



# DEPARTAMENTO DE CIÊNCIAS DA VIDA

FACULDADE DE CIÊNCIAS E TECNOLOGIA  
UNIVERSIDADE DE COIMBRA

Stable isotopes and regurgitations reveal differential consumption of fishery discards by yellow-legged and Audouin's gulls breeding in sympatry.

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Joana Filipa Gomes Calado

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## Abstract

Gulls are opportunistic seabirds that can take advantage of anthropogenic resources, such as discards produced by fisheries. Here we compared the feeding ecology of two gull species, the yellow-legged gull *Larus michahellis* (YLG) and the Audouin's gull *L. audouinii* (AG), breeding in sympatry at Barreta Island (South of Portugal). We assessed year-round inter-specific differences on the trophic (multi-tissue stable isotope analysis) and dietary (conventional techniques for diet identification) choices of both gull species, and estimated the importance of fishery discards in their diet. This represents the first study investigating resource partitioning between AG and YLG in Portugal. Overall, our results show segregation in habitat use, trophic ecology, and isotopic niche between these two sympatric gull species, and indicate marked inter-seasonal differences in their foraging strategies and resource partitioning. Pellets collected during the breeding season revealed a strong difference in the frequency of occurrence of epipelagic fish (*Belone belone*) in the diet of the two gull species (AG = 55.9% and YLG = 6.7%). The stable isotope mixing models estimated a higher proportion of demersal fish in the diet of YLG (43%) compared to AG (20%). The YLG generally showed a wider isotopic niche than AG, with significant differences in breast and eighth secondary feathers, representative of their diet during all year and the non-breeding period, respectively. AG chicks were highly segregated from their parents in their carbon signatures, meaning that AG adults fed their chicks with higher-quality prey captured in offshore waters (i.e. pelagic fish). Additionally, field observations revealed significant differences in the foraging strategies

between the two gull species, with higher numbers of YLG following the fishing boats and higher numbers of AG returning from offshore foraging locations.

Overall, YLG showed more generalist and opportunistic foraging strategies, and fed more on discards than AG. In this respect, the discard ban policy, implemented under the European Union Common Fisheries Policy in the next few years, will probably result in severe food shortage and, consequently, in negative interactions of the larger and more aggressive YLG on smaller sympatric seabird species. Therefore, future research should closely monitor the impact of the abundant YLG on the endangered AG.

**Keywords:** Yellow-legged gull; Audouin's gull; Diet; Stable isotopes; Discard ban.



## Resumo

As gaivotas são aves marinhas oportunistas que podem alimentar-se de recursos antropogénicos, tais como as rejeições produzidas pela pesca. Neste trabalho comparámos a ecologia alimentar de duas espécies de gaivotas (gaivota de patas-amarelas *Larus michahellis* e gaivota de Audouin *L. audouinii*) que se reproduzem em simpatria na Ilha da Barreta (Sul de Portugal). Utilizámos métodos convencionais e medimos as assinaturas isotópicas de diferentes tecidos para detetar diferenças entre as duas espécies ao longo do seu ciclo anual, que também nos permitiu estimar a importância dos peixes rejeitados pelas pescas na dieta de cada espécie. Este estudo é o primeiro a investigar a partilha de recursos entre as gaivotas de Audouin e de patas-amarelas em Portugal, utilizando a análise de isótopos estáveis, que fornece informações dietéticas integrativas. No geral, os nossos resultados indicam segregação no uso do habitat, ecologia trófica e nicho isotópico entre estas duas espécies simpátricas de gaivotas, e revelam diferenças nas suas estratégias de procura e partilha de recursos alimentares ao longo do ano. As egagrópilas recolhidas durante a época de reprodução revelaram uma grande diferença na frequência de ocorrência de peixes epipelágicos (*Belone belone*) na dieta das duas gaivotas (Audouin = 55,9% e patas-amarelas = 6,7%). Os modelos mistos de isótopos estáveis estimaram uma maior proporção de peixes demersais na dieta da gaivota de patas-amarelas (43%) em comparação com a gaivota de Audouin (20%). No geral, a gaivota de patas-amarelas possuiu um nicho isotópico mais amplo do que a gaivota de Audouin, com diferenças significativas nas penas do peito e na oitava pena secundária, representantes da dieta durante todo o ano e do período não reprodutivo,

respetivamente. As crias de Audouin apresentaram uma segregação importante em relação aos adultos nos valores isotópicos de carbono, o que indica que os progenitores alimentaram as suas crias com presas marinhas de maior qualidade (ou seja, peixes pelágicos). Além disso, observações de gaivotas que regressam das viagens de alimentação revelaram diferenças significativas nas estratégias de procura e captura de alimento entre as duas espécies, detetando-se um maior número da gaivota de patas-amarelas atrás dos barcos de pesca, e um maior número de gaivotas de Audouin a regressar do mar aberto.

No nosso estudo, a gaivota de patas-amarelas mostrou estratégias de procura e captura de alimento mais generalistas e oportunistas, alimentando-se mais das rejeições do que a gaivota de Audouin, o que está de acordo com estudos anteriores. A este respeito, a política de proibição das rejeições, a ser implementada no âmbito da Política Comum das Pescas da União Europeia nos próximos anos, irá, muito provavelmente, resultar numa grave escassez alimentar e, conseqüentemente, em interações negativas por parte da gaivota de patas-amarelas, espécie maior e mais agressiva, sobre aves marinhas simpátricas de menores dimensões. Por isso, pesquisas futuras deverão acompanhar de perto o impacto da gaivota de patas-amarelas na ameaçada gaivota de Audouin.

**Palavras-chave:** Gaivota de patas-amarelas; Gaivota de Audouin; Dieta; Isótopos estáveis; Proibição das rejeições.

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## **Chapter 1 – Introduction**

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## **1.1 Seabirds as indicators of marine ecosystems**

Seabirds are often at the top of food chains, responding to environmental changes, particularly those that occur at lower trophic levels, and have been proposed as indicators of the health and structure of marine ecosystems (Furness & Camphuysen 1997; Sydeman et al. 2007). Seabirds have several attributes which make them potentially suitable indicators of ecosystem status. They are large, wide ranging, highly visible, and conspicuous animals, with a high level of public interest (Burger & Gochfeld 2002). They also have the advantage of colonial terrestrial breeding, where large numbers of individuals concentrate at relatively few sites (Piatt et al. 2007a), and many species are philopatric (Brooke 2002), making them accessible and easy to study on a regular basis (Boyd et al. 2006). Because seabirds are long-lived species, with high adult survival rates and several years of deferred maturity (Schreiber & Burger 2002), their population numbers vary little among years, while prey species at intermediate trophic levels are short-lived or heavily fished, patchily distributed, highly mobile, show large fluctuations in recruitment, and, therefore, are difficult to survey (Montevecchi 1993; Cherel & Weimerskirch 1995; Furness & Camphuysen 1997). Thus, seabirds have been used as indicators of marine food supplies (Cairns 1988; Hamer et al. 2006; Harding et al. 2007; Piatt et al. 2007b). Shifts on seabirds' diet and reproductive success often reflect changes in prey abundance, distribution, and/or accessibility (Montevecchi et al. 2006; Shealer 2002).

Diet composition is a readily measured parameter, sensitive to stress and change, providing information about the food web structure and the abundance of different prey across spatial and temporal scales (Iverson et al. 2007).

Therefore, the foraging behaviour of seabirds reveal trophic and ecosystem dynamics (Montevecchi et al. 2006), and their response depend on the life-history traits of each species: in conditions of low food availability, generalist seabirds might switch to alternative prey species, whereas specialist seabirds may increase foraging effort (Furness & Camphuysen 1997; Enstipp et al. 2006; Asseburg et al. 2006).

Anthropogenic activities can have major impacts on the structure and dynamics of populations, communities, and overall ecosystems (Oro et al. 2012; Margarida et al. 2014; McCauley et al. 2015). Likewise, fisheries have profoundly altered marine ecosystems, and shaped many populations of seabirds (Trites et al. 2006; Votier et al. 2013; Pala 2013). These top predators are well known to follow fishing vessels, especially scavenging species like gulls, which can rely heavily on fishery waste as their main food source (Garthe et al. 1996). Therefore, they can be used to monitor the impacts of fisheries in the ecosystem (Furness & Camphuysen 1997). Fishing activities can directly deplete food stocks, and cause seabird mortality (i.e. bycatch) (Belda & Sánchez 2001; Igual et al. 2009; Ramos et al. 2012). On the other hand, some fishing fleets can generate large amounts of fishery waste by discarding to the sea undersized, non-targeted, or damaged fish and post-harvest waste (offal), thereby increasing the carry-capacity of the ecosystem (Bellido et al. 2011; Condie et al. 2014). However, this apparent beneficial effect for scavenging species can lead to detrimental long-term effects due to the consumption of low energy prey, such as demersal fish (i.e. junk food; Grémillet et al. 2008; Osterblom et al. 2008), and also to the loss of natural foraging skills (Tyson et al. 2015).

In the European Union (EU), the Common Fisheries Policy (CPF) has been in place since the 1970s with successive updates to promote sustainable fisheries within EU waters. One of the main objectives of the new CPF reform, is a discard ban policy which will be implemented in a phased manner until 2020 ([http://ec.europa.eu/fisheries/cfp/fishing\\_rules/discards/index\\_en.htm](http://ec.europa.eu/fisheries/cfp/fishing_rules/discards/index_en.htm)). For seabirds, the substantial reduction in the availability of discards should lead to a mid- to long-term decrease of scavengers' population numbers (Bicknell et al. 2013). Hence, the discard ban will lead to a food shortage for these species, especially for gulls, which are known to be affected by a decreased availability of discards (Oro et al. 1996a; Stenhouse & Montevecchi 1999). Therefore, it is crucial for management and conservation purposes to know the current importance of discards in gulls' diet. Additionally, if the discard ban is fully implemented in the future, diet composition and foraging behaviour of scavenging species such as gulls may be used as biological indicators of its effective implementation and efficiency, and also of its effects on the community structure and functioning (Heath et al. 2014).

Overall, seabird dietary studies are key to understand their own ecology and population dynamics, as well as the structure and change of marine ecosystems over time (Iverson et al. 2007)

## 1.2 Resource partitioning

Competition can be one of the major processes structuring avian communities (Ronconi et al. 2014). According to the “principle of competitive exclusion”, species that exploit similar niches are expected to use their food resources differently in order to reduce competition. This partitioning of resources allows the coexistence of ecologically-similar species through niche divergence (Navarro et al. 2009a; Navarro et al. 2013). Nevertheless, partitioning of resources can be a consequence not only of avoidance of competition but also of intrinsic mechanisms, such as foraging abilities and habitat preferences (Quillfeldt et al. 2013). In this respect, body size plays an important role in niche segregation, where the larger species can outcompete the smaller ones, and also feed on larger prey or have greater diving capacity (Mancini & Bugoni 2014). Species can segregate in their use of resources by selecting different prey within the same habitat, using distinct foraging areas or foraging at different times of the day (Amarasekare 2003). The degree of segregation among species may change seasonally, due to the different constraints experienced throughout the annual cycle (Bearhop et al. 2006; Navarro et al. 2009a). Nonetheless, a lack of resource partitioning and competition may arise from a superabundance of resources, allowing a large overlap in trophic niches between species (Mancini & Bugoni 2014; Afán et al. 2014).

Seabirds are suitable models to study resource partitioning, especially during the breeding season when they gather in discrete, usually, mixed-species colonies (Phillips et al. 2009; Kappes et al. 2011; Weimerskirch 2013). At this time of the annual cycle, seabirds become central-place foragers,

constrained in their foraging ranges by the need to visit their colony frequently to incubate their clutch or feed their growing chicks. Furthermore, this period is energetically demanding not only during the courtship and egg-laying but also throughout the chick-rearing phase, due to the need of adults to provide food for themselves and their chicks (Ramírez et al. 2010; Mackley et al. 2011). Thus, during the breeding season competition for resources is likely to be particularly intense (Afán et al. 2014).

Gulls are among the seabirds with greater behavioural plasticity, being highly opportunistic and feeding on a wide range of prey (Kubetzki & Garthe 2003). Moreover, some scavenger species feed in association with human activities, like fisheries, and the conditions at these sites are highly competitive and birds often steal food from each other (i.e. kleptoparasitism) (Sotillo et al. 2014). Therefore, gulls are suitable ecological models to study dietary flexibility and resource partitioning among closely-related species (Ronconi et al. 2014).

### **1.3 Assessment of seabird diet**

Traditional dietary methods have been widely used to study seabird diets and foraging habitats, and all used techniques have different advantages and caveats (Votier et al. 2003). Direct observations on birds carrying prey, usually fish, in their bill (e.g. terns) when returning to the colony is biased towards the most conspicuous prey, though the diet can be assessed without causing any disturbance (Barrett et al. 2007). An alternative dietary sampling method is taking advantage of the natural response to stress of some species (e.g. gannets and gulls) which regurgitate partly digested food when handled.

However, the success of such method depends on the timing, type and size of prey, amount of food in the stomach, bird age, and amount of stress generated on the individual (González-Solís et al. 1997a). Thus, the number of regurgitates obtained per sampling event is highly variable. Concerning species that do not spontaneously regurgitate (e.g. procellariiform species), their stomach content can be sampled using the water off-loading technique (Wilson 1984), but this method is more invasive (Votier et al. 2003). Pellet analysis is the dietary identification method most used in gull studies because pellets can be easily collected in the field (González-Solís et al. 1997a). However, pellets comprise indigestible parts of food, therefore, this analysis is biased towards prey that have large hard parts and underestimates soft-bodied or small prey (Ramos et al. 2009a; Moreno et al. 2010). These conventional methods allow great taxonomic detail, but reflect ingested prey over short time scales and are usually restricted to the breeding season (Inger & Bearhop 2008), when pellets and regurgitates can be easily collected. Moreover, these methods require exhaustive sampling and time-consuming identification, which often relies on extensive knowledge of diagnostic fragments of partially digested prey (Karnovsky et al. 2012; Alonso et al. 2013).

Intrinsic markers, such as stable isotopes, have become increasingly used to study the diet of marine top predators because they overcome most of the limitations associated with conventional methods (Forero & Hobson 2003; Ramos & González-Solís 2012). Stable isotope analysis (SIA) is a powerful tool to investigate foraging habitat (Ramírez et al. 2012), trophic relationships (Hobson et al. 1994; Hodum & Hobson 2000), and migratory movements of seabirds (Hobson 1999; Hedd et al. 2012). Naturally occurring stable isotopes

provide an integrative view on assimilated diets, and their use is based on the fact that isotope ratios pass from prey to consumer tissues in a predictable manner (Hobson & Clark 1992a; Post 2002). During ingestion, digestion, and assimilation of prey, isotopic concentrations change mainly due to a selective retention of the heavy isotope and excretion of the light in metabolic reactions (Inger & Bearhop 2008, Masello et al. 2013). The difference between isotopes ratios of consumers and their prey is a consequence of this discrimination against heavy isotopes (Fry 2006). In dietary studies the most used stable isotopes are nitrogen and carbon (Forero & Hobson 2003), which are discriminated differently. In marine environments, the nitrogen stable isotope ratios ( $\delta^{15}\text{N}$ ) of consumers exhibit a stepwise enrichment of 2.0 – 5.0‰ at each trophic level, thus, nitrogen can be used as a reliable proxy of trophic position (Caut et al. 2009). Carbon stable isotope ratios ( $\delta^{13}\text{C}$ ) are enriched to a lesser extent per trophic level, usually 0.7 – 1.0‰ (Inger & Bearhop 2008), showing little variation along the food chain. Therefore, carbon isotope ratios of consumers reflect the source of carbon at the base of the food web (Kelly 2000). Isotopic differences in the tissues of producers, caused by the different photosynthetic pathways used by terrestrial plants, macrophytes, or phytoplankton (Farquhar 1989), are passed throughout the food web to the consumers. Hence, carbon can be applied to identify foraging habitats, and marine food webs can be distinguished from terrestrial ones. Additionally, within marine ecosystems, carbon ratios also vary in particulate organic matter (POM), reflecting variation in sea-surface temperature (SST) and  $\text{CO}_2$  (Phillips et al. 2009; Paiva et al. 2010). Therefore, carbon ratios typically present a horizontal and a vertical enrichment gradients, allowing to distinguish offshore

from inshore and pelagic from benthic areas, respectively (Kelly 2000). Furthermore, both carbon and nitrogen isotope ratios can change geographically, reflecting latitudinal and longitudinal differences at the base of food webs. Thus, at a large scale, SIA can provide very useful insights into migratory movements and non-breeding areas of migratory seabirds (Ramos et al. 2009b and 2009c). However, at a regional scale marine isotopic landscapes (i.e. isoscapes) may be difficult to draw and can be masked by other sources of variation, such as dietary shifts (Roscales et al. 2011).

The time of dietary integration depends on the metabolic activity of the tissue sampled (Bearhop et al. 2006). Tissues that turn over at a fast rate, like plasma and liver, provide information of the past few days (Cherel et al. 2005a), whereas tissues with lower turnover rate, like blood cells and muscle, represent the diet of the last few weeks (Hobson & Clark 1992b). On the other hand, tissues that are metabolic inert after formation, such as feathers, claws, or eggs, integrate isotope ratios during the time of synthesis and maintain the isotopic record almost indefinitely (Bearhop et al. 2003). In seabird species with known moulting patterns, it is possible to sample specific feathers during the breeding season (when seabirds gather at their breeding colonies) that were moulted during the non-breeding season to study this comparatively less known period (Jaeger et al. 2009). Thus, handling the bird only once, it is possible to assess the feeding ecology at different periods of the seabirds' annual cycle by sampling different tissues with different turnover rates, with minimal detrimental effects for birds (Ceia et al. 2012; Ramos & González-Solís 2012). Additionally, eggshells can be collected opportunistically at the colony to study diet of females in a very concrete and important period of the breeding season (i.e.



egg formation; Kowalczyk et al. 2014). Energetically, this is a very demanding phase for females, and foraging on different prey may affect egg formation due to the lack of specific nutrients (Ramírez et al. 2013).

The isotopic niche width of a population (Newsome et al. 2007) is reflected in the isotopic variances in tissues of the individuals (Bearhop et al. 2004), and it can be calculated by plotting the isotopic data ( $\delta^{13}\text{C}$  vs  $\delta^{15}\text{N}$ ). A recent Bayesian approach (Stable Isotope Bayesian Ellipses in R: SIBER; Jackson et al. 2011) allows the measurement and comparison of isotopic niches by computing the area of the standard ellipse (SEA), the overlap between them, and test if they are different (Ceia et al. 2014).

Furthermore, by sampling prey species and applying the proper discrimination factors between consumer and prey isotope signatures, it is possible to estimate the relative proportion of each prey (i.e. sources) in the diet of the consumer with stable isotope mixing models (Karnovsky et al. 2012). The key parameter in these models is the discrimination factor for each isotope, which depends mainly on the consumer taxa, type of diet, and sampled tissue (Caut et al. 2009). Additionally, it can also vary according to the individual physiology (Bearhop et al. 2004), isotopic routing (Bearhop et al. 2002), high metabolic rate during growth (Bearhop et al. 2000), and water and nutritional stress (Hobson & Clark 1992b). Nevertheless, the latest mixing models available are based on a Bayesian framework, such as the Stable Isotope Analysis in R (SIAR; Parnell et al. 2010), which incorporate sources of variability into the inputted parameters (consumer and prey isotopic signatures, and discrimination factors). These models also take into account external sources of variation unrelated with isotopic variability and, ultimately, propagate sources of

uncertainty to posterior results, leading to robust estimates of diet composition and proportion of different prey sources (Karnovsky et al. 2012). A major drawback of SIA is that prey and/or foraging areas need to be isotopically distinct. This means that if birds are foraging on different prey/areas that have similar isotopic values, then this approach cannot discriminate between the two, leading to erroneous results (Inger & Bearhop 2008; Roscales et al. 2011; Paiva et al. 2010). Therefore, combining SIA with conventional dietary methods is recommended to interpret the isotopic values of consumers and use the isotopic signatures of prey in mixing models (Phillips et al. 2014). Additionally, traditional dietary techniques provide taxonomic detail that SIA cannot achieve.

In view of the recent technological and statistical developments, SIA is a powerful tool when using seabirds as indicators of marine ecosystems (Hobson et al. 1994; Ramos & González-Solís 2012). In applied ecology it has become an invaluable approach both in the conservation of threatened species (Sanpera et al. 2007a) as in the management of overpopulated populations (Ramos et al. 2011). Likewise, SIA can reveal the importance of fishery discards in the diet of scavenging species (Forero & Hobson 2003; Navarro et al. 2009b; Votier et al. 2010). Ultimately, SIA is a powerful method to unravel resource partitioning among sympatric taxa or multi-species assemblages (Cherel et al. 2005b), particularly when combined with conventional dietary approaches or tracking devices (Navarro et al. 2013; Mancini & Bugoni 2014), revealing important insights into the trophic choices of individuals both during the breeding and the non-breeding seasons (Navarro et al. 2009a; Jaeger et al. 2010).

## 1.4 Study rationale

The feeding ecology of Audouin's gulls (AG) and yellow-legged gulls (YLG) has been studied throughout their breeding ranges mostly with traditional methods, which have many biases and limitations. Concerning stable isotopes analyses, which provide a much deeper and accurate knowledge of the year-round feeding ecology of individuals, only a few studies were conducted, mainly focused on site-specific populations of YLG (Ramos et al. 2009a; Pedro et al. 2013; Ceia et al. 2014). Although these two closely-related species breed sympatrically in several places throughout their breeding range, only two studies investigated resource partitioning between them during the breeding seasons of 1993 - 1995 (González-Solís et al. 1997b; González-Solís 2003). These two gull species are of special interest to investigate ecological questions related with competition for resources, because they are closely related taxa, natural competitors, belong to the same trophic guild, have shown a great dependence on anthropogenic activities (i.e. fisheries), and both populations are increasing and expanding their geographical ranges.

Here, we investigate resource partitioning between AG and YLG breeding in sympatry at Barreta Island (South of Portugal), an area with high fishing activity in the its surroundings (Carvalho 2011). We applied a multi-tissue stable isotope analysis combined with conventional techniques. To our knowledge, this is a unique study using a multi-tissue stable isotope analysis to compare the feeding ecology of these two species. The present study arises as the first of its kind at this colony, and the first concerning the population of AG breeding in Portugal. We measured carbon and nitrogen isotope ratios in several tissues with different turnover rates that reflect dietary input over

different periods of the year. From breeding adults we sampled breast, eighth secondary, and first primary feathers, eggshell membranes, red blood cells, and plasma. We also analysed stable isotopes in blood and growing feathers of chicks to assess possible parent-offspring isotopic segregation within and between species. Conventional dietary methods were also performed to complement and compare with stable isotope values: we collected pellets to study the diet of breeding adults, and spontaneous regurgitates from both chicks and adults to assess their diet and also to create Bayesian stable isotope mixing models, which estimated the consumption of pelagic and demersal fish by each gull species. The isotopic niche width and overlap between the two gull species was analysed using Stable Isotope Bayesian Ellipses in R (SIBER). We also investigated short- (during the breeding season) and long-term (between seasons) individual consistency in foraging areas and trophic level by regressing stable carbon and nitrogen isotope ratios, respectively, between the tissues of each species. Additionally, to compare their foraging grounds during breeding, field observations were made at the colony to assess the numbers of individuals arriving from the sea or lagoon, and counts of each species following fishing boats were also performed.

Specifically, these methods were used to i) assess the diet composition of each gull species, ii) identify their foraging habitats, iii) estimate their trophic level, and iv) investigate parent-offspring dietary segregation. Given the location of the breeding colonies, fish was expected to be the main prey for both gull species, however, we predict AG should feed more on epipelagic fish. On the other hand, we expected YLG to have a more opportunistic and generalist diet, feeding on locally abundant and easily caught prey, such as demersal fish

discarded from trawlers and some tips from refuse dumps. Regarding foraging habitat, AG was predicted to forage more in the marine environment and more offshore than YLG, which was expected to forage in coastal areas and also inland, especially during the non-breeding season. Concerning trophic ecology, AG was predicted to have a lower trophic position, feeding on organisms at lower trophic levels (i.e. epipelagic fish), compared to YLG, which was expected to feed more on demersal fish from discards, which occupy higher trophic levels. Dietary segregation between adults and chicks was predicted for both species, with high-quality food in chicks' diet due to their higher nutritional and energetic requirements.

Additionally, the diverse array of tools applied in this study to investigate resource partitioning between these two closely related species, will also allow to assess the influence of fishing activities on these populations and forecast the potential consequences of a discard ban policy.



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## **Chapter 2 – Methods**

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## 2.1 Study area

The study was carried out in the South of Portugal, at Barreta Island (36° 57' 40" N, 7° 53' 20" W), during the 2014 breeding season, with an estimated breeding population of 900 and 700 breeding pairs of AG and YLG, respectively. This is one of the few breeding sites of AG outside of the Mediterranean basin and unique in Portugal, which was recently colonized most probably as a consequence of the growth of the western Mediterranean populations. The island has about 7 km long and lies about 5.5 km from mainland. It belongs to Ria Formosa Natural Park and it is one of the five barrier islands that form a narrow strip of dunes that separates the lagoon from the Atlantic Ocean (Ceia et al. 2010). An artificial inlet separates Barreta and another barrier island (Culatra), assuring the navigability of the fishing boats to the main fishing harbour of the region (Olhão). This area is characterized by high fishing activity (Carvalho 2011) dominated by small multigear boats, followed by purse-seiners, and then trawlers (Borges et al. 2001). Small multigear boats produce very few discards as opposed to trawlers that normally generate a high amount of discards and purse-seiners that can discard the total amount of the catch (Erzini et al. 2002).

Ria Formosa is a national protected area that covers approximately 18.000 ha along 55 km of coastline. Its ecological importance is also recognized internationally, being a Ramsar Site and also part of Natura 2000 network as a Special Protection Areas under the Birds Directive (ICNF 2015). Barreta Island stands out as a site with limited human disturbance, being an uninhabited site with elevated wooden pathways to allow the tourists to visit the island without walking randomly.

## 2.2 Study species

The yellow-legged gull (YLG), *Larus michahellis*, is one of the most abundant large gulls in the southwest Palearctic (Arizaga et al. 2010; Jordi et al. 2014). Like several other large gull species, its global population growth over the last 50 years is a consequence of the exploitation of anthropogenic resources, especially fishery discards that have increased in the last decades as a result of the industrialization of fisheries (Soldatini et al. 2005; Matias & Catry 2010). This is a generalist species that displays a highly opportunistic and plastic foraging behaviour, feeding on the most abundant/ available food sources (Munilla 1997; Ramírez et al. 2011). Owing to its great ability to adapt to human-modified habitats, discards or refuse tips are the major components of its diet, according to their local availability (Duhem et al. 2005; Ramos et al. 2009a). Furthermore, diet composition and diversity of this species largely depend on the presence of other alternative resources (Duhem et al. 2003). Moreover, they can broaden or narrow their trophic niche width according to fluctuations in their environment, such as changes in food availability and competition with related species (Duhem et al. 2003; Ramos et al. 2009d; Pedro et al. 2013). Consequently, in some locations this species has become superabundant and, therefore, problematic to human interests (Bosch et al. 2000), sympatric seabird species (Sanz-Aguilar et al. 2009), and flora (Vidal et al. 1998). In this regard, control measures have been applied mainly through culling eggs, young or adults. However, management of food supplies from human origin is considered the most effective and long-lasting way to control populations of large gulls (Sol et al. 1995; Martínez-Abraín et al. 2004; Oro & Martínez-Abraín 2007).

The Audouin's gull (AG), *Larus audouinii*, is a medium-sized gull breeding almost exclusively in the Mediterranean basin (BirdLife International 2015). In the 1970's it was one of the most endangered seabirds in the world, and regarded as a nocturnal specialist on epipelagic fish, mainly clupeiforms (Pedrocchi et al. 1996; Oro et al. 1999). However, an effective protection of a small colony (Ebro Delta, Spain) with high fishing activity in the surrounding marine area led to a shift in the species foraging behaviour (Oro & Ruxton 2001; Oro 2003). This species started to take advantage of the huge amount of discards produced by an intense trawling activity, which resulted in the exponential growth of its population (Oro & Ruxton 2001). The specialized traits of AG (predatory skills and nocturnal foraging activity) allow them to also feed in association with purse seiners that operate during the night, signaling the fish with powerful lamps, attracting it to the surface, and allowing the gulls to easily catch it while the net is being hauled (Arcos & Oro 2002). This kind of fishery also produce some discards when returning to the harbour, but much less when compared to trawlers (Borges et al. 2001). Presently, AG behaves as an opportunistic species, foraging both in marine and adjacent terrestrial habitats, during the day and night (Mañosa et al. 2004; Christel et al. 2012). Therefore, the amount of discards in their diet depend on the type and intensity of fishing activities surrounding the breeding colonies (Pedrocchi et al. 2002). AG is still classified as Near Threatened globally because most of its breeding population occur at only a few sites and is strongly influenced by fisheries (BirdLife International 2015). Therefore, the conservation of this species depends on the management of fishing activities (Cama et al. 2013; Bécares et al. 2015).

### **2.3 Habitat use**

From May to June ( $n = 15$  days) the foraging habitats (sea and lagoon) used by each gull species were identified by direct count (with binoculars and telescope) of the breeding adults arriving to the colony from foraging trips. The observations were made at each 10 minute periods between 4.30 and 6.30 p.m.. Only individuals with a straight flight to the colony to feed their chicks were considered. Additionally, the individuals of the two gull species following fishing boats ( $n = 24$ ), when returning to the main harbour of Olhão (usually from 7 to 10 a.m.), were also counted during May and June.

### **2.4 Sample collection**

A total of 171 pellets (AG:  $n = 111$ ; YLG:  $n = 60$ ) were randomly collected weekly around the nests during the incubation and chick-rearing periods (from late April to mid-June), every other week for each species. Only fresh pellets were sampled, to ensure that they were from a recent diet, and to exclude old pellets from previous weeks (Duhem et al. 2003). Regurgitates were collected from both adults (AG:  $n = 6$ ; YLG:  $n = 15$ ) and chicks (AG:  $n = 19$ ; YLG:  $n = 48$ ) that vomited spontaneously when handled. Only one chick per brood was sampled to avoid pseudoreplication, since parents usually feed their offspring with the same prey (Ramos et al. 2013a). The samples were placed in plastic bags and stored frozen until laboratory analysis.

Breeding adults (AG:  $n = 12$ ; YLG:  $n = 9$ ) were caught with nest traps. Although, in the study area, YLG breeds about two weeks earlier than AG and more asynchronously, we selected nests in similar stages, during late

incubation (7th and 8th May) to reduce the risk of desertion (Christel et al. 2012), and to better compare the adults' diet of the two species which were, thereby, experiencing the same constraints. We ringed and collected biometric measures from all caught birds. Biometric measurements included body mass, wing-length, tarsus length, culmen, and bill height at the gonys. We collected ca. 0.5 ml of blood from the brachial vein of the adults, and this was centrifuged within two hours to separate red blood cells (RBC) from plasma. RBC comprises dietary information from the previous 3-4 weeks until sampling (Bearhop et al. 2006), while plasma has a much faster turnover rate (about 7 days; Cherel et al. 2005a; Haug et al. 2015). Therefore, these tissues represent the diet during the laying and incubation periods, respectively. Blood samples were then frozen until preparation prior to Stable Isotope Analysis (SIA).

We randomly collected four breast feathers, the tips of the innermost primary (i.e. first primary, P1) and eighth secondary (S8), which were stored in labelled sealed plastic bags. Since breast feathers are moulted more or less continuously throughout the year, we assumed they represent the year round diet of the birds (Pedro et al. 2013). On the other hand, moulting patterns of wing feathers are constant and predictable, and, therefore, in these two gull species, P1 and S8 represent the diet during the previous breeding and non-breeding periods, respectively (Ramos et al. 2011). Chicks (ca. 3 weeks old) were captured by hand during the chick-rearing period (May-June; AG: n = 16; YLG: n = 17). Blood (ca. 0.2 ml) was collected either from the brachial or tarsal veins, and 3-4 growing mantle feathers were also sampled. Whole blood encompasses the diet from the previous 3-4 weeks, and feathers the diet assimilated during their growth, so both tissues represent the diet provisioned to

chicks during the chick-rearing period (Hobson & Clark 1992a; Sanpera et al. 2007b). Additionally, we opportunistically collected 17 eggshells from hatched eggs of each species, to study the diet of females during egg formation.

## **2.5 Diet analysis**

Pellets were examined using a stereomicroscope and the prey items were identified to the lowest possible taxonomic level using the reference collection from the National Museum of Natural History and Science (Lisbon) and published identification guides (Tuset et al. 2008; Xavier & Cherel 2009). Most of the items found were hard parts of prey (fish vertebrae and otoliths, crab chelae, and cephalopod beaks) and inorganic material from refuse tips (e.g. glass, plastic, paper). Inorganic items were probably ingested unintentionally, however, they give information about the foraging areas and strategies used by the gulls, and therefore, they were not excluded from the diet analysis. In fact, as these inorganic items have been included in published studies, this enables comparisons among studies. Prey items from regurgitates were weighed and identified to the lowest possible taxonomic level, and the best preserved were selected and prepared for SIA.

## **2.6 Stable isotope analysis**

In the laboratory, eggshell membranes were separated by hand and cleaned from other egg remains with water. We chose the membranes because they produce the same results as eggshells, but with less laboratory procedures, since it is not necessary to remove inorganic carbonate, and, more

importantly, their isotopic values are not affected by laying order (Polito et al. 2009).

Prior to SIA, plasma and prey samples (muscle) were thawed and, because high lipid concentrations can deplete  $\delta^{13}\text{C}$  values, lipids were extracted with successive rinses of a 2 chloroform: 1 methanol solution (Cherel et al. 2005a). The lipid content of whole blood and RBC is low and, therefore, they do not require lipid extraction (Cherel et al. 2005b). Indeed, C/N ratios of these tissues were low (less than 3.5). Additionally, plasma had similar C/N ratios between gull species and the same occurred between prey species (see “Results”) indicating that the lipid extraction was efficient (Kojadinovic et al. 2008).

Feathers were cleaned of surface lipids and contaminants also using a 2 chloroform: 1 methanol solution, oven-dried, and then cut with stainless steel scissors into small fragments. All tissue samples were dried in an oven for at least 48 h at 50 °C to a constant mass and homogenized. Dried eggshell membranes were grounded to a fine powder using an analytical mill. Sub-samples of approximately 0.50 mg for egg membranes and 0.35 mg for the other tissues were weighed in a microbalance, placed in a tin cup, and crimped for combustion. Isotopic ratios of carbon and nitrogen were determined by continuous-flow isotope ratio mass spectrometry (CF-IRMS). Results were expressed in the usual  $\delta$  notation as parts per thousand (‰) deviation from the international standards PeeDee Belemnite (PDB) for  $\delta^{13}\text{C}$  and atmospheric nitrogen ( $\text{N}_2$ ) for  $\delta^{15}\text{N}$ , according to the following equation:  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N} = [(R_{\text{sample}}/ R_{\text{standard}}) - 1] \times 1000$ , where  $R = {}^{13}\text{C}/{}^{12}\text{C}$  or  ${}^{15}\text{N}/{}^{14}\text{N}$ , respectively.

Replicate measurements of internal laboratory standards (acetanilide) indicate precision < 0.2‰ for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

## 2.7 Data analysis

A Kruskal-Wallis test was used to assess the effect of gull species (AG, YLG) and habitat type (sea, lagoon) on the number of individuals returning to the colony from foraging trips. A Mann-Whitney *U* test was used to assess differences in the number of each gull species following fishing boats.

To obtain information about fish availability, data on fishery landings (April to June) were gathered from the two main fishing harbours in the area (Olhão and Quarteira). Only potential prey species for gulls were taken into account, and the percentage of fish landed was correlated with their occurrence in the pellets of each gull species using the Spearman's rank correlation coefficient.

We calculated frequency of occurrence (FO), as the percentage of pellets with a certain prey type, and numeric frequency (NF) as percentage of the number of individuals of each prey type in relation to the total number of individuals (Alonso et al. 2013). Numeric frequencies were calculated by two methods: including all items or considering only fish prey. FO and NF of each prey type were used to perform Fisher's exact tests (efficient with low expected frequencies, to compare FO of each prey type between the two gull species) and to assess the overlap in diet composition between the two gull species by calculating the Horn's modification of Morisita's index:  $MH = 2 * \sum (x_{1i} * x_{2i}) /$



$((\lambda_1 + \lambda_2) * \sum(x_{1i}) * \sum(x_{2i}))$ , where  $\lambda_1 = \sum(x_{1i}^2) / (\sum(x_{1i})^2)$ . The Morisita-Horn index (MH) ranges from zero (no overlap) to one (100% overlap).

Regarding the diet of chicks, we also calculated FO and applied Fisher's exact tests to compare prey occurrence in the chick regurgitates between the two gull species. We applied the same procedures for adult regurgitates.

We adopted the Bayesian stable isotope mixing model SIAR (Parnell et al. 2010) to estimate the relative proportions of each prey group (i.e. sources) in the diet of each gull species. By grouping ecologically similar species, and removing the outliers (mean  $\pm$  1.5 SD), we created two dietary sources: pelagic fish (garfish *Belone belone*, sardine *Sardina pilchardus*, mackerels *Scombrus* spp. and *Trachurus* spp., and sandeel *Ammodytes* sp. (probably *A. tobianus*, which is the most common species in the area)) and demersal fish (bogue *Boops boops*, European conger *Conger conger*, and seabreams *Diplodus* spp.). Thus, we obtained two different ecological prey groups that also differed statistically in their isotopic values, as recommended by Phillips et al. (2005), which allowed us to estimate the consumption of discards (demersal fish) by each gull species. The discrimination factors applied in the model were 2.85 and 0.30‰ for nitrogen and carbon, respectively. These values were obtained from controlled experiments with four seabird species, available in literature (Hobson & Clark 1992b; Bearhop et al. 2002; Cherel et al. 2005b), which were previously used in isotopic mixing models with yellow-legged gulls (Ceia et al. 2014) and are similar to the values obtained for "birds" from a meta-analysis (Caut et al. 2009). We used a standard deviation of  $\pm$  1.0‰ to account for potential differences in discrimination factors among species and tissues.

In marine ecosystems,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are typically positively correlated, however, if seabirds forage across different habitats, such as brackish, freshwater or terrestrial, this correlation can disappear (Blight et al. 2014). Therefore we regressed the isotopic values ( $\delta^{13}\text{C}$  vs  $\delta^{15}\text{N}$ ) in the plasma of each gull species as an additional estimate of marine prey in their diet, using the Pearson's correlation coefficient.

The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were compared between gull species with a MANOVA (Wilk's lambda) and, when significant, followed by one-way ANOVAs, for carbon and nitrogen isotope ratios. We used the Levene's test to check for homogeneity of variances, which also provides an estimate of niche width (Bearhop et al. 2004). Moreover, to compare the isotopic niche between the two gull species throughout the year, we applied the isotopic signatures of each tissue in SIBER (Stable Isotope Bayesian Ellipses in R). We calculated the area of the standard ellipse corrected for sample size ( $\text{SEA}_c$ ) for each species (which represent their niche width), the niches overlap, and a Bayesian estimate of the standard ellipse area ( $\text{SEA}_B$ ) to test for differences between the two species' niche width (i.e. the proportion of ellipses in YLG that were lower than AG; see Jackson et al. 2011 for more details).

To investigate dietary consistency of individuals, we regressed isotopic ratios between tissues using the Pearson's correlation. For consistency in carbon source (i.e. foraging habitat) we used the standardized residuals of the relationship of  $\delta^{13}\text{C}$  with  $\delta^{15}\text{N}$  in the same tissue, eliminating the trophic component in  $\delta^{13}\text{C}$  (Ceia et al. 2014; Votier et al. 2010). When investigating consistency in trophic levels, we regressed nitrogen ratios. To assess short-term consistency we regressed stable isotope ratios in RBC and plasma

(between laying and incubation periods). To assess long-term consistency we regressed stable isotope ratios in RBC with S8 (between non-breeding and laying periods), RBC with P1 (between the previous breeding and laying periods), and S8 with P1 (between breeding and non-breeding seasons).

We examined if the adults' diet, reflected both in carbon and nitrogen isotope ratios, influenced their body condition. Therefore, we first regressed body mass with tarsus, wing, and bill length (culmen), to select the best model to calculate the standard residuals and estimate the body mass index (BMI). The relationship between body mass and wing length was the best model for both AG ( $r = 0.62$ ,  $P = 0.03$ ) and YLG ( $r = 0.88$ ,  $P < 0.002$ ) to estimate the BMI. Then, we correlated BMI with  $\delta^{15}\text{N}$  and residual  $\delta^{13}\text{C}$  in the plasma of gulls. However, no significant relationships were found (all  $r < 0.26$ ,  $P > 0.4$ ), indicating that diet did not influence body condition of the adults in either gull species.

Shapiro–Wilk tests were performed to assess the normality of the data, and non-parametric tests were used when there were strong deviations from normality. All analyses were performed with R software v. 3.1.2 (R Core Team 2015) with a significance level of  $P < 0.05$ .



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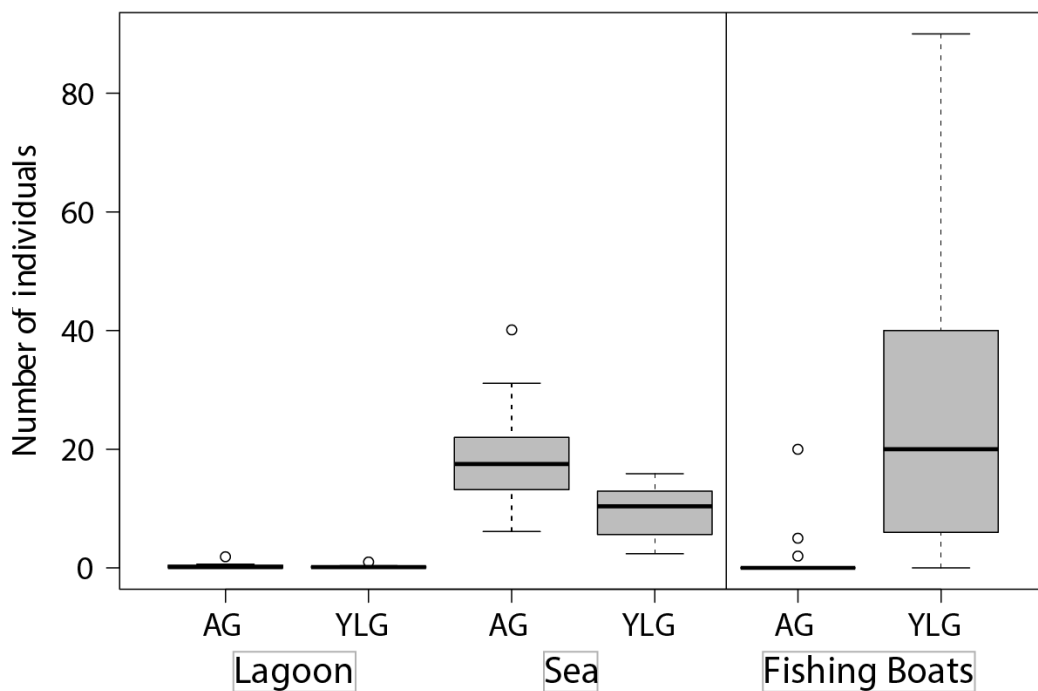
## **Chapter 3 – Results**

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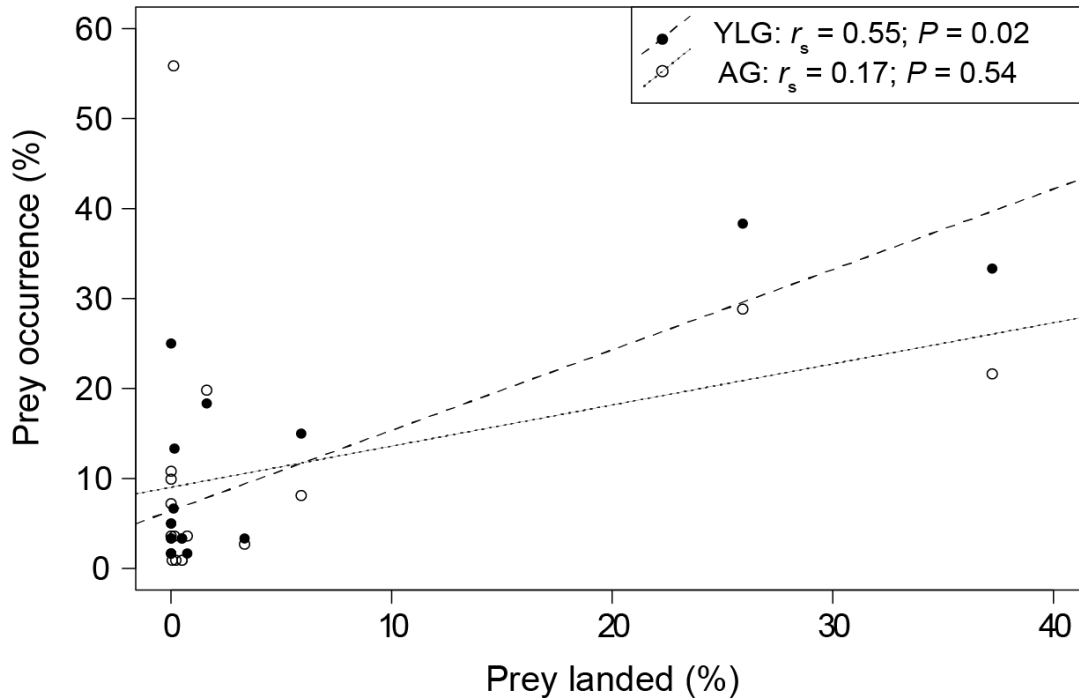
### 3.1 Habitat use and foraging behaviour

We found a significant effect of foraging area (sea or lagoon; Kruskal-Wallis test,  $H = 44.68$ ,  $df = 1$ ,  $P < 0.001$ ) but not of gull species ( $H = 1.95$ ,  $df = 1$ ,  $P = 0.16$ ) in the number of each gull species arriving to the colony. This indicates that the sea is predominantly used as a foraging ground by both gull species (Fig. 1). The two gull species differed in their foraging behaviour in association with fisheries (Mann-Whitney  $U$  test:  $Z = -5.04$ ,  $P < 0.001$ ), with higher numbers of YLG following the fishing boats (Fig. 1).



**Figure 1.** Number (median, 25–75% interquartile range, non-outlier range, and outliers) of Audouin's (AG) and yellow-legged (YLG) gulls counted arriving from each foraging habitat (lagoon and sea;  $n = 15$ ) and following fishing boats ( $n = 24$ ).

Additionally, we found a positive correlation between the frequency of occurrence (FO) of each prey in pellets and its percentage landed by local commercial fisheries for YLG ( $r_s = 0.55$ ,  $P = 0.02$ ) but not for AG ( $r_s = 0.17$ ,  $P = 0.54$ ; Fig. 2; Appendix 1).



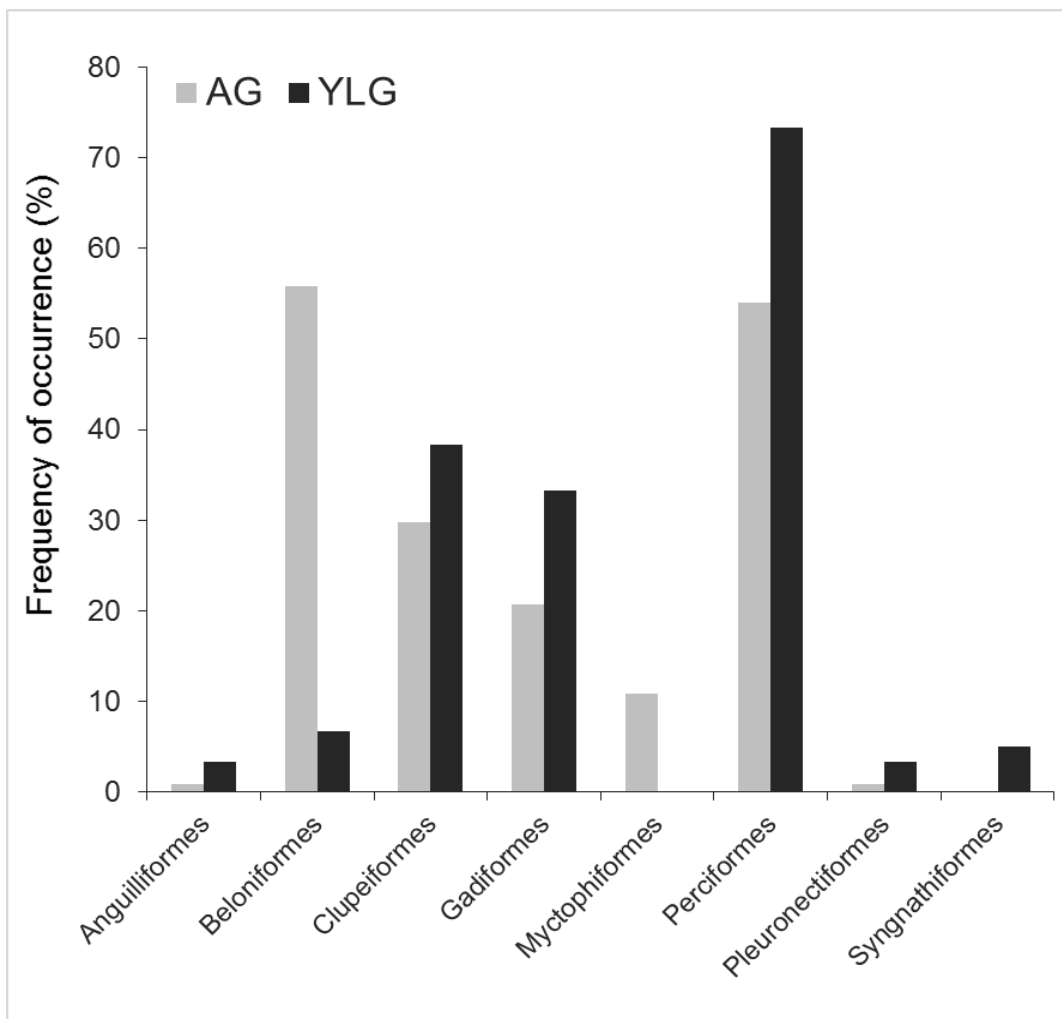
**Figure 2.** Correlation between the prey occurrence in the pellets (FO) of Audouin's (AG; n = 16; *open circles* and *dotted line*) and yellow-legged (YLG; n = 18; *black circles* and *dashed line*) gulls and the percentage of prey landed in the harbours of Olhão and Quarteira, using the Spearman correlation coefficient.

### 3.2 Diet of adults and chicks during the breeding season

Fish was the major component found in the pellets of both gull species. However, the two species differed in their consumption of fish (Fig. 3), with 100% of occurrence in AG pellets and 90% in YLG, and this difference was even more marked in their numeric frequencies: 91.8% in AG and 53.6% in YLG (Table 1). Overall, YLG samples presented more prey types, suggesting a more generalist diet. The main prey of AG was garfish (*Belone belone*), while it was only occasionally found in YLG. Sardines (*Sardina pilchardus*) were the main prey in YLG and the second most common prey in AG, with no significant differences between them neither in their occurrence nor numeric frequencies (Table 1). Mackerels (*Scomber* and *Trachurus* spp.) and seabreams (*Diplodus*



spp.) were also very common in the pellets of both gull species, with no significant differences between the two. On the other hand, spotted lanternfish (*Myctophum punctatum*) only appeared in AG pellets, and the occurrence of blue whiting (*Micromesistius poutassou*), bogue (*Boops boops*), refuse, and the numeric frequency of insects were significantly higher in YLG (Table 1).



**Figure 3.** Frequency of occurrence (FO; %) of the fish orders found in pellets of Audouin's (AG; n = 111; *light grey*) and yellow-legged (YLG; n = 60; *dark grey*) gulls.

**Table 1.** Frequency of occurrence (FO; %) and numeric frequency (NF; %) of all items present in the pellets of Audouin's (AG) and yellow-legged (YLG) gulls. Numeric frequency was also calculated considering only fish prey. *P* values (significant in bold) from the Fisher's exact test are also shown to assess differences in the consumption of each prey between the two gull species.

Prey	FO			NF				
	AG (n=111)	YLG (n=60)	<i>P</i>	All items			Fish	
				AG (n=318)	YLG (n=274)	<i>P</i>	AG (n=304)	YLG (n=147)
<b>Pelagic fish</b>								
<i>Belone belone</i>	55.9	6.7	<b>0.000</b>	31.1	1.8	<b>0.000</b>	32.6	3.4
<i>Engraulis encrasicolus</i>	0.9	—	—	0.3	—	—	0.3	—
<i>Gadiculus argenteus</i>	3.6	1.7	0.658	1.6	0.4	0.224	1.6	0.7
<i>Micromesistius poutassou</i>	9.9	25.0	<b>0.013</b>	4.7	9.5	<b>0.024</b>	4.9	17.7
<i>Myctophum punctatum</i>	10.8	—	—	9.4	—	—	9.9	—
<i>Pagrus sp.</i>	0.9	—	—	0.3	—	—	0.3	—
<i>Sardina pilchardus</i>	28.8	38.3	0.232	12.9	12.4	0.902	13.5	23.1
<i>Scomber spp.</i>	21.6	33.3	0.102	7.9	8.0	1.000	8.2	15.0
<i>Trachurus spp.</i>	8.1	15.0	0.194	2.8	5.8	0.099	3.0	10.9
<b>Demersal fish</b>								
<i>Boops boops</i>	3.6	13.3	<b>0.026</b>	1.6	3.3	0.187	1.6	6.1
<i>Capros aper</i>	—	3.3	—	—	0.7	—	—	1.4
<i>Cepola macrophthalma</i>	—	3.3	—	—	0.7	—	—	1.4
<i>Coelorinchus caelorinchus</i>	7.2	5.0	0.749	5.3	1.5	<b>0.013</b>	5.6	2.7
<i>Conger conger</i>	0.9	3.3	0.282	0.3	0.7	0.599	0.3	1.4
<i>Diplodus spp.</i>	19.8	18.3	1.000	7.5	4.0	0.081	7.9	7.5
<i>Macroramphosus scolopax</i>	—	5.0	—	—	2.2	—	—	4.1
<i>Merluccius merluccius</i>	3.6	1.7	0.658	1.6	0.4	0.224	1.6	0.7
<i>Microchirus variegatus</i>	0.9	3.3	0.282	0.3	0.7	0.599	0.3	1.4
<i>Pomatoschistus sp.</i>	—	1.7	—	—	0.4	—	—	0.7
<i>Serranus sp.</i>	—	1.7	—	—	0.7	—	—	1.4
Unidentified fish	11.7	23.3	—	4.1	5.8	—	—	—
Total fish	100.0	90.0	<b>0.002</b>	91.8	53.6	<b>0.000</b>	—	—
<b>Others</b>								
Brachyura <sup>a</sup>	3.6	6.7	0.453	1.6	4.0	0.078	—	—
Cephalopoda <sup>b</sup>	2.7	3.3	1.000	0.9	1.8	0.481	—	—
Insect <sup>c</sup>	10.8	10.0	1.000	5.0	35.4	<b>0.000</b>	—	—
<i>Rattus rattus</i>	—	3.3	—	—	0.7	—	—	—
Refuse	0.9	16.7	<b>0.000</b>	—	—	—	—	—
Unidentified	0.9	3.3	—	—	—	—	—	—

<sup>a</sup> Include *Polybius henslowii*.

<sup>b</sup> Include *Sepia officinalis*.

<sup>c</sup> Include Coleoptera, Hemiptera, Hymenoptera, and Diptera.

The numerical frequency (NF) of different prey species differed more between the two gull species than the frequency of occurrence (FO), even for the hollowsnout grenadier (*Coelorinchus caelorinchus*), which did not show significant differences in FO, but differed in NF. Concerning statistical differences in prey with higher occurrence in YLG pellets, some species showed higher differences in FO than in NF, such as the bogue, which was statistically different from AG in FO but not in NF. This indicates that, unlike AG, in YLG not all the species with higher occurrences had higher numeric frequencies. Additionally, the Morisita-Horn index revealed a high overlap in the overall diet composition between the two gull species in FO of prey (0.64) but not in NF (0.37).

In regurgitates, fish was the only prey type found, with one exception regarding insects (coleoptera) from one chick of YLG (Table 2). There were no statistical differences in prey occurrence between the two gull species neither in chicks (*Boops boops*,  $P = 0.67$ ; *Sardina pilchardus*,  $P = 0.07$ ; *Scomber* spp.,  $P = 1$ ; *Trachurus* spp.,  $P = 0.10$ ) nor adults (*Scomber* spp.,  $P = 0.32$ ).

**Table 2.** Frequency of occurrence (FO; %) of prey found in regurgitates from adults and chicks of Audouin’s (AG) and yellow-legged (YLG) gulls.

Prey	Adults		Chicks	
	AG (n=6)	YLG (n=15)	AG (n=19)	YLG (n=48)
Fish				
<i>Ammodytes sp.</i>	16.7	—	—	2.1
<i>Belone belone</i>	33.3	—	—	—
<i>Boops boops</i>	—	13.3	5.3	10.4
<i>Conger conger</i>	—	—	—	2.1
<i>Diplodus spp.</i>	—	—	—	4.2
<i>Sardina pilchardus</i>	—	—	15.8	2.1
<i>Scomber spp.</i>	50.0	26.7	26.3	25.0
<i>Trachurus spp.</i>	—	53.3	26.3	50.0
Unidentified	—	20.0	31.6	10.4
Insect <sup>a</sup>	—	—	—	2.1

<sup>a</sup> Coleoptera

### 3.3 Dietary estimations based on isotopic signatures of plasma

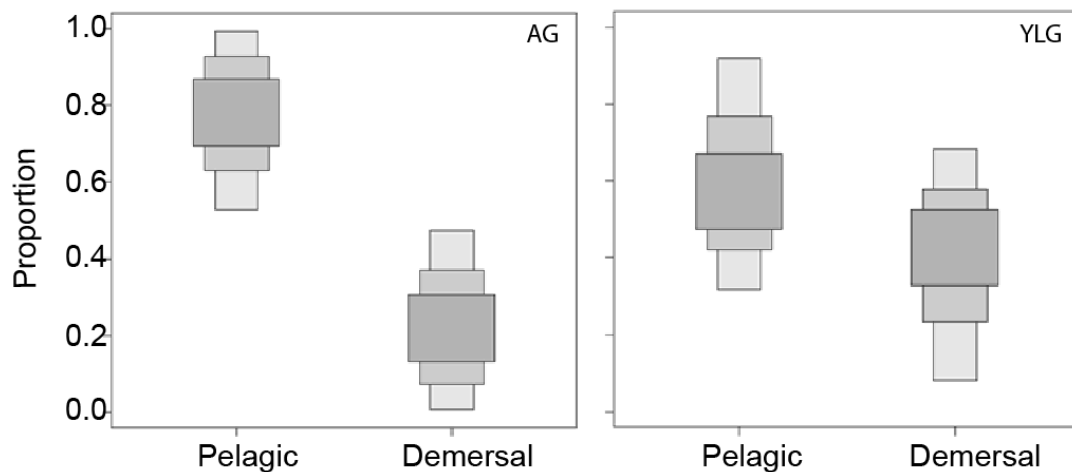
Prey isotopic composition was analysed from gull regurgitates and showed overall isotopic differences (MANOVA, Wilk’s lambda,  $F_{1,82} = 3.16$ ,  $P = 0.001$ ), and analysing each isotope separately, both nitrogen (one-way ANOVA,  $F_{1,40} = 3.96$ ,  $P = 0.004$ ) and carbon values ( $F_{1,40} = 3.40$ ,  $P = 0.009$ ) differed significantly between prey species (Table 3). However, Bonferroni corrected pairwise comparisons revealed significant differences only between seabreams and the other prey ( $P < 0.05$ ) in nitrogen values, with the exception of bogue ( $P = 0.48$ ), and concerning carbon, differences were found only between seabreams and garfish ( $P = 0.005$ ). Therefore, in order to obtain statistically and ecologically different sources to apply in SIAR, we created two groups, pelagic and demersal fish (see “Methods”), which differed in their isotopic signatures (MANOVA, Wilk’s lambda,  $F_{1,66} = 7.32$ ,  $P = 0.002$ ), both in  $\delta^{15}\text{N}$  (one-way ANOVA,  $F_{1,32} = 11.72$ ,  $P = 0.002$ ) and  $\delta^{13}\text{C}$  ( $F_{1,32} = 9.70$ ,  $P = 0.004$ )

signatures. SIAR mixing models estimated a higher proportion of demersal fish in the diet of YLG (43.4%) compared to AG (20.0%; Fig. 4).

**Table 3.** Stable isotope ratios of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) and C/N mass ratios in prey from gull regurgitates. Values are means  $\pm$  SD.

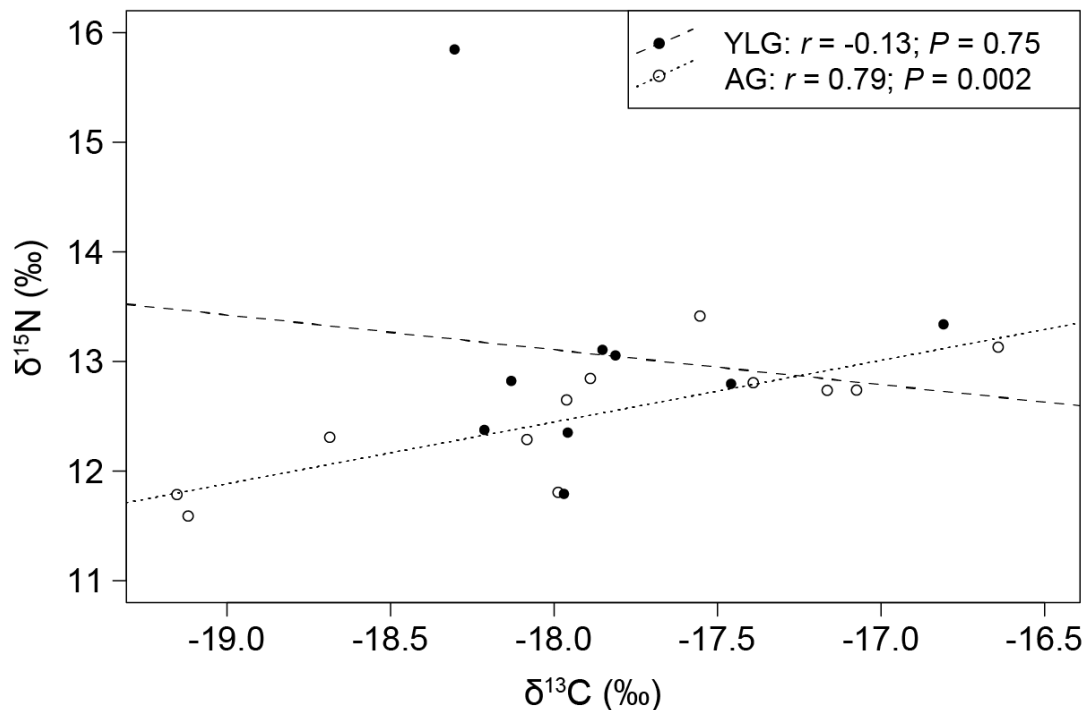
Prey	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C/N ratio	n
Pelagic fish				
<i>Ammodytes sp.</i>	-19.1 $\pm$ 0.5	9.7 $\pm$ 1.9	3.14 $\pm$ 0.07	2
<i>Belone belone</i>	-19.9 $\pm$ 0.2	9.0 $\pm$ 0.7	2.94 $\pm$ 0.05	2
<i>Sardina pilchardus</i>	-17.5 $\pm$ 0.6	10.0 $\pm$ 0.3	3.03 $\pm$ 0.05	4
<i>Scomber spp.</i>	-18.4 $\pm$ 1.1	10.0 $\pm$ 1.1	3.07 $\pm$ 0.09	13
<i>Trachurus spp.</i>	-18.7 $\pm$ 1.2	10.2 $\pm$ 0.6	3.07 $\pm$ 0.09	12
Demersal fish				
<i>Boops boops</i>	-17.5 $\pm$ 0.4	10.9 $\pm$ 0.6	3.01 $\pm$ 0.04	7
<i>Conger conger</i>	-16.7	10.4	3.05	1
<i>Diplodus spp.</i>	-16.7 $\pm$ 0.8	12.5 $\pm$ 0.8	3.03 $\pm$ 0.06	2
Coleoptera	-23.1	4.8	3.31	1*

\* 4 individuals pooled



**Figure 4.** Estimated proportions of the two main prey groups (pelagic and demersal fish) in the diet of Audouin's (AG; n = 12) and yellow-legged (YLG; n = 9) gulls, based on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of plasma. Decreasing bar widths represent 50, 75 and 95% Bayesian credibility intervals computed by SIAR.

As expected, we found a strong significant positive relationship between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of marine prey ( $r = 0.73$ ,  $P < 0.001$ ). Regarding gulls, AG presented significant positive correlations in plasma signatures ( $r = 0.79$ ,  $P = 0.002$ ) but not YLG ( $r = -0.13$ ,  $P = 0.75$ ; Fig. 5), suggesting a diet mainly from marine origin in AG.



**Figure 5.** Correlations between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of plasma of Audouin's (AG;  $n = 12$ ; *open circles and dotted line*) and yellow-legged (YLG;  $n = 9$ ; *black circles and dashed line*) gulls, using the Pearson's correlation coefficient.

### 3.4 Isotopic signatures and niche width of adults throughout the year

Stable isotope analysis (Table 4) revealed that breeding adults of AG and YLG had similar isotopic signatures of plasma (MANOVA, Wilk's lambda,  $F_{1,40} = 1.01$ ,  $P = 0.38$ ) and RBC ( $F_{1,40} = 0.61$ ,  $P = 0.55$ ). There were also no significant differences in the isotopic signatures of eggshell membranes ( $F_{1,66} = 1.56$ ,  $P =$

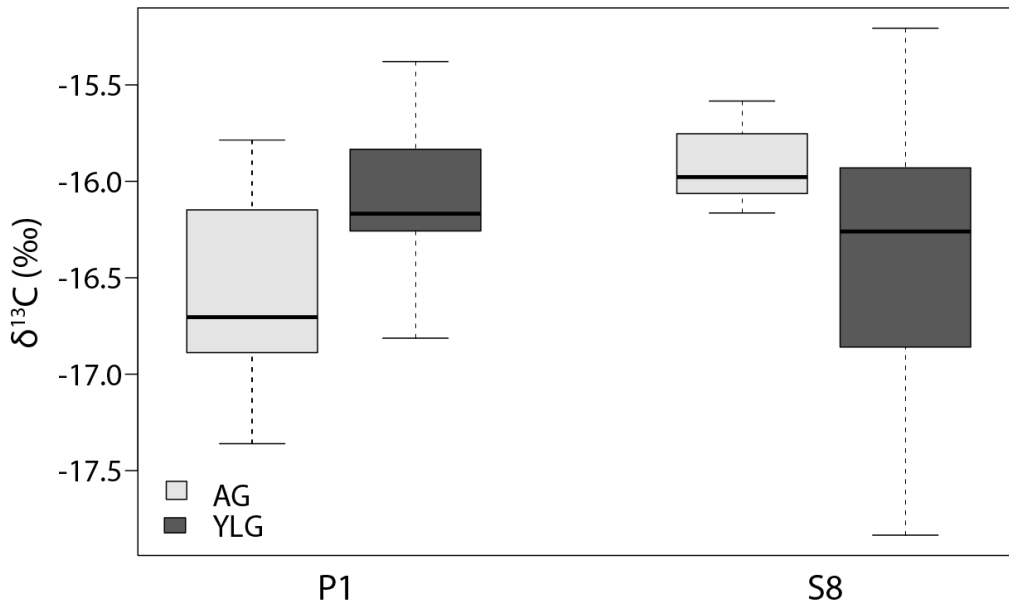
0.23), suggesting an overall isotopic overlap between the two gull species during the breeding season. Regarding carbon and nitrogen isotope ratios of feathers (Br, P1 and S8), we found no significant effect of species ( $F_{1,124} = 1.72$ ,  $P = 0.19$ ) or feather type ( $F_{1,124} = 2.32$ ,  $P = 0.06$ ) but only of their interaction ( $F_{1,124} = 3.88$ ,  $P = 0.005$ ). Analysing the results from the two isotopes separately, we only found a significant effect of the interaction between species and feather type in carbon ratios (two-way ANOVA,  $F_{1,61} = 5.02$ ,  $P = 0.01$ ). Removing breast feathers from the analysis to investigate inter-seasonal differences, there was a significant effect of feather type (MANOVA, Wilk's lambda,  $F_{1,82} = 3.51$ ,  $P = 0.04$ ) but not of gull species ( $F_{1,82} = 0.02$ ,  $P = 0.98$ ), but there was a significant interaction between them ( $F_{1,82} = 4.57$ ,  $P = 0.02$ ) in the isotopic composition of feathers.  $\delta^{15}\text{N}$  values were influenced by feather type (two-way ANOVA,  $F_{1,40} = 6.95$ ,  $P = 0.01$ ) and its interaction with gull species ( $F_{1,40} = 4.11$ ,  $P = 0.05$ ). On the other hand, for  $\delta^{13}\text{C}$  values we only found a statistical significance for the interaction gull species: feather type ( $F_{1,40} = 8.98$ ,  $P = 0.005$ ). However, Levene's test revealed significant differences in the homogeneity of variances of carbon values between P1 and S8 in AG ( $F_{1,22} = 7.19$ ,  $P = 0.014$ ) and between species in S8 ( $F_{1,19} = 7.00$ ,  $P = 0.016$ ), suggesting that AG is more specialist in their foraging habitat during the non-breeding season, compared to the breeding period and to YLG (Fig.6). Indeed, SIBER analysis revealed a significantly lower isotopic niche with S8 (i.e. non-breeding period;  $\text{SEA}_B$ ,  $P = 0.003$ ) and Br (i.e. all year;  $P = 0.004$ ) of AG compared to YLG (Fig. 7). There were no significant differences in isotopic niche for the other tissues, although niche width (i.e.  $\text{SEA}_c$ ) of YLG tended to be

wider than AG (Table 5). Additionally, SIBER revealed important isotopic niche overlap between the two gull species throughout the year (Table 5; Fig. 7).

**Table 4.** Stable isotope ratios of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) and C/N mass ratios in breast (Br), 1<sup>st</sup> primary (P1), and 8<sup>th</sup> secondary (S8) feathers, eggshell membranes (eggshell), red blood cells (RBC), and plasma of Audouin's (AG; n = 12) and yellow-legged (YLG; n = 9) gulls. Values are means  $\pm$  SD.

Tissue	Period	$\delta^{13}\text{C}$ (‰)		$\delta^{15}\text{N}$ (‰)		C/N ratio	
		AG	YLG	AG	YLG	AG	YLG
Br	All year	-16.0 $\pm$ 0.5	-17.0 $\pm$ 1.7	13.8 $\pm$ 0.7	14.7 $\pm$ 1.9	3.00 $\pm$ 0.04	2.98 $\pm$ 0.04
P1	Breeding	-16.6 $\pm$ 0.5	-16.1 $\pm$ 0.4	13.3 $\pm$ 0.6	13.8 $\pm$ 0.5	3.04 $\pm$ 0.02	3.04 $\pm$ 0.03
S8	Non-breeding	-15.9 $\pm$ 0.2	-16.4 $\pm$ 0.8	14.2 $\pm$ 0.5	13.8 $\pm$ 1.1	3.02 $\pm$ 0.04	3.03 $\pm$ 0.04
Eggshell*	Pre-laying	-16.1 $\pm$ 0.4	-16.1 $\pm$ 0.5	13.0 $\pm$ 0.4	12.8 $\pm$ 0.7	3.03 $\pm$ 0.02	3.00 $\pm$ 0.03
RBC	Laying	-18.0 $\pm$ 0.5	-18.3 $\pm$ 0.5	12.2 $\pm$ 0.8	11.9 $\pm$ 0.8	3.17 $\pm$ 0.06	3.13 $\pm$ 0.04
Plasma	Incubation	-17.9 $\pm$ 0.8	-17.8 $\pm$ 0.5	12.5 $\pm$ 0.6	13.1 $\pm$ 1.1	3.17 $\pm$ 0.08	3.16 $\pm$ 0.05

\* Eggshell n = 17



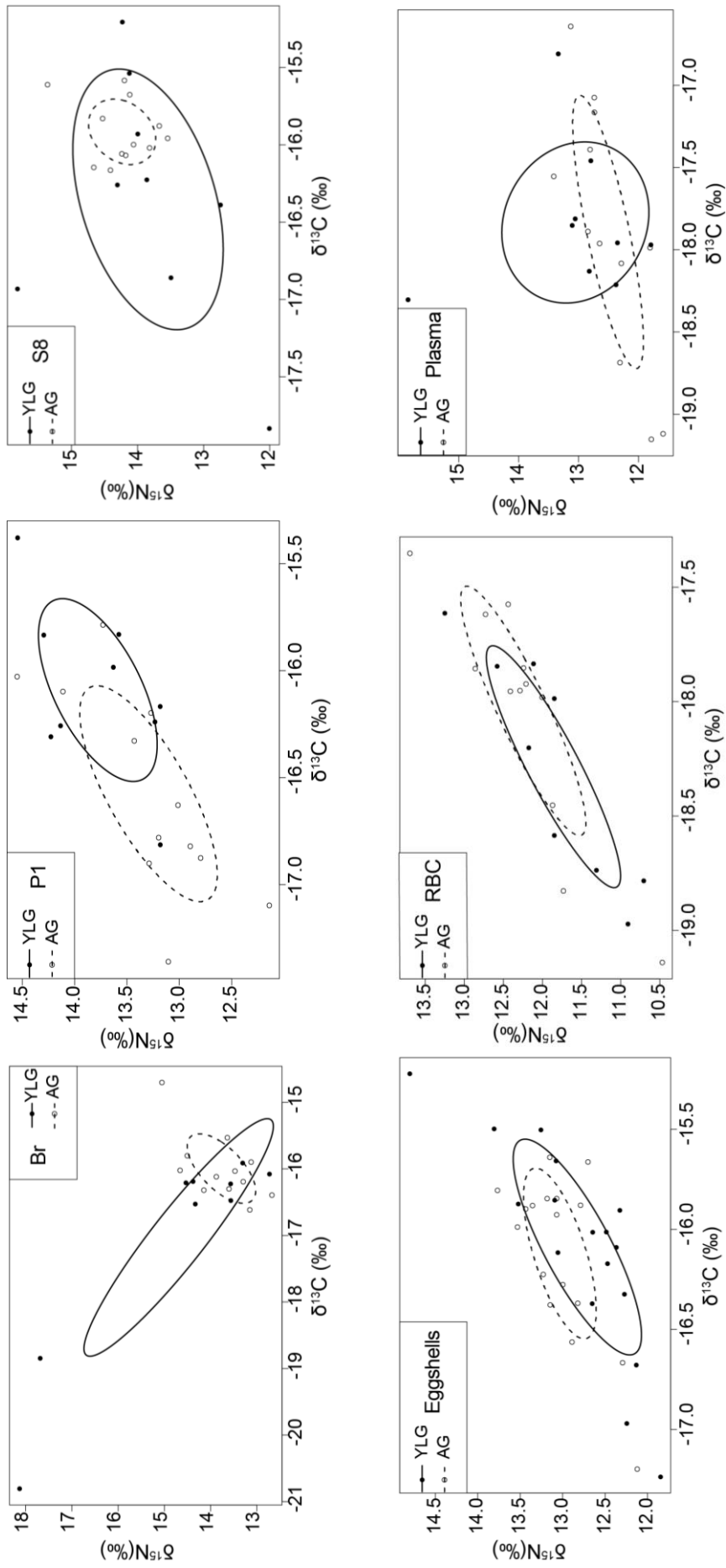
**Figure 6.** Carbon isotopic signatures ( $\delta^{13}\text{C}$ ) of 1<sup>st</sup> primary (P1) and 8<sup>th</sup> secondary (S8) feathers of Audouin's (AG; n = 12; *light grey*) and yellow-legged (YLG; n = 9; *dark grey*) gulls (median, 25–75% interquartile range, and non-outlier range).



Additionally, by regressing  $\delta^{15}\text{N}$  and residual  $\delta^{13}\text{C}$  between tissues, we found no significant positive correlations for either species, suggesting that there is neither short-term (RBC vs plasma, all  $r < 0.4$ ,  $P > 0.1$ ) nor long-term (S8 vs RBC, P1 vs S8, and P1 vs RBC, all  $r < 0.5$ ,  $P > 0.1$ , with exception of P1 vs RBC in AG residual  $\delta^{13}\text{C}$ :  $r = -0.76$ ,  $P = 0.004$ ) individual consistency.

**Table 5.** SIBER outputs: area of the standard ellipse ( $\text{SEA}_c$ ) of Audouin's (AG) and yellow-legged (YLG) gulls, overlap between the two,  $P$  value (significant in bold) based on Bayesian estimates of standard ellipses ( $\text{SEA}_B$ ) to assess possible niche width differences, and the layman metric of convex hull area (TA). Values are means  $\pm$  SD.

Tissue	$\text{SEA}_c$			$\text{SEA}_B$	TA	
	AG	YLG	Overlap	$P$	AG	YLG
Br	0.93	4.05	0.56	<b>0.004</b>	1.88	6.60
P1	0.69	0.61	0.24	0.503	1.56	0.92
S8	0.33	2.78	0.33	<b>0.003</b>	0.63	4.64
Eggshell	0.43	0.74	0.32	0.121	0.96	1.67
RBC	0.60	0.61	0.43	0.364	1.39	0.95
Plasma	0.94	1.87	0.57	0.170	1.88	3.01



**Figure 7.** Stable isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) in breast (Br), 1<sup>st</sup> primary (P1), and 8<sup>th</sup> secondary (S8) feathers, eggshell membranes (Eggshells), red blood cells (RBC), and plasma of Audouin's (AG;  $n = 12$ ; empty circles and dashed lines) and yellow-legged (YLG;  $n = 9$ ; black circles and solid lines) gulls. The represented standard ellipses areas corrected for small sample size (SEAc) were constructed using the Stable Isotopes Bayesian Ellipses package in R (SIBER, Jackson et al. 2011).

### 3.5 Parent-offspring isotopic segregation

Regarding isotopic signatures of chicks (Table 6), we found strong significant differences between the two species, both in blood (MANOVA, Wilk's lambda,  $F_{1,64} = 16.04$ ,  $P < 0.001$ ) and feathers ( $F_{1,64} = 26.69$ ,  $P < 0.001$ ). Analysing each isotope separately, we found that significant differences in blood were present only in  $\delta^{13}\text{C}$  (one-way ANOVA,  $F_{1,31} = 27.09$ ,  $P < 0.001$ ) and not in  $\delta^{15}\text{N}$  ( $F_{1,31} = 1.22$ ,  $P = 0.28$ ), while in feathers both nitrogen ( $F_{1,31} = 9.29$ ,  $P = 0.005$ ) and carbon ( $F_{1,31} = 54.90$ ,  $P < 0.001$ ) isotope ratios were significantly different.

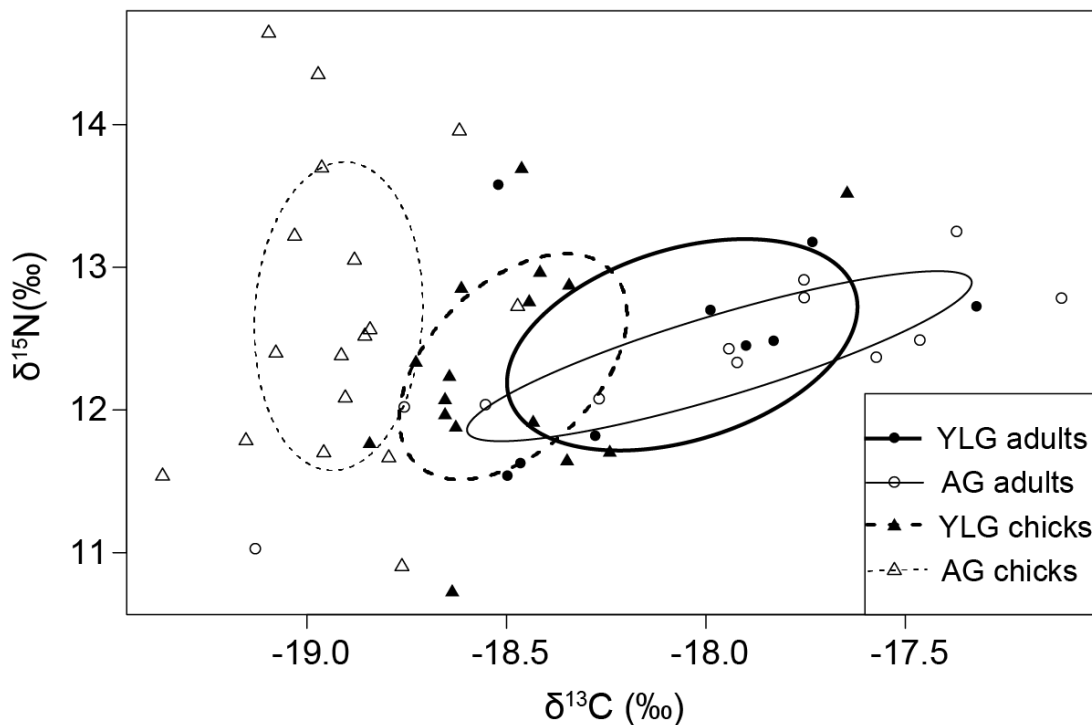
**Table 6.** Stable isotope ratios of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) and C/N mass ratios in feathers and whole blood of Audouin's (AG;  $n = 17$ ) and yellow-legged (YLG;  $n = 16$ ) gulls. Values are means  $\pm$  SD.

Tissue	$\delta^{13}\text{C}$ (‰)		$\delta^{15}\text{N}$ (‰)		C/N ratio	
	AG	YLG	AG	YLG	AG	YLG
Feathers	$-17,3 \pm 0,2$	$-16,7 \pm 0,2$	$12,3 \pm 0,4$	$12,9 \pm 0,7$	$3,03 \pm 0,04$	$3,04 \pm 0,04$
Blood	$-18,9 \pm 0,2$	$-18,5 \pm 0,3$	$12,7 \pm 1,0$	$12,3 \pm 1,0$	$3,55 \pm 0,06$	$3,42 \pm 0,13$

We found an isotopic enrichment (feather-blood) of 1.6‰ and 1.8‰ in  $\delta^{13}\text{C}$  for Audouin's and yellow-legged gulls, respectively, and 0.6‰ in  $\delta^{15}\text{N}$  for YLG. For AG there was a 0.4‰ blood-feather enrichment in nitrogen isotope ratios.

When investigating parent-offspring isotopic segregation, we found significant differences in whole blood isotope signatures (MANOVA, Wilk's lambda,  $F_{2,104} = 9.31$ ,  $P < 0.001$ ). However, these differences were significant only in  $\delta^{13}\text{C}$  (one-way ANOVA,  $F_{2,50} = 18.49$ ,  $P < 0.001$ ) but not in  $\delta^{15}\text{N}$  ( $F_{2,50} =$

0.57,  $P = 0.64$ ). Regarding carbon ratios, pairwise multiple comparisons with Bonferroni correction showed that AG chicks were highly segregated from their own parents ( $P < 0.001$ ), and from both adults ( $P < 0.001$ ) and chicks ( $P = 0.01$ ) of YLG. On the other hand, YLG chicks differed significantly from AG adults ( $P = 0.005$ ) and almost from their own parents ( $P = 0.06$ ). SIBER analysis did not detect any significant differences in isotopic niche width among adults and chicks from both YLG and AG (all  $SEA_B$ ,  $P > 0.2$ ), however confirmed the high segregation of AG chicks, which had almost no overlap with the other groups (Fig. 8).



**Figure 8.** Stable isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) in whole blood of Audouin's gull (AG) chicks ( $n = 17$ ; *empty triangles and dotted line*) and adults ( $n = 12$ ; *empty circles and thin solid line*), and yellow-legged gull (YLG) chicks ( $n = 16$ ; *black triangles and dashed line*) and adults ( $n = 9$ ; *black circles and thick solid line*). The represented standard ellipses areas corrected for small sample size ( $SEA_C$ ) were constructed using the Stable Isotopes Bayesian Ellipses package in R (SIBER, Jackson et al. 2011).

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## **Chapter 4 – Discussion**

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Our study shows segregation in habitat use, trophic ecology, and isotopic niche between Audouin's gulls (AG) and yellow-legged (YLG) gulls. During the breeding season, pellet analysis revealed important differences in their diet composition: while AG relied almost exclusively on marine fish, YLG showed a more generalist diet. On the other hand, isotopic values indicate trophic niche segregation at the end of the breeding season, and a shift in feeding strategies for both gull species during the non-breeding period, in opposite ways: YLG broaden their isotopic niche, revealing a highly generalist diet, whereas AG showed a more specialist feeding strategy. Moreover, we found a clear parent-offspring segregation in carbon isotope ratios in AG, indicating different foraging areas of adults for self-feeding and chick provisioning, but not in YLG, suggesting that this species maintains their opportunistic feeding habits during the chick-rearing period.

#### **4.1 Inter-specific dietary segregation during the breeding season**

Contrary to most AG colonies in the western Mediterranean (Oro et al. 1996b; Pedrocchi et al. 1996; Pedrocchi et al. 2002), clupeiformes were not the main prey of AG in the Ria Formosa. Although they were the third most common group in their samples, and sardines were actually the second most common species, garfish (*Belone belone*) was clearly the most consumed prey. The only previous record of this fish species in the diet of AG is from a study published in 1981 (Witt et al. 1981).

It is well known that opportunistic seabirds have a high feeding plasticity, and thus their diet reflect prey abundance at several spatial and temporal scales

(Montevecchi & Myers 1995). Therefore, even though garfish is not a frequent prey of AG breeding in the Mediterranean, its higher abundance in the study area may explain its higher presence in the gulls' diet, since it is an easily caught epipelagic fish species that lives close to the surface (Froese & Pauly 2014). In the Ria Formosa lagoon system, garfish is also an important prey for little terns *Sternula albifrons* (Catry et al. 2009; Ramos et al. 2013b). Garfish is not commonly targeted by fisheries, nevertheless, published data recorded garfish as one of the main species discarded in purse seiners in the South of Portugal, which encompasses our study area (Borges et al. 2001; Erzini et al. 2002). Therefore, this species could partly be caught by AG in association with purse seine fisheries, either actively, when the boats attract the fish to the surface with powerful lamps and when they encircle the net, or by exploiting its discards (Arcos & Oro 2002; Pedrocchi et al. 2002). This would also explain the low occurrence of garfish in YLG pellets, since this gull species does not present nocturnal foraging activity, and therefore is not expected to attend purse seiners (Arcos & Oro 2002).

Another important prey revealed by the pellet analysis was the myctophid *Myctophum punctatum* in the diet of AG. To our best knowledge, myctophids were not found previously in their diet. This fish species is highly oceanic and nyctoepipelagic (Froese & Pauly 2014), and therefore could indicate natural nocturnal foraging activity by this gull species, however, myctophids are taken by YLG and roseate terns (*Sterna dougallii*) breeding in the Azores Islands, probably fish that die during their vertical migrations and are easily caught by the birds during the day (Ramos et al. 1998a, 1998b). Additionally, at Ria



Formosa, given their bathypelagic habitats, *M. punctatum* are not targeted by fisheries nor commonly discarded (Borges et al. 2001; Erzini et al. 2002).

In our study area, the diet of the YLG appears to be strongly dependent on fishery activities. Their prey occurrence was highly correlated with local fishery landings, and their diet composition was also related with the most discarded fish species in the area. They consumed both heavily fished and discarded species (*Scomber* spp. and *Sardina pilchardus*), and also species not actually landed on the harbours of the area but discarded, such as bogue (*Boops boops*), blue whiting (*Micromesistius poutassou*), and snipe-fish (*Macroramphosus scolopax*) (Erzini et al. 2002). The consumption of other prey, such as terrestrial invertebrates and refuse, is of much less importance compared to marine prey. The same results were found at Chafarinas (González-Solís et al. 1997b) and Columbretes (Ramos et al. 2009d) Islands in the Mediterranean, where this gull species also feeds largely on discards generated by the surrounding fishing activities. However, dietary studies performed on several YLG populations revealed that their diet is highly variable throughout their breeding range (Ramos et al. 2009a), being influenced by the most abundant and easily accessible resources, such as those resulting from human activities, as well as by the presence of alternative food resources, such as marine and terrestrial invertebrates (Munilla 1997; Duhem et al. 2005; Moreno et al. 2010). In the southeast coast of France, refuse is their main resource, while marine prey is the least consumed prey type due to the very limited fisheries operating in the area, and the high accessibility to refuse dumps (Duhem et al. 2003). At Selvagem Grande Island in the northeast Atlantic, the small colony of YLG has no access to either discards or refuse,

thus their diet is mainly composed of seabirds and land snails, whereas fish was nearly absent from their pellets, with a frequency of occurrence of 0.3% (Matias & Catry 2010). Similarly, in the 1920's, YLG in Azores should have had a diet typically dominated by other seabird species (Pedro et al. 2013).

Interestingly, seabreams (*Diplodus* spp.) were frequently consumed by both gull species, being the fourth most commonly encountered species in their pellets. Seabreams are demersal species frequently discarded in purse seiners and trawlers (Borges et al. 2001), indicating feeding in association with fisheries for both gull species. In agreement with the pellet analysis, the SIAR mixing models also revealed that the two gull species fed on demersal fish species, although their relative proportion was higher in the diet of YLG. Demersal species are available to surface feeders, such as gulls, only through fishery discards, however, pelagic species can also be discarded at fishing boats. Indeed, the most discarded species in the area is the pelagic Atlantic chub mackerel (*Scomber colias*), and sardines and other pelagic species can be also discarded (Borges et al. 2001; Erzini et al. 2002). Therefore, inferring the consumption of discards by the proportion of demersal species in the gulls diet will almost certainly be underestimated, as pointed out by Votier et al. (2010) when estimating the consumption of demersal fish by northern gannets (*Morus bassanus*) with isotopic mixing models, and also by Oro et al. (1996b) when assessing the importance of discards in the diet of AG through pellet analysis. Nevertheless, the isotope mixing models gave important insights regarding the diet of each gull species and revealed a higher consumption of discards by YLG than AG.

In accordance with our results, González-Solís et al. (1997b) and González-Solís (2003), the only studies available on resource partitioning between YLG and AG, also found a great dependence on fishing activities by both gull species. Additionally, González-Solís (2003) revealed that the two gull species had different patterns of activity, with YLG showing clearly a diurnal pattern, while AG were both diurnal and nocturnal, as also suggested by the prey we identified through pellet analysis. However, contrary to our data, the two gull species presented a high dietary overlap in the period with normal fishing activity, feeding mainly on clupeiformes (González-Solís et al. 1997b; González-Solís 2003). This may be explained by the fact that clupeiformes were the most abundant and captured prey in their western Mediterranean study area (Martínez-Abraín et al. 2002).

#### **4.2 Year-round feeding strategies revealed by stable isotopes**

The isotopic signatures of YLG and AG largely overlapped during the breeding season. This could mean either an actual overlap in their diet and foraging areas, or that the two species foraged in distinct areas and/or consumed different prey but with similar isotopic signatures (Bearhop et al. 2004). However, since these results contrast with those found by conventional methods, which revealed differences in the diet between the two gull species, the most likely explanation seems to be that their prey did not differ isotopically. Marine fish was by far the most consumed prey by the two gull species, and the pellet analysis provided a high taxonomic resolution that revealed dietary differences between the two gull species. Stable isotope ratios

strongly differ between marine and terrestrial food webs, with higher values of carbon and nitrogen in the marine environment (Inger & Bearhop 2008; Kelly 2000). However, within this environment, isotopic differences may not be detected, especially at small spatial scales (Barrett et al. 2007; Bearhop et al. 2004). As pointed out by Karnovsky et al. (2012), different dietary species, such as forage fish, can have a high overlap in their isotopic values. Indeed, the stable isotope signatures of fish support this explanation since only seabreams differed significantly from a few fish species, indicating that indeed this marine system does not present a high isotopic heterogeneity, and differences in isotopic signatures of organisms are, probably, only revealed between highly distinct areas (Mancini & Bugoni 2014). Nonetheless, more isotopic variability might have been found if more individuals had been sampled (Roscales et al. 2011). Another way to augment isotopic variability and possibly detect isotopic differences between the two consumers, could be the use of sulphur isotope ratios ( $\delta^{34}\text{S}$ ,  $^{34}\text{S}/^{32}\text{S}$ ). Similarly to carbon,  $\delta^{34}\text{S}$  values are used to identify sources of primary production and, therefore, foraging areas (Ramos et al. 2009a). This stable isotope seems to have a greater discrimination power, distinguishing among terrestrial, freshwater and marine prey, but also different marine species (Sanpera et al. 2007a; Moreno et al. 2010; Arizaga et al. 2013). Thus, the inclusion of  $\delta^{34}\text{S}$  in addition to the widely used carbon and nitrogen isotope ratios in SIA is often recommended, especially when consumers have a generalist diet.

Additional, the difference found between the two dietary methods could be related with a temporal mismatch, since eggshells and RBC signatures reflect the dietary input during the pre-laying and laying period, respectively,

and the majority of pellets were collected during the chick-rearing period. Plasma overlapped with conventional dietary sampling, but represents diet over a short time period (i.e. few days prior sampling). In Pedro et al. (2013) there were also differences between stable isotope and pellet analyses owing to the fact that the tissue sampled (i.e. breast feathers) reflected the year-round diet of YLG, while pellets represented their diet over a shorter time period (i.e. breeding season). However, in our study, although it might have occurred a shift in the gulls' diet, their prey were also sampled during the chick-rearing phase, suggesting that the isotopic overlap between the two gull species was most probably related with a foraging environment and isotopically homogeneous diet. Nevertheless, more studies are needed to properly assess the isotopic signatures within this marine environment.

Contrastingly, the isotopic signatures in the first primary feather (P1) of the two gull species, which reflect their diet at the end of the previous breeding season, had the lowest isotopic overlap than any other tissues. This difference in their isotopic niches suggest that they exploited different trophic resources (Bearhop et al. 2004; Jackson et al. 2011). AG isotopic signatures of both carbon and nitrogen, although not statistically significant, tended to be lower than YLG, suggesting that they foraged more offshore and feed mostly on epipelagic fish, which occupy lower trophic levels compared to mesopelagic and demersal species (Sanpera et al. 2007a; Navarro et al. 2009b). Nevertheless, differences in seabird isotopic values could also be related with variation in isotopic baseline values (Quillfeldt et al. 2015). In our study we do not have isotopic signatures of prey from the previous breeding season, however, it seems unlikely that annual variation in baseline isotope values would

completely change our results (Phillips et al. 2009), and inter-annual differences in foraging ecology has been found for several seabird species (Thaxter et al. 2012). Unrelated to isotopic values, at Ria Formosa, Catry et al. (2009) found that little tern diet varied among years due to changes in the availability of prey. In the Mediterranean, Ramírez et al. (2011) also detected inter-annual differences in the diet of YLG during the pre-laying period by the analysis of the isotopic composition of albumen. In a population of YLG breeding in Portugal, Ceia et al. (2014) found inter-annual differences in the foraging ecology of gulls during both the pre-laying and incubation periods, related with the availability of resources; however, despite this difference, there was short- and long-term consistency in the feeding ecology of individuals. This result contrast with ours, since we found greater variation within individuals than among individuals, indicating that there is no isotopic individual consistency for either gull species (Votier et al. 2010; Ceia et al. 2012).

Inter-seasonal isotopic variations were expected in AG because they are migratory seabirds and therefore, after the breeding season, they move to non-breeding areas in the northwest African coast (i.e. Morocco, Mauritania, Senegal), which, presumably, have different isotopic baseline values from the breeding colony (Oro et al. 2011). Latitudinal variations in baseline isotopic values have revealed very important insights into the spatial ecology of several marine predators. However, knowledge concerning geographic gradients in stable isotopes (i.e. isoscapes) is scarce, and not available for our study area. Therefore, it is not possible to determine the non-breeding areas of AG based on their isotopic signatures, as noted by Sanpera et al. (2007a) when studying the Chafarinas Islands and Ebro Delta breeding populations. Nevertheless,

higher carbon ratios found in the eighth secondary feathers of AG, which reflect dietary input during the non-breeding season, compared to other tissues that reflect their diet during the breeding season, and also to their resident congener YLG, could mean foraging in a more productive area. The marine system of the Canary Current in the northwest African coast (12° to 43° N) is a highly productive upwelling area (Aristegui et al. 2009) and it is an important non-breeding ground for several seabird species (Ramos et al. 2013c), including AG (Bécares et al. 2012). Higher baseline  $\delta^{13}\text{C}$  values have been found in this marine system compared to northern temperate Atlantic areas, related to a higher photosynthetic activity (Graham et al. 2010; McMahon et al. 2013). Thus, as found in another breeding population (Ebro Delta) with ring recoveries and resightings (Oro & Martínez-Vilalta 1994), our isotopic data indicate that the northwest African coast can be a non-breeding area for AG breeding at Barreta Island. Sanpera et al. (2007a) is the only study so far that also compared isotopic signatures of AG in feathers formed during the breeding and non-breeding seasons, and they also found an isotopic enrichment in the feathers grown during the non-breeding period. Furthermore, they revealed a common non-breeding area for the two different breeding colonies.

Additionally, we found that AG seems rather specialist in their foraging ecology during non-breeding season, revealing the smallest isotopic niche width of the year during this period. In contrast, YLG showed the widest isotopic niche, indicating a highly generalist feeding ecology during the non-breeding season. Different nutritional requirements, prey availability, and constraints between the breeding and non-breeding seasons can lead to changes in the feeding ecology of seabirds, switching to more generalist diets (Hobson et al.

1994). Ramos et al. (2011) studied the feeding ecology of YLG in several breeding populations in the western Mediterranean, and, in some colonies, the isotopic signatures of eighth secondary feathers also revealed a more diverse diet during the non-breeding season compared to the breeding period. In accordance, our results also revealed a shift in the feeding ecology of YLG in the non-breeding season, when they no longer behave as central-place foragers and thus have access to more dietary opportunities, notably the fact that outside the breeding season, YLG can also forage inland and exploit a wider range of food sources, such as landfills or agriculture fields.

#### **4.3 Parent-offspring isotopic segregation**

Stable carbon isotope ratios revealed strong intra-specific segregation between AG adults and their chicks, but a clear parent-offspring segregation was not found in YLG. Additionally, isotopic inter-specific differences were found between chicks during the breeding season. However, this result does not agree with that found with the conventional dietary sampling, due to the relatively small sample size of regurgitates.

Intra-specific segregation found in AG indicate that adults used different foraging strategies for self-feeding and chick provisioning. Lower  $\delta^{13}\text{C}$  in chicks could mean that the adults performed longer marine trips, or that they selected more pelagic than demersal fish, or a combination of both, for provision their chicks, as found in other seabird species (Forero et al. 2002; Alonso et al. 2012). Parent-offspring dietary segregation has been revealed in several studies and linked to their different food requirements (Hodum & Hobson 2000;



Forero & Hobson 2003; Ronconi et al. 2014). Chicks need high energy prey for their growth, and pelagic fish has a higher lipid content than demersal fish (Anthony et al. 2000; Eder 2005; Grémillet et al. 2008).

Changes in physiology and metabolic rate can be responsible for differences in isotopic signatures between adults and chicks. Although these issues can sometimes be difficult to tease apart from dietary differences, some studies revealed that chicks have a lower  $\delta^{15}\text{N}$  in blood compared to adults due to their rapid growth (Bearhop et al. 2000; Sears et al. 2009). Protein catabolism is higher in growing chicks, and its waste products (uric acid or urea) are typically depleted in  $^{15}\text{N}$  (Peterson & Fry 1987; Hodum & Hobson 2000; Forero et al. 2002). However, as pointed out by Sears et al. (2009), this difference is small (less than a trophic level enrichment) and particularly important when the isotopic variation is small. This was not the case in our samples, because parent-offspring isotopic segregation and inter-specific differences in blood isotopic signatures of chicks were only detected for stable isotope carbon ratios. Therefore, we can conclude that the age-related isotopic differences revealed by this study mean an actual segregation between the foraging areas used by adults for self-feeding and those areas used for chick provisioning.

In accordance with our results, Pedrocchi et al. (1996) also found age-related differences in diet composition of AG. Additionally, Navarro et al. (2010) performed SIA and also revealed differences in the feeding ecology between chicks and adults in this gull species, with the adults provisioning more pelagic fish than demersal to their offspring. Contrastingly, and in agreement with our data, Arizaga et al. (2013) did not found differences between isotopic signatures

of chicks and adults in YLG, suggesting that this species maintains a highly opportunistic feeding strategy also during chick-rearing period.

Interestingly, regarding isotopic signatures of blood and feathers of chicks, we detected differences in both gull species, between these two tissues that were formed at the same time and at the breeding colony, and therefore, should record the same dietary information. However, we found higher  $\delta^{13}\text{C}$  in feathers compared to whole blood of chicks. This difference could be related to different discrimination factors among tissues (Caut et al. 2009), but relatively few studies sampled simultaneously blood and feathers of chicks. However, in a recent study, Cherel et al. (2014) sampled 22 seabird species and reviewed the existing studies in order to compare the isotopic signatures between these two tissues, and revealed that feathers were consistently enriched in  $^{13}\text{C}$  compared to blood, as found in our study. Additionally, they detected that feathers were also  $^{15}\text{N}$  enriched, however to a much less extent, and in some species the opposite ( $^{15}\text{N}$  enrichment in blood) was true. Sanpera et al. (2007b) also sampled blood and feathers of AG chicks and detected 1.6‰ feather-blood enrichment for  $\delta^{13}\text{C}$  and 0.9‰ for  $\delta^{15}\text{N}$ . In agreement in their results, we found very similar values for  $\delta^{13}\text{C}$  in both AG and YLG, however, nitrogen ratios were more variable. Overall, our data agrees with the most important findings found so far, and contributes to further knowledge concerning tissue-specific isotopic discrimination factors.

#### **4.4 Influence of fisheries on gull population dynamics**

Similarly to most gull studies so far, our study suggests that commercial fisheries provide a superabundance of resources (i.e. discards), allowing these two populations of closely related species to coexist and even augment annually (authors' personal observations). However, the upcoming discard ban policy will lead to severe reductions in food availability for these populations.

Fishing activities have led to a global population increase and expansion for both AG and YLG. In accordance with the optimal foraging theory, these gull species can decrease the time and energy spent foraging during the breeding season by feeding on abundant and predictable food resources provided by fisheries, allowing them to feed and guard their offspring, thereby increasing their breeding success (Oro et al. 1996b). Furthermore, these resources may also be important over the non-breeding period, allowing a higher survival of juveniles and a better body condition of adults, thus potentially increasing recruitment in future reproductive seasons (Bicknell et al. 2013).

The major ecological impacts of a discard ban are expected to occur during their breeding season, because during the non-breeding period, AG migrates to the African coast, and YLG already exhibits a wide dietary plasticity. Furthermore, both gull species have been negatively affected by a decreased fishing activity (i.e. trawling moratorium to protect fish stocks), showing their great dependence on fishery discards during this energetically demanding season (Oro et al. 1996a; Oro & Ruiz 1997). Moreover, this food shortage also led to changes in resource partitioning and trophic niche overlap between these two closely related species breeding in sympatry (González-Solís et al. 1997b).

The niche overlap between the two gull species decreased, and the changes in their foraging behaviour occurred in agreement with their evolutionary and life-history characteristics. The YLG broaden their niche and showed a dietary shift towards terrestrial habitats, as expected for a generalist species. The AG increased foraging effort, maintaining epipelagic fish as its main prey and the same trophic niche, in accordance with their more marine and specialist behaviour (González-Solís et al. 1997b).

In agreement with these data, in our study area YLG might increase the consumption of alternative prey, such as terrestrial prey, increasing their trophic niche (Ramos et al. 2009d). The more specialised AG may also feed on a wider range of fish species and other coastal prey, possibly from the adjacent lagoon, but are also expected to increase their foraging effort and change their distribution at sea (Cama et al. 2013). However, these changes in diet composition and foraging behaviour might not be sufficient to meet their requirements and therefore, will lead to breeding failures in both gull species, as previously recorded during trawling moratoriums in the western Mediterranean (Oro et al. 1996b; Oro & Ruiz 1997). Consequently, in these fasting circumstances, predation events of YLG upon AG are likely to appear and increase (González-Solís 2003). In normal conditions of food availability, these interactions do not affect significantly AG (Martínez-Abraín et al. 2003) since YLG act as a natural predator, i.e. removing individuals with low reproductive outcome, and predating mainly the damaged or lost eggs and the less frequently attended chicks (Oro & Martínez-Abraín 2007). However, a severe decrease in their main prey can increase the rates of kleptoparasitism and predation of eggs, chicks and even adults, thus creating a negative scenario for

AG populations (Arcos et al. 2001). Likewise, in some colonies where habitat and food availability are limited, the YLG has outcompeted and negatively affected the sympatric AG (Paracuellos & Nevado 2010). Additionally, Stenhouse & Montevecchi (1999) found that gull predation on sympatric storm-petrels was influenced by a decreased availability of discards. Therefore, we predict that competition for resources will increase and shape these two gull populations, and expect increases in disturbance, kleptoparasitism, and predation of YLG upon AG (Ramos et al. 2011). Additionally, YLG could also affect other species breeding nearby, such as little terns. Ultimately, this management policy will lead to overall reductions in gull populations, however, because food shortage affects fecundity before survival in long-lived species as seabirds, reductions are only expected in the long-term for both gull species (Oro et al. 1996a; Sotillo et al. 2014). Possibly, a greater overall population decline is expected for YLG since this species is highly opportunistic and presents little natural foraging skills (Duhem et al. 2003; Matias & Catry 2010).

Nevertheless, the degree of both immediate and long-term indirect consequences of such policy for scavenging seabirds is still poorly understood and long-term seabird monitoring is needed, especially for gull species that show a great dependence on discards (Bicknell et al. 2013, Bécares et al. 2015). This study provides only a snapshot of the foraging ecology and resource partitioning between AG and YLG breeding at Barreta Island. Additionally, not all individuals within the population are expected to respond equally due to differences in their competitiveness or nutritional requirements (Votier et al. 2010; Masello et al. 2013). In fact, Navarro et al. (2010) revealed that the diet of AG adults varied according to their age, with older individuals

relying more on marine fish, and García-Tarrasón et al. (2015) also found sex-specific differences in foraging behaviour when fishing activities decreased dramatically on weekends. Individual specialization has been also recorded for YLG (Sanz-Aguilar et al. 2009; Ceia et al. 2014). Predicting the consequences of such a major resource decline in the dynamics of this ecosystem is indeed quite challenging, and future research should closely monitor the impact of YLG on the sympatric AG.

#### **4.5 Conclusion**

In our study we detected some differences between conventional methods and stable isotope analysis related with the taxonomic detail achieved by the former, which revealed dietary segregation between the two gull species. Therefore, as found in other studies (e.g. Ramos et al. 2009a; Moreno et al. 2010; Pedro et al. 2013) our data indicates that the isotopic approach performs best when combined with conventional dietary methods.

Overall, our results indicate strong inter-seasonal differences in the foraging strategies and resource partitioning between AG and YLG breeding at Barreta Island. This niche differentiation can be explained by their foraging habitat selection and dietary preferences, as found in other gull species breeding sympatrically in the north-west Atlantic (laughing gull, herring gull, great black-backed gull, and ring-billed gull; Washburn et al. 2013) and in the southern North Sea (black-headed gull, mew gull, herring gull, and lesser black-backed gull; Kubetzki & Garthe 2003; Sotillo et al. 2014). AG exhibit predominantly marine feeding habits and their longer and narrower wings in

proportion to body size may allow a greater foraging range in the marine system (Martínez-Abraín 2003). Indeed, we found that AG foraged more in the marine environment and more offshore than YLG, also as recorded by Arcos et al. (2001) in the western Mediterranean, and adopted both natural and opportunistic (i.e. in association with fisheries) feeding strategies, also revealed by several studies (Pedrocchi et al. 2002; Navarro et al. 2010; García-Tarrasón et al. 2015). Furthermore, their diet composition suggest diurnal and nocturnal foraging activity that allowed them to attend two main types of fishing fleets operating during daylight (trawlers) and at night (purse seiners), as found in other studies (Christel et al. 2012; Bécares et al. 2015). Trawlers generate a huge amount of discards, mostly demersal species. Purse seiners produce a smaller amount of discards but target the same species as AG (i.e. epipelagic fish), which can also actively capture live fish when it becomes more accessible upon closure of the fishnet.

On the other hand, the YLG is a coastal seabird with generalist and high opportunistic feeding habits. Indeed, we found that this species fed mainly in association with fisheries, taking advantage of the abundant food resource produced (i.e. discards). Additionally, the YLG may exclude smaller species, such as AG, from competitive feeding grounds (Oro & Ruiz 1997; Oro et al. 2009). In fact, fishing boats can be highly competitive during the discarding process (Votier et al. 2010; Bicknell et al. 2013; Sotillo et al. 2014), and interference competition between AG and YLG have been reported at these foraging grounds (Arcos et al. 2001). Therefore, AG may avoid trawlers, which attract high numbers of YLG, in order to reduce inter-specific competition. Indeed, as found in other gull (Ronconi et al. 2014) and diving seabird (Bearhop

et al. 2006) species, there are several mechanisms that explain partitioning of resources between AG and YLG.

Our results agree with previous findings on the feeding ecology of both gull species, and contribute to further knowledge throughout their geographical breeding ranges, since there are no previous studies at our study area. Additionally, this study stands out as the first to investigate resource partitioning between AG and YLG using stable isotope analysis, which provide integrative dietary information. Moreover, our data revealed that both gull species fed on discarded species, especially YLG, suggesting that a discard ban will result in severe food shortage, and consequently an increase of negative interactions of the larger and more aggressive YLG on the sympatric, smaller and endangered AG is likely to occur.



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## Appendix 1

**Table 7.** Frequency of occurrence (FO; %) of prey species in the pellets of Audouin's (AG) and yellow-legged (YLG) gulls, and the percentage of prey landed in local harbours (Olhão and Quarteira).

Prey	FO		% Landed
	AG	YLG	
<i>Belone belone</i>	55.9	6.7	0.112
<i>Boops boops</i>	3.6	13.3	0.157
<i>Capros aper</i>	0	3.3	0
<i>Sepia officinalis</i>	1.8	3.3	3.333
<i>Cepola macrophthalma</i>	0	3.3	0
<i>Coelorinchus caelorinchus</i>	7.2	5.0	0
<i>Conger conger</i>	0.9	3.3	0.498
<i>Diplodus spp.</i>	19.8	18.3	1.618
<i>Engraulis encrasicolus</i>	0.9	0	0.217
<i>Gadiculus argenteus</i>	3.6	1.7	0
<i>Macroramphosus scolopax</i>	0.0	5.0	0
<i>Merluccius merluccius</i>	3.6	1.7	0.738
<i>Microchirus variegatus</i>	0.9	3.3	0.491
<i>Micromesistius poutassou</i>	9.9	25.0	0.002
<i>Myctophum punctatum</i>	10.8	0	0
<i>Pagrus sp.</i>	0.9	0	0.058
<i>Pomatoschistus sp.</i>	0	1.7	0
<i>Sardina pilchardus</i>	28.8	38.3	25.923
<i>Scomber spp.</i>	21.6	33.3	37.228
<i>Serranus sp.</i>	0	1.7	0
<i>Trachurus spp.</i>	8.1	15.0	5.896