items to a consumers' diet (Caut et al. 2008; Ramos et al. 2009). However, such models require prior conventional dietary analysis to allow the selection of potential prey to include in the modelling. In this study, potential prev were determined from previous conventional dietary studies from Mar Chiquita Coastal Lagoon (Berón 2003; Berón et al. 2007) and direct observations of Gulls associated with semi-commercial (J. P. Seco Pon, unpubl. data) and commercial (Seco Pon and Favero, in press) fisheries. Isotopic data for crab and fish species (potential prey) were obtained from other studies conducted in the same coastal area as the study site (Botto et al. 2005, 2011; Mariano-Jelicich et al. 2008). Preliminary studies have shown that there are no significant differences in isotopic signals of crabs between years (F. Botto, pers. comm.), so we assumed that baseline isotopic signatures do not change between years and have thus used the published data in our study.

To compare the contribution of fish caught either naturally or in association with human activities, species were grouped in three trophic guilds: (1) pelagic fish, which are a natural food resource (Argentine Anchovy, Engraulis anchoita; Cornalito Silverside. Odontesthes incisa; Pejerrey Silverside, O. argentinensis); (2) demersal fish, which are an anthropogenic food resource (Stripped Weakfish, Cynoscion guatucupa; Whitemouth Croaker, Micropogonias furnieri; flounder, Paralichthys orbygnianus) and (3) benthic-demersal fish, which is also an anthropogenic food resource (Southern Eagle Ray, Myliobatis goodei; Rio Skate, Rioraja agazzizi; Smallnose Fanskate, Squa-

71

tina bonapartii; Spotback Skate, Atlantoraja castelnaui). Classification of fish into the three guilds was based on published literature (Cousseau and Perrota 2004; Botto et al. 2011). Data for Argentine Anchovy were corrected for lipid content (Logan et al. 2008), yielding changes in δ^{13} C of 0.59–1.29% (as determined by C: N).

Bayesian stable-isotope mixing models allow for the inclusion of known variability in sources, as well as fractionation and elemental concentration and other unquantified variability, within the model and so offer many advantages over previous mixing models (Jackson et al. 2009). Moreover, the outputs of stableisotope mixing models represent true probability density functions, rather than a range of possible solutions as in earlier mixing models (Votier et. al. 2010). In order to use the Bayesian stableisotope mixing model, the isotopic values for food sources must be adjusted by appropriate fractionation factors (enrichment factor Δ_{dt}) of N and C during digestion and assimilation (Gannes et al. 1998). We obtained the most appropriate fractionation factor from the literature; for δ^{13} C the used enrichment factor was -0.3% and for δ^{15} N the diet-blood enrichment factor used was 3.1% (both factors were calculated as the average value for Δ_{dt} diet-blood from lipid-free fish prey and whole blood of different seabird species, Hobson and Clark 1992; Bearhop et al. 2002).

Abundance of Olrog's Gull

We estimated the abundance of Olrog's Gull in Mar Chiquita Coastal Lagoon (n = 12 counts) and Mar del Plata Harbour (n = 6) during the non-breeding season (April-September) of 2007. Abundance was determined using point counts (Bibby et al. 1997). One or more land-based counts were conducted per month at both study sites (Mar Chiquita and Mar del Plata Harbour) and each census took ~1 h. Mean abundance was determined for each season (autumn, winter and spring) using the maximum values of each count. We recognised three ageclasses based on plumage characteristics (see Harrison 1983): juveniles (1–2 years old), subadults (3 years old) and adults (>4 years old, and potential breeders).

During the non-breeding season of 2007 the abundance of Olrog's Gulls attending semi-commercial coastal vessels operating in waters off Mar del Plata Harbour was recorded as part of a broader study on the interaction of top-order predators and smallscale coastal fisheries. Two different fisheries operations were observed during the study: (1) fishing operations using purseseine nets without leadlines (locally known as 'lampara nets') and targeting Chub Mackerel (Scomber japonicus) (counts were made during May-December 2007 on 22 trips) and (2) fisheries using bottom otter-paired trawl-nets, targeting Argentine Shrimp (Pleoticus muelleri) (June; 5 trips) (Seco Pon et al. 2012). Purseseine operations discard few fish (<2% of monitored hauls), mainly Rough Scad (Trachurus lathami) during the sampling period, whereas trawl-net operations discarded larger numbers of pelagic, demersal (Scianidae, Carangidae) and benthic fish (Paralichthydae, Rajidae) and crabs (Platyxanthidae, Portunidae) (Seco Pon et al. 2012). In general, monitored fisheries operated within ~18.5 km (10 nautical miles) of Mar del Plata Harbour in shallow waters 3.4-40 m deep. At-sea abundance of Gulls was estimated by seabird observers on the vessels in counts at intervals of 30 min during the duration of each fishing operation. Counts

were made during every fishing operation on each fishing trip; a total of 122 counts were conducted on a total of 27 trips (22 purseseine operations, 5 trawl-net operations, as above). The observers stood in the stern corner of the vessel, which afforded the least obstructed view of the fishing gear and area behind the vessels. At the beginning of each survey, the observer scanned repeatedly within 5 min a 270°-arc astern of the ship (the area obstructed by the wheel-house being excluded) and counted within a 30-m radius. Maximum numbers of Olrog's Gulls were obtained by pooling the data for each fishing trip. Owing to the difficulty of accurately assessing plumage differences at sea, Olrog's Gulls were classified only as adult or young (juvenile and subadult combined).

Statistical analysis

Values of stable isotope ratios of prey types were compared by means of a one-way analysis of variance (ANOVA) with Levene's test used to check for homoscedasticity. Posterior pairwise differences were tested using Tuckey's procedure (Zar 1999). Isotopic signatures of trophic guilds were then compared using a one-way ANOVA (Zar 1999). All analyses included the Bayesian mixing model (SIAR; Parnell et al. 2008) and the IsoSource model and were fitted using R software ver.2.13.1 (R Development Core Team 2011). Kruskal-Wallis tests were used to examine differences among adults and juveniles associated with fishing vessels (Corder and Foreman 2009).

Results

Stable isotope signatures for predator and prey

Mean values (and s.d.) for the isotopic signatures of adult, subadult and juvenile Olrog's Gulls were 13.32% (0.4), 14.81% (0.6) and 13.03% (0.3) for $\delta^{15}N$ and -16.30% (0.3), -15.92% (0.3) and -15.37% (0.6) for δ^{13} C, respectively. The δ¹⁵N values of prey in the diet of Olrog's Gulls ranged from 8.56 to 17.59%; N. granulata had the lowest values, A. castelnaui the highest. The δ^{13} C of prev in the diet of Olrog's Gulls showed values between -18.40 and -12.64%; C. angulatus had the lowest values, N. granulata the highest (Fig. 1). Values of δ¹³C differed significantly between the demersal and pelagic fish and the benthic-demersal fish ($F_{2,9}$ =43.34, P<0.001). These differences were a result of carbon enrichment of benthicdemersal species compared with demersal (Tuckey test, P 0.001) and pelagic (Tuckey test, P < 0.001) fish, and carbon depletion in pelagic fish compared with demersal fish (Tuckey test, P < 0.05). Isotopic nitrogen signatures tended to be higher in benthic-demersal species compared with demersal and pelagic fish, but differences were not statistically significant ($F_{2,9} = 3.3$, P = 0.08). Fig. 1 shows variations in isotopic nitrogen signatures of subadult Gulls feeding at different trophic levels.

The stable-isotope analysis in R Bayesian mixing model showed that Gulls of all ages mainly ate crabs (95% credibility interval: adults, 14-49% N. granulata, 10-45% C. angulatus; subadults, 19-42% N. granulata, 4-37% C. angulatus; juveniles, 28–71% N. granulata, 3–50% C. angulatus). Adult and subadult Gulls consumed similar proportions of demersal fish (95% credibility interval: adults, 0-32%; subadults 0-37%), whereas juveniles consumed smaller amounts (0-23%). Stable-isotope analysis indicated that pelagic and benthic-demersal fish were the

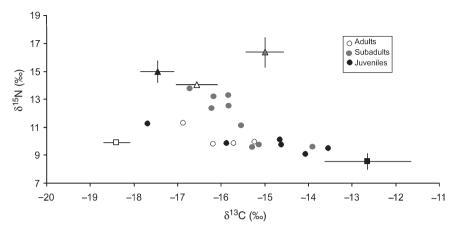


Fig. 1. Mean values of δ^{13} C and δ^{15} N (\pm s.d.) for Olrog's Gulls and their potential prey. Values for Olrog's Gull adjusted for fractionation. Cirlces, Olrog's Gulls; white triangles, demersal fish; black triangles, pelagic fish; grey triangles, benthic-demersal fish; white squares, Cyrtograpsus angulatus; black squares, Neohelice granulata.

prey types least consumed by all ages of Olrog's Gull (95% credibility interval: adults, 0-30% pelagic fish, 0-26% benthicdemersal fish; subadults, 0-34%, 0-31%; juveniles, 0-20%, 0-16%; Fig. 2).

Association of Olrog's Gull with coastal fisheries

The abundance of different ages of Olrog's Gulls in Mar Chiquita Coastal Lagoon and Mar del Plata Harbour was similar, with juveniles being more abundant and occurring more regularly than subadults and adults. At sea, both adults and young (subadults and juveniles combined) were recorded from fishing vessels. In Mar Chiquita Coastal Lagoon and Mar del Plata Harbour and at sea, numbers were greater in autumn (21 March–20 June) (Table 1).

Olrog's Gulls regularly attended fishing vessels, usually with other seabirds and marine top predators, such as Kelp Gulls (Larus dominicanus), Brown-hooded Gulls (Chroicocephalus maculipennis), South American Terns (Sterna hirundinacea), Common Terns (S. hirundo) and South American Sea-lions (Otaria flavescens). Olrog's Gulls comprised ~16% of total seabird numbers attending the fishing operations (both types of fisheries operations combined). Most Olrog's Gulls (62%) were observed attending trawl-net operations, whereas only 38% attended purse-seine operations. Olrog's Gulls attended coastal vessels throughout the non-breeding season (Kruskal-Wallis $H_{6,122 \text{ counts}} = 76.393, P < 0.001$).

Discussion

In Olrog's Gulls, as all organisms, diet plays an important role in determining the condition and survival of individuals, in both their wintering and breeding ranges, as well as influencing breeding success and recruitment. In seabirds, fish are often considered a preferred resource (Lewis et al. 2001), whereas invertebrates are considered a lower-quality resource owing to their lower energetic content and higher proportion of hard indigestible material compared with fish (Ciancio et al. 2007). However, because they are abundant, accessible and their occurrence predictable, crabs can form a large proportion of the diet of gulls, and particularly Olrog's Gulls during the breeding season (Yorio et al. 2005). The crabs N. granulata and C. angulatus are known to be a food resource extensively exploited by all ages of Olrog's Gulls during the non-breeding season in Mar Chiquita Coastal Lagoon (see Berón et al. 2011, and references therein). The stable-isotope mixing model showed that crabs contributed more than fish to the diet of Gulls of all ages.

Considering only crabs, N. granulata and C. angulatus formed similar proportions of the diet of adult and subadult Gulls, whereas N. granulata formed a larger proportion of the diet of juvenile Gulls, consistent with earlier studies showing differences in the diet relating to foraging skills of individuals of different ages (see Berón et al. 2011). Although previous dietary data based on analysis of regurgitated pellets showed that the diet of Olrog's Gulls during the non-breeding season was dominated by crabs (e.g. Berón and Favero 2010), the present study highlights the likely importance of demersal and pelagic fish in the diet. Moreover, the analysis of stable isotopes indicated some individual foraging plasticity, with subadults feeding at different trophic levels than adults and juveniles (Fig. 1). These differences have been observed in other species of seabird and, thus, highlight the utility of stable-isotope analysis (Bearhop et al. 2006; Cherel et al. 2006, 2007; Votier et al. 2010). Based on observations of Olrog's Gulls attending coastal fishing operations, we suggest that the contributions of fish to the isotopic signatures in the Gulls may derive from prey taken in association with, and facilitated by, the fisheries operations, particularly with bottom otter-paired trawling operations. Isotopic signatures of demersal and benthic fish in Olrog's Gulls may also be, at least in part, a product of the consumption of fish remains or byproducts of sport fishing activities common in the study area (Berón and Favero 2009). Although it has been suggested (but not quantified) that Olrog's Gulls exploit anthropogenic food resources (Martínez et al. 2000), our results indicate that, at least during the non-breeding period, fish are taken regularly and, at times, form as important a component of the diet as some crab species (see isotopic signatures of subadult Gulls in Fig. 2). The occurrence of fish in the diet may have been underestimated or undetected in previous

73

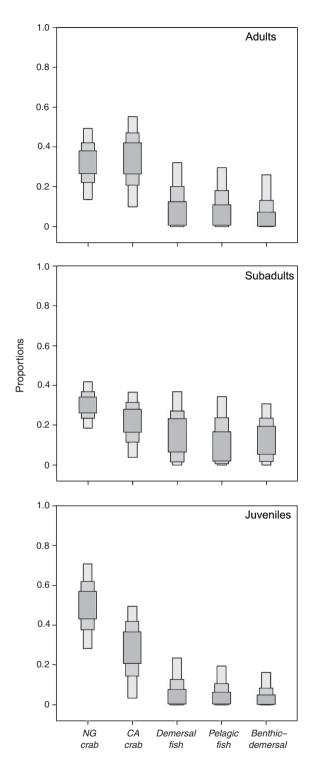


Fig. 2. Results of the Bayesian stable-isotope mixing model (95, 75 and 50% credibility intervals shown), indicating estimated contribution of prey items to the diet of adult, subadult and juvenile Olrog's Gulls sampled in Mar Chiquita Coastal Lagoon during the non-breeding period (April-September) 2007. NG crab, Neohelice granulata; CA crab, Cyrtograpsus angulatus; Demersal fish: Cynoscion guatucupa, Micropogonias furnieri and Paralichthys orbygnianus; Pelagic fish: Engraulis anchoita, Odontesthes incisa and O. argentiniensis; Benthic-demersal fish: Myliobatis goodei, Rioraja agazzizi, Squatina bonapartii and Atlantoraja castelnaui (see Methods).

Table 1. Mean abundance per count (±s.d.) of adult, subadult and juvenile Olrog's Gulls at Mar Chiquita Coastal Lagoon (MCH), Mar del Plata Harbour (MDP) and attending semi-commercial coastal fishing vessels (FV) April-September 2007

Number of counts are in parentheses. At-sea observations from fishing vessels combined subadult and juvenile Gulls in a single age-class 'Young' (see Methods)

Age	Site	Season		
		Autumn	Winter	Spring
Adults	MCH	0.6 ± 0.7 (3)	1.7 ± 4.5 (6)	1.0 ± 0.3 (3)
	MDP	_	5.0 ± 1.4 (2)	_
	FV	$10.5 \pm 10.3 \ (6)$	-	0.1 ± 0.4 (7)
Subadults	MCH	2.3 ± 0.6 (3)	2.8 ± 1.7 (6)	2.0 ± 1.0 (3)
	MDP	_	2.5 ± 0.7 (2)	1.0 ± 0.7 (2)
Juveniles	MCH	5.0 ± 1.0 (3)	6.2 ± 3.4 (6)	6.0 ± 3.0 (3)
	MDP	1.0 ± 1.4 (2)	4.0 ± 1.4 (2)	0.5 ± 0.7 (2)
Young	FV^A	$10.8 \pm 14.1 \ (6)$	_	_

^ASubadult and juvenile individuals pooled.

studies owing to the limitations of earlier methodologies (see Introduction).

The use of fishery discards may benefit Olrog's Gulls by providing an additional and predictable source of food, enhancing their conservation (Martínez et al. 2000). However, fisheries activities are known to have long-term negative effects on some seabird populations via adverse effects on marine food webs (e.g. through destruction of habitat and overexploitation; Pauly et al. 1998; van Gils et al. 2006) and mortality of seabirds associated with fishing operations (Anderson et al. 2011, and references therein). The availability of supplementary food resources, in the form of fishery discards or escaping fish, can also lead to complex interactions between birds and fisheries activities by modifying the population biology of species through enhanced breeding success and survival (Tavecchia et al. 2007). Further, such interactions can favour some species over others; for example, increases in populations of Kelp Gulls can increase rates of predation on other species and colonisation of sites previously used by smaller and threatened populations of species such as Olrog's Gull (García-Borboroglu and Yorio 2007).

Olrog's Gulls have previously been reported associating with sport-fishing activities in their non-breeding grounds and making use of fishing by-products, sometimes resulting in birds being seriously injured or killed through entanglement in fishing lines and nylon bags and the ingestion of discarded baited hooks (Berón and Favero 2009). Because the risk of injury to or mortality of seabirds appears to be linked to the abundance of seabirds associating with a fishery operation (Abraham et al. 2009; Pierre et al. 2010) it is of concern that Olrog's Gulls are regularly attending Argentine coastal fisheries. However, during our at-sea observations we did not record any incidental mortality of Gulls associating with fishing vessels or any serious adverse interactions with fishing gear. Our work suggests that more detailed studies of the interaction between Olrog's Gulls and both sport and small-scale commercial fishing are needed in order to understand how these activities affect the conservation of this threatened South American gull.

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75

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76 Emu M. P. Berón et al.

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