

**THE IMPACT OF REFUSE ON THE KELP GULL (*LARUS DOMINICANUS*) IN THE
RÍO DE LA PLATA ESTUARY, URUGUAY**

by

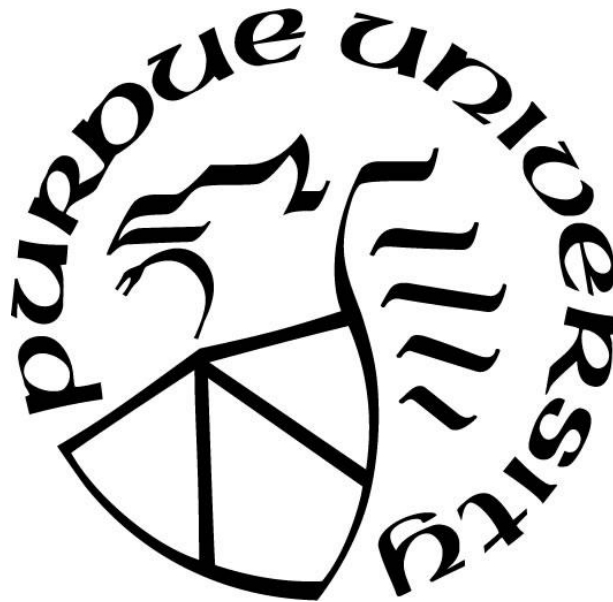
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ABSTRACT

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Title: The Impact of Refuse on the Kelp Gull (*Larus dominicanus*) in the Río de la Plata Estuary, Uruguay

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Modern economic activities, like industry and agriculture, as well as household activities, generate an important amount of refuse. The way we collect, transport, and dispose it will determine the level of environmental contamination. Many animals exploit refuse as a food source (i.e., anthropogenic food subsidy) and gulls are the most important group. Refuse subsidizes energetically gull populations, which impacts on their acquisition and allocation of resources, as well as on the environment, with ecological and evolutionary consequences are not well understood. In this dissertation we evaluated potential impacts of refuse on gulls by doing a literature review as well as empirical research on the Kelp Gull (*Larus dominicanus*) in the Río de la Plata Estuary in South America. Direct and indirect impacts of refuse on gull species and the environment have been observed during the review process. We have detected positive impacts of refuse on body size, chick growth, fecundity, reproductive success, and population dynamics. However, negative impacts were also found focusing on fecundity, reproductive success, and population dynamics. Indirect negative impacts on other species, water bodies, and airport security were also found. Refuse produces numerous impacts on gulls at the individual, population, and species levels, with indirect negative consequences on ecosystems. There is a need to reduce the access of gulls to sources of refuse to mitigate the existing and potential conflicts with human activities and other species, especially those that are threatened and endangered. During our empirical research we found that refuse was ingested and assimilated by Kelp Gull chicks during the chick rearing period and that the ecological niche width increased with the age of the chick. We propose that parents incorporate isotopically unique food sources to nestling's diet during their growth, increasing isotopic diversity of nestlings. Additionally, we found that refuse could affect foraging decisions of females during the pre-incubation period, which could positively affect future fecundity and negatively impact reproductive success. We

found also that refuse consumption on fecundity and reproductive success of gulls is generally studied at the colony level, using conventional diet techniques, but not much has been done using stable isotopes at the individual level, making comparisons among studies and conclusions difficult to address. We encourage other researchers to continue incorporating the isotopic ecology perspective to study the effect of food subsidies on gulls. Additionally, we found that Kelp Gull on the coast of the Rio de la Plata Estuary ingest plastic debris. We conclude that plastic bags and plastic films might be the most important source of contaminants for the Kelp Gull on the coast of the estuary. Main findings of this dissertation suggests the need for an improvement of waste management practices and a regulation of plastic production and use in Uruguay to reduce plastic ingestion by gulls. Finally, next steps for research are provided in this important area of environmental science and natural resource management.

1. GENERAL INTRODUCTION

1.1 Background

A significant environmental consequence of industrialization and agriculture has been the generation of massive waste. The World Bank (Kaza et al., 2018) has recently estimated that 2.0 billion tons of waste is produced each year globally and this will rise to 3.4 billion tons by 2050. This problem is heightened in the developing world where management of waste has been largely challenged by the unfavorable economic, institutional, legislative, technical, and infrastructural constraints (Imam et al., 2008; Leitmann et al., 1992; Srivastava et al., 2015). These strains are reflected in the fact that as much as 20% of the budget of municipalities in developing countries is devoted to waste management, with only 39% of the waste actively collected and effectively disposed (Kaza et al., 2018).

Waste has affected wildlife in a variety of ways. For example, numerous species of terrestrial and marine animals have been reported exploit refuse as a new food source (Boarman et al., 2006; Fedriani et al., 2001; Oro et al., 2013; Schneider et al., 2011). These allochthonous food inputs are known as “anthropogenic food subsidies” (Leroux and Loreau, 2008; Polis et al., 1997). Anthropogenic food subsidies might have a diversity of consequences at different levels of biological organization, altering ecological processes including habitat suitability, food availability, individual fitness, disease, and, *inter alia*, movement, (Marzluff, 2001; Marzluff et al., 2001).

Among the species that have long been exploiting anthropogenic food subsidies, gulls are one of the most notorious (Oro et al., 2013). This group of birds has also been recognized as synanthropic, which means that they exhibit patterns of both commensalism or mutualism mediated by humans (Johnston, 2001). As a result, gulls have increased in abundances and distributions during the first half of the twentieth century (Coulson, 2015). Specific underlying causal mechanisms have been presented to explain population expansion of gulls, most focus on inadequate waste management and commercial fishing practices (Giroux et al., 2016; Pons, 1992; Rome and Ellis, 2004; Skórka et al., 2005; Wilhelm et al., 2016).

It has been observed that gull populations that show positive population growth forage on refuse, although evidence is still limited. For instance, the increase of population abundance of

Herring Gull (*Larus argentatus*) and Lesser Black-back (*L. fuscus*) from 235 pairs to 19,000 in 30 years on the west coast of England, has been attributed to their association with a large human population of more than 7 million people (Brown, 1967; ONS, 2011). In the Mediterranean, Yellow-legged Gull (*L. cachinnans*) populations have been expanding at a rate of 7-9% over the second half of the 20th century (Thibault et al., 1996) and in recent years its annual population growth has been estimated to be around 2% (Duhem et al., 2008; Oro and Martinez-Abraín, 2007). Bosch et al. (1994) found that populations of Yellow-legged Gull that foraged more on refuse showed a historical increase in their population sizes than those with a more diverse diet. Conversely, when refuse dumps were closed, negative population growth was observed, as was the case of the Herring Gull in Finland between 1993 and 1997 (Kilpi and Öst, 1998).

However, it should be pointed out that refuse is not composed entirely of organic matter, but also of harmful materials like plastic debris that could have a detrimental effect on animals that interact with it. Plastic has become a major component of modern debris because it is versatile, resistant, and cheap to produce (Andrady and Neal, 2009; Lechner et al., 2014). Plastic manufacturing started in the 1950's with 1.5 million tons per year produced, and current production has risen to 322 million tons a year (Lechner et al., 2014; Plastics Europe, 2016). Considering that a piece of plastic can take centuries to degrade, every piece of plastic that has ever been produced may be still in that form on Earth (Hopewell et al., 2009). Thus, it is fair to ask the fate of all this plastic at the end of the life cycle? It has been estimated that only 5.7% of plastics are recycled and that the rest is discarded (Barnes et al., 2009). If around 50% of the plastics produced are single-use (Hopewell et al., 2009), then 141 million tons of only single-use plastics are discarded annually. Hence, a large amount of plastic await their degradation in landfills. However, during the waste management process, from collection to final deposition, part of this plastic waste finds its way to natural ecosystems like waterways and coastal oceanic areas (Lozoya et al., 2015; Moore et al., 2011). There, it becomes available for biota producing entanglement and consequently asphyxia, lacerations, injuries, and death. In addition, plastics can also be ingested with many negative consequences for animals.

Gulls that forage on refuse can accidentally ingest plastic debris; regrettably, this has been rarely documented in the literature (Lindborg et al., 2012). In contrast, in the marine environment, plastic ingestion by other seabirds like shearwaters, albatrosses, and petrels have been extensively evaluated because they often die from obstruction of their digestive tract and

most of them face conservation challenges (Acampora et al., 2014; Avery-Gomm et al., 2013; Blight and Burger, 1997; Codina-García et al., 2013; Jiménez et al., 2015; Lavers et al., 2013; e.g., Ryan and Fraser, 1988). Although gulls may not die, ingestion of plastic debris may decrease their foraging efficiency and reduce consumption, negatively affecting chick growth and survival (Ryan, 1989). In addition to the negative effects of plastic ingestion, many toxic chemicals are also attached to plastic materials and the most problematic are persistent organic pollutants (POPs) (Colabuono et al., 2010; Hirai et al., 2011).

The effects of refuse on gull populations has been analyzed most notably in the northern hemisphere (e.g., Belant et al., 1993; Duhem et al., 2005; Hunt, 1972; Ortiz and Smith, 1994; Pons, 1992; Weiser and Powell, 2010; Weiser and Powell, 2011) where landfill and refuse management reflects practices of more developed societies. Unfortunately, effects of refuse on gull populations have been much less studied in the southern hemisphere (Bertellotti et al., 2001) where refuse patterns in these developing countries and gull feeding behaviors may be different. Particularly, the Rio de la Plata estuary in South America has unique characteristics that make it an accessible and opportunistic place to analyze the interaction between gulls and refuse in a developing society. This estuary is one of the largest in the Americas (35,000 km²) supporting the largest human settlements of Argentina and Uruguay, with more than 12 million inhabitants (Boschi, 1988). These settlements produce more than 12,775 metric tons of refuse daily from household activities. Additionally, a great diversity of industries like slaughterhouses, tanneries, sawmills, crop mills, among others, generate almost 300,000 metric tons of refuse per year with most located along the Uruguayan coast (FICHTNER-LKSUR-Asociados, 2004). Additionally, it has been estimated that recreational activities account for 74% of the 4.5 metric tons of refuse collected during the International Coastal Cleanup Day by Ocean Conservancy (Lozoya et al., 2015). Therefore, refuse production in the Rio de la Plata estuary has the potential to affect animal populations as an alternative food source increasing the risk of harmful materials entering the food chain (Acurio et al., 1997; Bertellotti et al., 2001; López et al., 2008; Lozoya et al., 2015).

Four gull species extensively use the Rio de la Plata estuary, but the most ubiquitous is the Kelp Gull (*Larus dominicanus*) (Escalante, 1970). This species is widely distributed in the southern hemisphere (Bertellotti and Yorio, 1999; Ludynia et al., 2005; Yorio et al., 2016). Its breeding distribution extends to South America, Australia, New Zealand, nearly all Sub Antarctic

islands, and the Antarctic Peninsula (Burger and Gochfeld, 1996). Studies in other geographic contexts, like marine ecosystems of South America, Antarctica, and Tasmania, have shown that kelp gulls forage on a wide variety of prey such as marine invertebrates, fish, insects, carrion (e.g., waste from the poultry industry and fisher discards), and refuse (e.g., cow and pork meat, and synthetic products like plastic, metal, and glass, among others) (Coulson and Coulson, 1993; Giaccardi et al., 1997; Silva et al., 2000; Yorio et al., 1998). Besides the characteristics of its natural history, the Kelp Gull is a suitable species to study interactions between gulls and refuse in the Rio de la Plata for several reasons. Firstly, it is highly abundant on the Uruguayan coast (Sarroca et al., 2006), and, unlike other gull species, we know precisely where the breeding colonies are (Yorio et al., 2016). Secondly, a great amount of information about its foraging behavior can be obtained in a short period of time using the traditional techniques for quantifying food intake by birds (Barrett et al., 2007).

The overarching objective of this dissertation is to study the extent of the impacts of refuse on the Kelp Gull in the Rio de Plata estuary of Uruguay. Specific objectives include: (1) to review the direct and indirect impacts of refuse on gulls, (2) to study the variation in the ecological niche of Kelp Gull chicks during their growth on a reproductive colony in the Rio de la Plata Estuary, (3) to analyze the fitness consequences of foraging on refuse during the pre-incubation period of Kelp Gull females that reproduce in the Rio de la Plata Estuary, and (4) to evaluate the ingestion of plastic debris by the kelp gull in the Rio de la Plata Estuary.

1.1 Organization of the Thesis

Here I provide a general overview of the research conducted as part of this dissertation. Chapters 2-5 were written as stand-alone papers for publishing in peer-reviewed journals. The thesis begins with a review paper about the direct impacts of refuse on gulls from the individual to species level. Direct and indirect impacts of refuse on other species that coexist and interact with gulls, as well as the indirect impacts on human activities and ecosystems, were analyzed as well. Positive impacts of refuse on body size, chick growth, fecundity, reproductive success, and population dynamics were documented in the literature. However, negative impacts were also found focusing on fecundity and reproductive success. Indirect negative impacts on other species, water bodies, and airport security were also found and summarized. We conclude that refuse, as an anthropogenic food subsidy, produces numerous impacts on gulls at the

individual, population, and species levels, with indirect consequences on the rest of the ecosystem. We argue that there is a need to reduce the access of gulls to sources of refuse to mitigate the existing and potential conflicts with human activities and other species, especially those that are threatened and endangered.

Additionally, to study the potential impact of refuse on the Kelp Gull in the Rio de la Plata Estuary, we conducted three studies each presented as a separate chapter (Chapters 3-5). In the third chapter, we characterized the variation of the ecological niche of Kelp Gull chicks during their growth in response to foraging on refuse, combining conventional diet analysis (pellets) and stable isotopes of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of blood and feathers. We found that refuse was ingested and assimilated by Kelp Gull chicks during the chick rearing period and that the ecological niche width increased with the age of the chick. We propose that parents incorporate isotopically unique food sources to chick's diet during their growth, increasing isotopic diversity.

In the fourth chapter, using pellet analysis and stable isotopes of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of down feathers of Kelp Gull chicks, we assessed the fitness consequences of foraging on refuse during the pre-incubation period of Kelp Gull individual females. We found that refuse could affect foraging decisions of females during the pre-incubation period, which could positively affect future fecundity and negatively impact reproductive success. Refuse consumption on fecundity and reproductive success of gulls is generally studied at the colony level, using pellet analysis, but not much has been done using stable isotopes at the individual level, making comparisons among studies and conclusions difficult to address. We encourage other researchers to continue incorporating the isotopic ecology perspective to study the effect of food subsidies on gulls.

In the fifth chapter we quantified plastic ingestion by the Kelp Gull on the coast of the Rio de la Plata Estuary using pellet analysis. We concluded that plastic bags and plastic films might be the most important source of contaminants for the Kelp Gull on the coast of the estuary. In addition, polyethylene and polypropylene were the most important polymers found in the diet of this bird. Based on the nature of the debris, we suggest that most of it is consumed in landfills. We call for an improvement of waste management practices and a regulation of plastic production and use in Uruguay to reduce plastic ingestion by gulls.

A brief concluding chapter summarizes the main findings of this dissertation and suggests next steps for research in this very important area of environmental science and natural resource management.

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2. A REVIEW OF THE IMPACT OF REFUSE ON GULLS

2.1 Introduction

Clearly, human activities generate refuse, and the way we collect, transport, treat and dispose of it determines its level of environmental impact to water, plants and animals (Zurbrugg, 2002). Households, and economic activities like construction, mining, energy, manufacturing, services, hospitals, agriculture, forestry, among others (Karak et al., 2012; Pipatti et al., 2006) all generate refuse. Each type of waste has its own methods by which it is collected, transported, treated, and disposed, and the efficiency of this process relates positively with socio-economic factors (Bandara et al., 2007; Hoornweg and Bhada-Tata, 2012). Inefficiencies along the waste management system, especially in developing countries, may result in the contamination of land, air, and water causing detrimental consequences to human health and to that of wildlife (Hamer, 2003; Teuten et al., 2009; Thompson et al., 2009; Wright et al., 2013).

One of the poorly understood consequences of inadequate waste management is refuse consumption by animal populations. Refuse could easily be used by animals because it is highly predictable in space and time (Newsome et al., 2015; Oro et al., 2013). Thus, animals could use refuse as an alternative source when natural food is not available, or as a regular source and use natural sources as a substitute (Plaza and Lambertucci, 2017). For example, mammals such as wolves (*Canis lupus*) (Meriggi and Lovari, 1996), Australian dingo (*Canis lupus dingo*) (Newsome et al., 2014), coyotes (*Canis latrans*) (Fedriani et al., 2001), grizzly bears (*Ursus arctos*) (Peirce and Van Daele, 2006), and red fox (*Vulpes vulpes*) (Basuony et al., 2005) use refuse as an alternative food source. On the other hand, hyenas (*Crocuta crocuta*) compensate reductions in anthropogenic food sources, their regular food, by foraging on natural food (Yirga et al., 2012). In birds, it has been observed in many species like rooks (*Corvus frugilegus*) (Olea and Baglione, 2008), kites (Blanco, 1997), skuas (*Stercorarius longicaudus*) (Julien et al., 2014), and gulls (Sol et al., 1993) forage on refuse as an alternative food source. Among birds, gulls may be the most common family (Laridae) that exploit refuse as an alternative food source (Oro et al., 2013), however, the consequences of this outcome is still unknown.

Undoubtedly, foraging on refuse alters the natural resource acquisition processes of gulls (Lowry et al., 2013). Thus, direct positive or negative impacts on individual gulls are expected to occur in human-dominated landscapes. For example, foraging behavior, chick diet, body size and condition, demographic parameters, and population dynamics, could potentially be directly impacted by refuse. Additionally, considering that most gull species and populations are expanding and that gulls have an aggressive behavior, negative impacts could be related to the increase of predation, competition, and kleptoparasitism of other sympatric species (Plaza and Lambertucci, 2017). Moreover, refuse is contaminated with several pathogens and when ingested by gulls they might get infected causing indirect detrimental impacts to other individuals, other species, water bodies, humans and/or cattle (Kim and Monaghan, 2006). In some geographical contexts, sources of refuse are close to airports, and the presence of gulls could increase the risk of strikes with aircrafts. Thus, in a scenario of increasing gull populations, understanding the effects of refuse on gulls and the potential conflicts with co-occurring species, ecosystems, and human activities, is therefore of key concern.

In this study, we conducted a review of the extent to which refuse is used by gulls. We start with previous reviews on the impact of anthropogenic food subsidies on animals (Oro et al., 2013), as well as the impact of garbage dumps on vertebrates (Plaza and Lambertucci, 2017), and a review of refuse on terrestrial mammals (Newsome et al., 2015). We desire here to build on these reviews providing a detailed analysis in order to inform the role of refuse on gull population dynamics and behavior. We aim to determine which gull species use refuse as a food source, and assess the impacts that it has at the individual, population and species levels. Additionally, we address the direct and indirect impacts on other species that do not forage on refuse but are affected by ecological interactions with gulls, and what might be the potential conflicts with humans and the environment.

2.2 Material and Methods

Four bibliographic searches of scientific articles were performed using the Web of Science and Google Scholar using a combination of key words without restricting the search to any year or geographic location. We used the word “gull” in every search followed by any of the following key words: “refuse”, “garbage”, “waste”, or “debris”. We revised the first 1,000 results of Google Scholar and all the references from the Web of Science. Once these four searches

were performed, we also searched for those references that were cited in the articles reviewed. All the references were downloaded and inspected; depending on the information in the title and abstract, the full article was read and its principal outputs systematized in a database.

We incorporated articles to the database that analyzed explicitly the direct impacts of refuse on: fecundity, reproductive success, population growth, movement patterns, pathogen infection, chick's diet, and habitat use. We also attempted to identify the indirect impacts of refuse on any of the following: water quality, impact on other species, and airport security. We additionally incorporated those observational and comparative articles that analyzed the diet of the gulls to determine to what extent refuse composed a food source in these studies.

2.3 Results

We found 140 studies that focused, in totality, on 22 species of gulls that use sources of on refuse during their life cycle (Table 2.1, Appendix). Twenty species are from the *Larus* genus and the remaining two are from the *Pagophila* and *Rhodostethia* genus (Table 2.1). The species that has been mostly studied was the Herring Gull (36%, 51 studies), and the second most frequently studied species was the Kelp Gull (15%, 21 studies, Table 2.1). Almost half of the reviewed studies (48%, 68 studies) did not analyze direct impacts of refuse, but they quantified its consumption as observational studies (30%, 42 studies) or compared diets spatially and/or temporally (19%, 26 studies) (Appendix).

Among those studies which analyze impacts of refuse (52%, 73 studies), positive impacts on fecundity, reproductive success, and population growth, were recorded in 10% of the articles (14 studies). On the other hand, negative impacts were documented to affect fecundity, reproductive success, and population growth, but were less commonly (3%, 4 studies) reported than positive impacts. Additionally, only two studies (1%) found no impacts of refuse on fecundity or on reproductive success (Appendix). Some researchers also observed impacts of refuse on movement patterns (6%, 8 studies), pathogen infection (14%, 19 studies), water quality (2%, 3 studies), impacts on other species (4%, 6 studies), chick diet composition (9%, 12 studies), habitat use (6%, 8 studies), and even some examined airport hazards (4%, 5 studies). Other impacts (11%, 16 studies) like age-related foraging differences, adult survival, and body weight, and the presence of persistent organic pollutants, were also documented. These patterns

synthesize the importance of refuse during many aspects of the life cycle of gulls, as well as the potentiality for environmental conflicts that this interaction could have.

Most of the species were registered foraging on landfills and refuse dumps, although we did not find suitable definitions for these disposal and treatment methods of refuse (see Figure 2.1) (73%, 102 studies). This highlights the importance of the last link of the waste management systems around the world as are the disposal sites. Additionally, gulls also used recreational areas, sewage offal, poultry farms, restaurants, food outlets, intertidal zones, and the urban habitat (6%, 8 studies). Around 4% of the studies addressed their research questions at the individual level (5 studies), 50% (71 studies) at the colony level, 3% (4 studies) at the population level, and 36% (51 studies) at the species level. It is important to underline that the levels of biological organization that are key to study ecological and evolutionary processes, like individual and population levels, are very much underrepresented in the revised investigations. This should be a wake-up call for the researchers to incorporate individual and population levels in their investigations, to be able to properly address the many ecological and evolutionary processes affected by this interaction.

Among those studies that addressed diet, pellet analysis was the most used technique with 15% of the studies (21 studies). Regurgitations was used by 6% (8 studies), stable isotope analysis combined with pellet analysis by 3% (4 studies), and 1% (2 studies) added GPS tracking with other techniques. Focal observations of individuals were used by 19% of the studies (21 studies), stomach contents and digestive tract from dead birds or from culling by 8% (11 studies), and focal observation and video recording in 18% of the studies (25 studies). The use of a variety of techniques follows objectives that are principally tools used to analyze diets at the colony and species levels, such as pellet analysis. The use of stable isotopes and GPS tracking is a relatively new set of tools that have been used lately with very good results to address the use of sources of garbage by individual gulls, allowing researchers to address patterns of acquisition and allocation of resources (e.g., Caron-Beaudoin et al., 2013; Ceia et al., 2014).

2.3.1 Impacts of refuse on gulls

Direct impacts

We found that refuse positively impacts individual parameters like body weight, size, and condition. For instance, Steigerwald et al. (2015) found that after the closure of a refuse dump in

Spain, male and females of Yellow-legged Gulls (*Larus michahellis*) decreased their body mass by 10.4 and 7.8%, respectively. Additionally, after the closure of a landfill in France, Pons and Migot (1995) found that Herring Gull males and females decreased in weight by 4.6% and 4.7%, respectively. In addition, Auman et al. (2008) compared individuals of Silver Gulls (*Larus novaehollandiae*) from an urbanized area and a remote region, where males from the urbanized area were found to be heavier and of better body condition, an index estimated as body mass/(head length + keel length). Another investigation by these authors confirmed that the Silver Gulls from the urban area foraged more on refuse, and the individuals of the remote region foraged on foods of natural origin (Auman et al., 2011). Thus, when individual gulls forage on refuse, they tend to increase their body condition and weight.

Fecundity and reproductive success generally are affected positively by foraging on refuse. After the closure of refuse dumps in Europe, following European Union directives, colonies of Herring Gull decreased their reproductive investment and success (Kilpi and Öst, 1998; Pons, 1992; Pons and Migot, 1995). Moreover, after the closure of a landfill in Spain, Steigerwald et al. (2015) found that clutch size and egg volume of a Yellow-legged Gull colony significantly decreased, indicating that refuse had positively affected these reproductive parameters. In addition, Hunt (1972) found that colonies (on outer islands) distant from sources of refuse tended to exhibit less reproductive success than those colonies (inner islands) closer to waste sources in the coast of Maine (United States).

Other studies, conversely, have detected mixed impacts of refuse on fecundity and reproductive success. For instance, Pierotti and Annett (2001) analyzed the diet of Western Gulls (*Larus occidentalis*) and found that reproductive success was lower in those colonies closer to sources of refuse. However, they found no impact of refuse on fecundity (i.e., clutch size). Additionally, these authors performed an experiment where they fed a group of chicks with fish and another group of chicks with refuse. Their results indicated that refuse-fed chicks were not capable of growing properly, as fish-fed chicks did.

Negative impacts of refuse on fecundity and reproductive success were also recorded. Pierotti and Annet (1991) observed that Herring Gulls that specialized on refuse did not produce as many eggs, chicks, and fledglings, as other individuals that specialized in other natural diets like mussels and petrels, even those generalist individuals. In addition, Belant et al. (1998) analyzed clutching success of Herring Gulls and Ringed-billed Gulls in the Laurentian Great

Lakes. These authors found that urban colonies had on average > 40% less hatching success than the colonies located at a large distance from urban areas.

Positive impacts of refuse on population growth have been detected in several gull species. For example, along the Mediterranean coast of Spain and on the coast of Maine in the United States, colonies of Herring Gull and Great Black-backed Gull, were respectively associated to sources of refuse, and had shown to increase in abundance since the establishment of refuse dumps (Bosch et al., 1994; Drury, 1973). Recently established colonies of Yellow-legged Gulls in Poland associated with human-made habitats exponentially increased their abundances, doubling their population sizes every 1.2 years (Skórka et al., 2005). In South America, Lisnizer et al. (2011) analyzed population dynamics of the Kelp Gull along an important sector of the Argentinean coast. These authors found that most of the colonies (74%) increased their abundances, and the overall population is increasing at a rate of 2.7% per year. Those colonies associated with urban refuse dumps showed positive population increase ($\lambda > 1$). On the other hand, those colonies associated with no sources of refuse showed negative population growth ($\lambda < 1$) (Lisnizer et al., 2011).

Refuse has been shown to impact chick growth and development as well, positively affecting their survivorship (Coulson and Porter, 1985). When parents are growing their chicks, they shift their diet during chick rearing, which commonly initially is based on refuse, to a more nutritious prey, like fish or invertebrates. For example, Neves et al. (2006) found that in two colonies of Yellow-legged Gull in the Azores Archipelago, the proportion of refuse in pellets, and regurgitations of adults and chicks, was more frequent during the incubation period than during the chick rearing period. Additionally, Moreno et al. (2010) also found, using stable isotope analysis, that reproductive colonies of Yellow-legged Gull in the north of Spain incorporated a smaller proportion of refuse during the chick rearing period in relation to natural food, like fish. The same pattern was observed in Western Gull breeding on Alcatraz Island, where adults heavily fed on refuse; however, once their eggs hatched, they changed their foraging preferences to fish (Annett and Pierotti, 1989).

Chicks' diet also was studied along the chick rearing period at a finer temporal scale. Pedrocchi et al. (1996) analyzed the diet of different age classes of Audouin's Gull chicks and found that the amount of fish increased with chick age. In addition, younger chicks were fed with terrestrial food, which increased feeding rates, likely due to shorter foraging trips. In addition,

chicks older than 20 days were fed with refuse. However, it is difficult to determine if temporal patterns in diet shifts could have been related to nutritional requirements during the chick rearing period or to time constraints imposed by foraging.

One of the consequences of refuse as an anthropogenic food subsidy is its effect on animal movement patterns. It has been sometimes observed that foraging distances are reduced during specific periods of the life cycle of individual gulls. After the commencement of a deterrence program using birds of prey at a landfill area, Arizaga et al. (2014) found that Yellow-legged Gulls started to forage in alternative areas further away, suggesting a change in movement patterns. Additionally, Belant et al. (1993) found that Herring Gulls used the landfills more as a foraging habitat during the post fledging period. In addition, Belant et al. (1998) found that the post-fledging period was an important period of the life cycle for the Ring-billed Gull breeding close to urban areas. Additionally, Camphuysen et al. (2011) observed that the dispersion of Herring Gulls to different locations in the Netherlands was affected by the presence of refuse dumps at different distances of the breeding colonies, and depending on the period of the year. These authors also found that temporal changes in post-breeding dispersal were also detected, affecting differently individuals of different age classes. For instance, Herring Gull juveniles spent more time on refuse dumps than adults, especially during the reproductive season, when adults are constrained by reproduction.

During the first 70 years of the twenty century, it has been noted that Herring Gull and Great Black-backed Gull populations have dramatically increased due to both their protection and to the prevalence of anthropogenic food subsidies (Anderson et al., 2016). However, during the past decades, populations have decreased, most notably due to the potential negative effects of culling programs and the preponderance of new pathogen diseases as a consequence of foraging on refuse (Coulson, 2015). One of the most important diseases found in gulls is botulism, which is caused by the bacteria *Clostridium botulinum* through the botulinum toxin, a neurotoxic protein that generates flaccid paralysis (Shapiro et al., 1998). This bacteria develops in anaerobic conditions where organic matter accumulate, like animal carcasses or garbage (Coulson, 2015; Erbguth, 2004). Many articles have analyzed the potential botulism transmission from environmental sources, including refuse dumps and landfills. For example, Ortiz and Smith (1994) have analyzed botulism in refuse dump soil samples and found that 63% of the sites were contaminated with spores of *C. botulinum*. An outbreak of botulism in the British Isles in the

summer of 1975 affected mostly Herring Gulls, and some authors proposed that refuse dumps were a potential source of pathogens. Additionally, the high temperature of the summer of 1975 may have improved the environmental conditions for the development of the outbreak (Lloyd et al., 1976; Macdonald and Standring, 1978). Analyzing the symptoms of botulism, like paralysis of the limbs and neck, ocular disturbances, and respiratory distress, Kim and Monaghan (2006) found that adults and chicks of Herring and Lesser Black-backed Gulls died of this disease in a colony subjected to various feeding conditions, including refuse at a local dump. These researchers also discovered that mortality caused by botulism was similar in both species, suggesting that this may not be a potential consequence of a differential reduction of Herring Gull populations as suggested by Coulson (2015).

Salmonella is another common pathogen found in gull individuals. For example, in England, Herring Gulls have been detected carrying salmonella at potential sources like sewage outfalls and refuse dumps and landfills (Butterfield et al., 1983). These researchers found that the proportion of gulls infected with *Salmonella* increased with time and that young birds were proportionally more infected than adults during the summer. In the winter, on the other hand, the proportion of infected young gulls decreased, possibly because mortality of fledglings occurred during the summer.

Indirect impacts

Some studies have also compared the incidence of infected gulls with the incidence of infected humans and cattle (Fenlon, 1981). For instance, Monaghan et al. (1985) found a positive relationship between infected humans and infected gulls in refuse dumps during an outbreak of salmonella in Scotland. These researchers also found that Herring Gull, Lesser Black-backed Gull, and Black-headed Gull were infected by 30 different serotypes of *Salmonella*. Additionally, the analysis of refuse samples showed five serotypes of salmonella, two of which were also recorded in gull samples captured in the same day at the same time. In addition, six of the most frequent salmonella serotypes carried by gulls were also detected in humans, cattle, and sheep. Moreover, carriage was positively associated with higher human population densities, which were also positively associated to a higher density of refuse.

In addition to salmonella, other pathogens like *Listeria* and *Campylobacter* have been analyzed in gulls, humans, and cattle associated to refuse dumps and landfills. Twenty percent of individuals of Ringed-billed Gull caught at refuse dumps, nesting sites and river shores were

infected with *Salmonella* spp., *Campylobacter* spp. or *Listeria monocytogenes* near Montreal in Canada (Quessy and Messier, 1992). *Campylobacter* was the most prevalent genus found in 16% of the gulls analyzed, followed by *Listeria* with 10 %, and salmonella with 9%. Although some studies have found correlations between pathogen serotypes and human infections, as mentioned above, these authors argue that the Ringed-billed Gull did not play a major role in human epidemiology and cattle contamination with *Campylobacter*, *Salmonella*, and *Listeria*. Additionally, Whelan et al. (1988) reported a carriage rate of *Campylobacter* between 30-90% in Herring Gulls and also found between 0.2% and 24.3% of campylobacter serotypes from humans in Scotland. Besides this finding, authors concluded that the more frequent *Campylobacter* serotypes present in gulls did not correlate with those commonly present in humans. Thus, evidence of gulls as a potential vector of this pathogen in Scotland might be not so conclusive.

Several studies have analyzed the differences in pathogen infection between sexes, age classes and time of the year. Frere et al. (2000) analyzed 100 individuals of Kelp Gull of different ages to determine the presence of ten species of bacteria of eight genus (*Escherichia*, *Proteus*, *Citrobacter*, *Salmonella*, *Hafnia*, *Shigella*, *Enterobacter*, and *Yersinia*). These researchers found the ten species of bacteria in the analyzed sample, with *Escherichia coli* as the most prevalent (96%), followed by *Proteus* sp. (28%). Both sexes presented similar rates of infection for all the pathogenic species, as well as between adults and juveniles. Infection by *Proteus* and *Citrobacter* were more prevalent during spring and summer.

Water contamination by gulls has not been addressed much, despite the fact that they are carriers of pathogens, and that water bodies are a potential source of transmission to humans and cattle. Pathogens carried by gulls acquired from refuse can contaminate water bodies like reservoirs, wetlands, and ponds. For example, Anza et al. (2014) analyzed the potential of pathogens carried by several bird species, including gulls, to study if dead carcasses could be able to trigger outbreaks in wetland communities. After analyzing several matrices like sediment, water, feces, and invertebrates, and several physicochemical parameters, these authors found that *Escherichia coli* and *Botulism* were more prevalent when wetlands receive waste water. Temperature, oxygen, chlorophyll a, inorganic carbon, and sulfate levels were positively related with *Botulism* outbreaks. In addition, botulism in bird feces was detected before the outbreaks, suggesting that bird carcasses might be vectors and their carcasses could serve as further sources of pathogens (Anza et al., 2014).

Converse et al. (2012) evaluated the impacts of gulls on microbial water quality scaring away gull individuals from a sector of the Lake Michigan coast using trained dogs. They found that gull abundance decreased from hundreds to less than 20 individuals, and *Enterococcus* spp. and *Escherichia coli* densities were also significantly reduced in the lakeshore. In addition, refuse from refuse tips, surface water, and cloacal samples from Herring, Lesser Black-backed, and Black-headed, were analyzed for *Salmonella* by Girdwood et al. (1985) in Scotland. These researchers found 20 different serotypes in gulls, six of which were also found in humans, cattle and sheep. Additionally, they found one serotype found from surface waters, which was also isolated from gull samples captured in the same site the same day. Also, positive results from the refuse tips were more important during summer and fall months, when temperature is high.

The increase in gull populations has had secondary impacts, many consequential. Predation and competition are often cited as important interspecific interactions, and some investigators have examined how these are affected by the presence of refuse. For instance, in Poland, the Yellow-legged Gull population size is expanding, which Skórka et al. (2005) attributed to the incorporation of refuse to their diets during a part of the reproductive cycle. These authors also analyzed the negative effects of the Yellow-legged Gull on the Black-headed Gull, as the first is aggressive and outcompetes the second successfully. Moreover, they found a negative correlation between the numbers of breeding pairs of both species in those colonies where they reproduce sympatrically. Additionally, terns and cormorants; many of these preyed species are endangered, with declining population sizes (e.g., Emslie et al., 1995; Hernández and Ruiz, 2003; Lenzi et al., 2010; Martínez-Abraín et al., 2003; O'Connell and Beck, 2003; Voorbergen et al., 2012; Yorio et al., 1998; Yorio and Quintana, 1997). In addition, in South America, gulls feed on the skin and blubber of living southern right whales, particularly on mother-calf pairs, which reduce calf health and survivorship (Fazio et al., 2012; Groch, 2001; Rowntree et al., 1998; Sironi et al., 2009).

In landfill sites, Audouin's Gull has been observed kleptoparasiting Black-headed Gulls that are almost half of their body size. Likewise, Lesser Black-backed Gulls have been observed aggressively displacing individuals of the smaller Audouin's Gull (Blanco and Marchamalo, 1999; Dunning, 2008). In addition, Skórka and Wójcik (2008) found that conspecifics, Black-headed Gulls, and Jackdaws (*Corvus monedula*) were kleptoparasited by the Caspian Gull, which is a bigger species (Dunning, 2008). These interactions principally occurred by juvenile birds

during the non-reproductive period, possibly when food was limited (Oro, 1996). The role of refuse on these kinds of interactions lacks proper measurements however, suggesting that this is an area that requires further investigation.

Gulls are also one of the main threats to airport security and the most common of the birds identified in air strikes (Sodhi, 2002). When airports are geographically close to refuse dumps, those gull species that use these landscapes could be a potential hazard to airport security (Chilvers et al., 1997b) because their flight courses could interfere with aircraft routes during landing or take off (Burger, 1983). In several cities worldwide, interactions between gulls and aircraft are aggravated because landfills are often close to airports, as well as the reproductive colonies (Belant et al., 1993; Buckley and McCarthy, 1994; Chilvers et al., 1997a; Dolbeer et al., 1997; Dolbeer et al., 1990; Lisnizer et al., 2015; Yorio et al., 1998); thus the close proximity of all three increase hazards. Gulls are attracted to airports because they find suitable habitat (open and flat), food, and fresh water (Brown et al., 2001). Additionally, gulls might be habituated to noise and traffic, which could also contribute to reduce the presence of predators (Burger, 1983). Closing or management of refuse dumps and landfills have been reported to reduce the numbers of gulls at airports. For instance, among other management practices, a reduction of household refuse near Sydney airport (Australia) reduced the number of bird strikes of Silver Gulls, (Van Tets, 1969). On the other hand, in Ontario (Canada) the incinerator near the airport was replaced by a sanitary landfill, which have brought more than 2,000 Herring Gulls to the area of the airport (Thomas, 1972). Later, the dump was closed and the number of gulls in the area decreased to previous levels. Thus, the presence of refuse near airports could be an important factor that increase the risk to airport security.

In summary, we found that refuse directly and indirectly impacts several aspects of gulls' ecology and behavior, with important implications for other species, human health, water quality, and airport security (Figure 2.2).

2.4 Conclusions

Refuse produces a variety of direct and indirect impacts on gulls (Figure 2.2). We found that positive impacts were more frequently detected in body size, fecundity, reproductive success, and population dynamics. However, some investigations have reported mixed or negative impacts on these parameters. These contradictory observations highlight the need to

further address these topics and find the mechanisms that drive the positive and negative impacts. Additionally, other direct impacts such as modifications in chick's diet, movement patterns, and pathogen infection were detected, which might impact positively on reproductive success, and negatively on foraging effort and survival of populations, respectively.

Indirect impacts of refuse were negative for other species, the quality of water bodies, and airport security. These significant effects are the last links of the chain of impacts that refuse produce on gulls and the environment. These impacts are less frequently addressed in the literature, which suggest the need to investigate them, because the high risk of poisoning and bacterial infection might place refuse as an ecological trap (Battin, 2004). Thus, to establish a link between refuse with impacted sympatric species could be useful to improve the management of those species with conservation challenges and/or protected areas. Additionally, water quality and bird strikes in airports are a research topic that deserve more attention because they are directly related to human health and security, respectively.

Production of waste is projected to increase in the following years (Hoornweg et al., 2013). Therefore, humanity needs to establish modern and adequate waste management practices in order to prevent future conflicts between humans and nature. The array of direct and indirect impacts of refuse in gulls need to be addressed by more research that establish a) whether refuse is being used when natural food is scarce, or it is regularly used independently of the variability of natural food, b) the mechanisms responsible for the positive and negative impacts of refuse on fecundity, reproductive success, body size, chick growth, and population size, c) the contribution of refuse and the mechanisms for the pathogen contamination of water bodies, vertebrates, and humans, and d) the contribution of sources of refuse to population parameters of reproductive colonies that are close to airports. Given the current increase of waste production and the lack of management actions, especially in the southern hemisphere, we need to better understand the impacts of refuse of animal populations and human induced ecosystems in order to improve decision and policy making processes.

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Table 2.1. Species studied by the articles reviewed, and the number of studies and proportion that address each of them.

Species	Number of studies	Proportion (%)
<i>Larus argentatus</i>	51	36
<i>Larus canus</i>	7	5
<i>Larus ridibundus</i>	12	9
<i>Larus delawarensis</i>	11	8
<i>Larus fuscus</i>	10	7
<i>Larus marinus</i>	11	8
<i>Larus atricilla</i>	7	5
<i>Larus audouinii</i>	5	4
<i>Larus cachinanns</i>	6	4
<i>Larus californicus</i>	3	2
<i>Larus glaucescens</i>	3	2
<i>Larus dominicanus</i>	21	15
<i>Larus maculipennis</i>	2	1
<i>Larus cirrocephalus</i>	1	1
<i>Larus hyperboreus</i>	2	1
<i>Larus michahellis</i>	12	9
<i>Larus novaehollandiae</i>	5	4
<i>Larus pacificus</i>	2	1
<i>Larus philadelphia</i>	1	1
<i>Larus schistisagus</i>	2	1
<i>Pagophila ebrunea</i>	1	1
<i>Rhodostethia rosea</i>	1	1

Box. Dumping and landfilling of refuse, some definitions

Refuse dumps are open cavities in the earth where trash is disposed without any pre-classification or treatment, and no management actions are performed. Thus, all types of wastes are disposed in dumps, like municipal solid waste and hazardous materials. The bottom of open dumps is not waterproofed, so leachates are filtered to the soils polluting it as well as underground water reservoirs.

Landfills are well engineered cavity in the earth that receives non-hazardous wastes only. It is covered daily with an impermeable layer in the bottom that prevents leachates to drain into the soil, which are collected and treated. Methane gas is also collected and can be used to produce electricity or is burnt converting it to CO_2 and water. This prevents it to be released to the atmosphere as it is a greenhouse gas more powerful than CO_2 .

Figure 2.1. Difference between dumps and landfills.

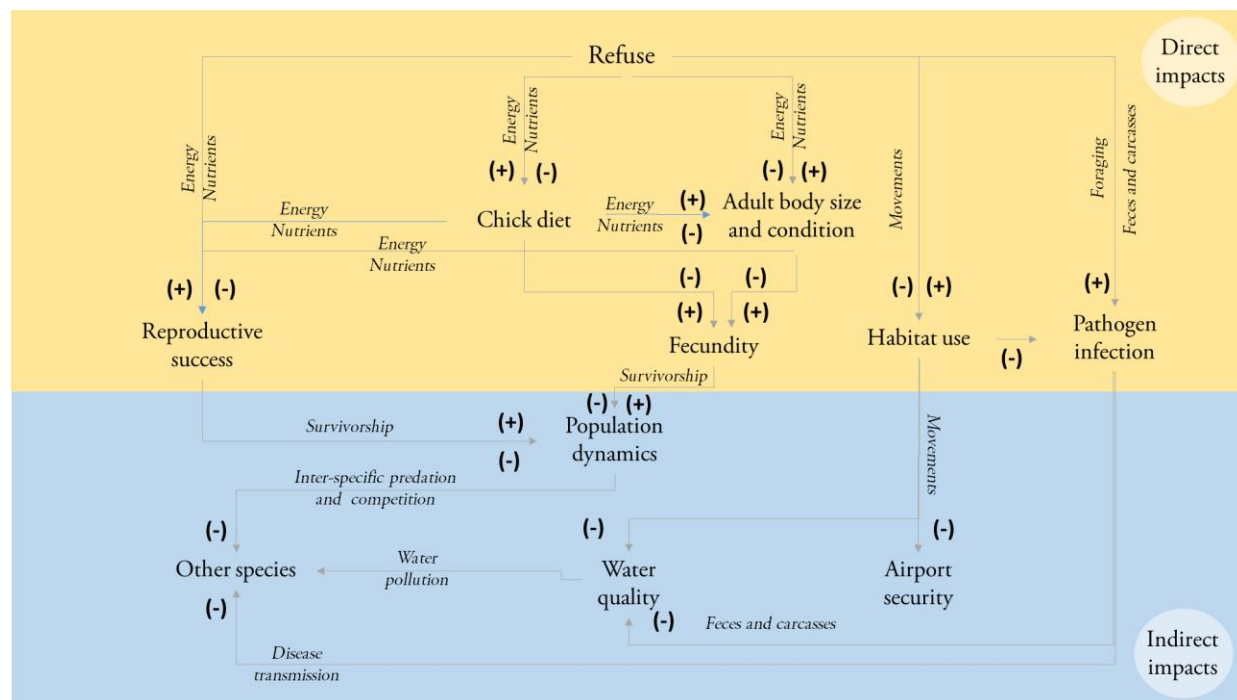


Figure 2.2. Scheme of the direct (orange) and indirect (light blue) impacts, found in the reviewed literature, of refuse and their positive (+) or negative (-) effects on gulls, the environment, and human activities. In italics are represented the mechanism responsible for the impacts produced

2.6 Supplementary Material

Articles analyzed for this review and the topics that they address.

Reference	#	Species	Source of refuse	Level of biological organization	Impacts on Fecundity (+, -, 0)	Impacts on rep success (+, -, 0)	Impacts on pop growth	Impacts on movement patterns	Pathogen Infection	Wat qual	Impacts on other species	Chick's diet	Habitat use	Airport security	Other impacts	Method	Obs.
Ramos et al 2009a	1	Larus michahellis	Dumps	Colony												Pellets	Relative to chick age
Ramos et al 2009b	2	Larus michahellis	Dumps	Colony												Both	
Weiser & Powell 2010	3	Larus hyperboreus	Landfill	Colony		+										Pellets	
Weiser & Powell 2011	4	Larus hyperboreus	Landfill	Colony												Pellets	Before and after incineration
Abdennadher et al 2014	5	Larus michahellis	Dumps	Colony												Both	
Annet & Pierotti 1999	6	Larus occidentalis	Dumps, picnic areas, or intertidal zones	Individual	-	-										Pellets	
Annet & Pierotti 1989	7	Larus occidentalis		Individual/colony													Diet switching.
Arizaga et al 2013	8	Larus michaellis	Dumps	Colony												SIA	Diet variability
Arizaga et al 2014	9	Larus michaellis	Landfill	Individual				Yes									
Auman et al 2008	10	Larus novaehollandiae	Garbage dumps, cans, recreational areas, restaurants and fast food outlets	Colony											mass, size and body cond.		Non-urbanized vs urbanized and temporal variation

Reference	#	Species	Source of refuse	Level of biological organization	Impacts on Fecundity (+, -, 0)	Impacts on rep success (+, -, 0)	Impacts on pop growth	Impacts on movement patterns	Pathogen Infection	Wat qual	Impacts on other species	Chick's diet	Habitat use	Airport security	Other impacts	Method	Obs.
Auman et al 2011	11	Larus novaehollandiae	Garbage dumps, cans, recreational areas, restaurants and fast food outlets	Colony												Both	Non-urbanized vs urbanized and temporal variation
Belant et al 1993	12	Larus argentatus	Landfill	Individual/Colony		-		Yes								Pellets	Temporal variation
Belant et al 1998	13	Larus argentatus, L. delawarensis	Landfill	Colony				Yes								Pellets	Temporal variation
Belant et al 2013	14	Larus argentatus	Landfill	Landscape				Yes						Yes			
Bertellotti & Yorio 1999	15	Larus dominicanus	Landfill	Colony												Pellets	Temporal and spatial variation, distance to landfill
Bertellotti et al 2001	16	Larus dominicanus	Landfill	Colony				Yes									
Blight et al 2015	17	Larus glaucescens	-	Population	-	-	+,-										Unclear link between refuse and population parameters
Bosch et al 1994	18	Larus cachinnans	Landfill	Colony			+									Pellets	
Braune 1987	19	Larus philadelphia		Colony												Stomach content	Consumption of plastic
Brousseau et al 1996	20	Larus delawarens	Landfill, poultry farms	Colony								Yes				Pellets, regurgitates	Variation of chick's diet

Reference	#	Species	Source of refuse	Level of biological organization	Impacts on Fecundity (+, -, 0)	Impacts on reproduction success (+, -, 0)	Impacts on population growth	Impacts on movement patterns	Pathogen Infection	Water quality	Chick's diet	Habitat use	Airport security	Impacts on other species	Other impacts	Method	Obs.
Brown & Ewins 1996	21	Larus delawarensis	Landfill	Colony							Yes					Regurgitations, Pellets	Chicks and adult diets
Buckacinska et al 1996	22	Larus argentatus		Individual/Colony		0										Pellets	Breeding pairs
Burger & Goschfeld 1983	23	Larus atricilla	Landfill	Individual											Age-related foraging success	Obs.	
Burger 1988	24	Larus delawarensis, L. canus, L. argentatus, L. marinus, L. glaucescens, L. atricilla, L. ridibundus	Dump, sewage offal	Species												Obs.	Habitat use, foraging behavior
Chilvers et al 1997	25	Larus sp.	Dump										Yes			Obs.	Refuse dump close to airport
Chudzik et al 1994	26	Larus delawarensis, L. argentatus		Species		0					Yes						
Coulson & Coulson 1993	27	Larus pacificus, L. dominicanus		Species												Pellets	Compares diets
Coulson & Coulson 1998	28	Larus pacificus, L. dominicanus, L. novaehollandiae	Landfill	Species			+							Reduction of L. novaehollandiae		Obs.	
Coulson & Coulson 2008	29	Larus fuscus	Landfill	Species												Pellets	
Coulson et al 1983	30	Larus argentatus	Landfill	Species					Salmonella					Cattle		Obs.	

Reference	#	Species	Source of refuse	Level of biological organization	Impacts on Fecundity (+, -, 0)	Impacts on rep success (+, -, 0)	Impacts on pop growth	Impacts on movement patterns	Pathogen Infection	Wat qual	Chick's diet	Habitat use	Airport security	Impact s on other species	Other impacts	Method	Obs.
Coulson 2015	31	Larus argentatus	Landfill	Species	0	0	-		Botulism						Culling	Review	
Coulson et al 1987	32	Larus argentatus	Landfill	Species												Mark / recapt.	Activity patterns
Coulter 1975	33	Larus occidentalis		Species				Yes								Mark / recapt.	
Davis et al 2015	34	Larus argentatus	Landfill	Colony												Pellets	Spatial and temporal variation in diet
Divoky 1976	35	Pagophila ebrunea, Rhodostethia rosea		Species												Stomach content	13 and 7 stomachs
Dosch 1997	36	Larus atricilla	Towns, tourist sites, landfills	Colony												Regurgitates	Temporal variability
Drury 1973	37	Larus argentatus. L. marinus	Dumps	Species			+									Review	
Duhem et al 2003a	38	Larus michahellis	Landfill	Colony												Pellet	Spatial and temporal patterns in diet of adults
Duhem et al 2003b	39	Larus michahellis	Landfill	Colony												Pellet	Spatial patterns in diet of adults
Duhem et al 2005	40	Larus michahellis	Landfill	Colony							Yes					Regurgitates	Spatial patterns in diet of chicks
Duhem et al 2008	41	Larus michahellis	Landfill	Colony			+										
Ewins et al 1994	42	Larus argentatus		Colony													Spatial and temporal variation in diet
Fordham 1968	43	Larus dominicanus	Dump	Population								Yes				Obs. Mark / recapt.	Spatial and temporal variation in diet

Reference	#	Species	Source of refuse	Level of biological organization	Impacts on Fecundity (+, -, 0)	Impacts on rep success (+, -, 0)	Impacts on pop growth	Impacts on movement patterns	Pathogen Infection	Wat qual	Chick's diet	Habitat use	Airport security	Impacts on other species	Other impacts	Method	Obs.
Fordham 1970	44	Larus dominicanus	Dump	Population		+											
Giaccardi & Yorio 2004	45	Larus dominicanus	Landfill	Species								Yes				Obs.	
Giaccardi et al 1997	46	Larus dominicanus	Landfill	Species								Yes					Temporal patterns
Gilliard et al 2004	47	Larus marinus		Species		0											
Greenhalgh 1952	48	Larus californicus	Urban dumps	Species												Stomach content	
Gwiazda et al 2011	49	Larus cachinnans, L. argentatus		Species												Pellet	Analyzes fish content
Pierotti 1987	50	Larus argentatus		Colony											Time budgets	Obs.	Studies in Avian Biol.
Hebert et al 1999	51	Larus argentatus	Landfill	Population/ Species												Pellet, SIA	Spatial and temporal variability in diet
Hillstrom et al 1994	52	Larus argentatus		Colony		0									Foraging frequency	Obs.	
Hunt & Hunt 1976	53	Larus occidentalis		Colony												Regurgitation	Diet
Hunt 1972	54	Larus argentatus	Landfill, recreation	Colony	0	+										Regurgitation	
Jehl et al 1983	55	Larus californicus		Species											Sex differences	Stomach content, Regurgitates	
Haycock & Threlfall 1975	56	Larus argentatus	Dump														
Kilpi & Ost 1998	57	Larus argentatus	Dump	Colony	+	+										Closure of dump	
Kim & Monaghan 2006	58	Larus argentatus, L. fuscus	Landfill	Colony					Yes							Pellets	Diet and reproduction separately

Reference	#	Species	Source of refuse	Level of biological organization	Impacts on Fecundity (+, -, 0)	Impacts on rep success (+, -, 0)	Impacts on pop growth	Impacts on movement patterns	Pathogen Infection	Wat qual	Chick's diet	Habitat use	Airport security	Impacts on other species	Other impacts	Method	Obs.
Knoff et al 2002	59	Larus atricilla		Colony												Pellets and SIA	Spatial and temporal variation in diet
Kubetzki & Garthe 2003	60	Larus argentatus, L. ridibundus, L. canus, L. fuscus		Species												Pellets	Niche overlap
Kubetzki & Garthe 2007	61	Larus canus	Canteen clients	Colony												Pellets	Urban colonies
Kubetzki et al 1999	62	Larus canus		Colony												Pellets, feces	
Ludynia et al 2005	63	Larus dominicanus	Landfill	Colony												Pellet	Spatial and temporal variation in diet
Butterfield et al 1983	64	Larus argentatus	Landfill	Species					Salmonella								% infection
Monaghan et al 1985	65	Larus argentatus	Landfill	Species					Salmonella								% infection
Moreno et al 2010	66	Larus michahellis	Dump	Colony							Yes					Pellets, SIA	Spatial variation in diet
Neves et al 2006	67	Larus michahellis		Colony							Yes						
Osterback et al 2015	68	Larus occidentalis	Landfill	Species													Retrospective stable isotope analysis
Pedrocchi et al 2002	69	Larus audouinii	Landfill	Colony							Yes					Regurgitations	Spatial variation in diet
Pedrocchi et al 1996	70	Larus audouinii	Landfill	Colony							Yes					Regurgitations	Temporal variation in diet

Reference	#	Species	Source of refuse	Level of biological organization	Impacts on Fecundity (+, -, 0)	Impacts on rep success (+, -, 0)	Impacts on pop growth	Impacts on movement patterns	Pathogen Infection	Wat qual	Chick's diet	Habitat use	Airport security	Impacts on other species	Other impacts	Method	Obs.
Petracci et al 2004	71	Larus dominicanus	Landfill, poultry farms	Colony												Pellets, regurgitations	Observational study
Pierotti & Annett 1991	72	Larus argentatus		Individual	-	-										Pellets, regurgitations, focal observation	
Pierotti & Annett 2001	73	Larus occidentalis		Colony	0	-		Yes			Yes					Focal observation, chick rearing experiments	Also, spatial and temporal variation in diet. ENSO effects on diet and foraging trips.
Pons & Migot 1995	74	Larus argentatus	Landfill	Colony	+	+	+								Adult survival (0), Body weight (-),		Closure of landfill, mark-capture-recapture
Pons 1992	75	Larus argentatus	Landfill		+	+									Breeding population	Regurgitations	Closure of landfill
Garthe et al 2003	76	Larus argentatus		Colony													Temporal variation in diet
Rail & Chapdelaine 2000	77	Larus argentatus		Colony													
Sibly & McCleery 1983a	78	Larus argentatus	Landfill	Colony												Obs.	Sex diff, temporal variation in diet
Sibly & McCleery 1983b	79	Larus argentatus	Landfill	Colony												Obs.	Temporal variation in diet
Silva et al 2000	80	Larus dominicanus	Landfill,	Species												Pellets	Spatial variation in diet

Reference	#	Species	Source of refuse	Level of biological organization	Impacts on Fecundity (+, -, 0)	Impacts on rep success (+, -, 0)	Impacts on pop growth	Impacts on movement patterns	Pathogen Infection	Wat qual	Chick's diet	Habitat use	Airport security	Impacts on other species	Other impacts	Method	Obs.
Skorka et al 2005	81	Larus cachinnans	Dump	Colony			+							Displacement of Larus ridibundus and Sterna hirundo			Spatial and temporal patterns in diet
Sol et al 1993	82	Larus cachinnans	Landfill	Species												Counts	Temporal use of landfill
Spaans 1971	83	Larus argentatus	Landfill	Species												Counts	Spatial and temporal use of landfill
Steele & Hockey	84	Larus dominicanus	Landfill	Species											Kleptoparasitism	Obs.	
Steele 1992	85	Larus dominicanus, L. hartlaubii	Landfill	Species												Pellets, obs., remains, stomach	Spatial and temporal variation in diet
Steigerwald et al 2015	86	Larus michahellis	Landfill	Colony	+										Body mass (+)	Regurgitation	Landfill closure
Verbeek 1979	87	Larus marinus		Colony												Regurgitations, food remains	Observational study
Vermeer 1982	88	Larus glaucescens	Dumps	Colony												Pellets and regurgitations	Spatial variation in diet
Washburn 2012	89	Laridae	Transfer stations	Species												Obs.	Temporal and spatial use of transfer stations
Washburn 2013	90	Larus atricilla, L. argentatus, L. marinus y L. delawarensis		Species												Stomach contents from culled individuals	Temporal variation, sex differences, and chick growth variation in diet

Reference	#	Species	Source of refuse	Level of biological organization	Impacts on Fecundity (+, -, 0)	Impacts on rep success (+, -, 0)	Impacts on pop growth	Impacts on movement patterns	Pathogen Infection	Wat qual	Chick's diet	Habitat use	Airport security	Impacts on other species	Other impacts	Method	Obs.
Watanuki	91	Larus schistisagus		Individual												Obs, regurgitations	
Welham 1987	92	Larus delawarensis	Dumps	Species												Stomach content, regurgitations	Spatial and temporal patterns in diet
Whelan et al 1988	93	Larus argentatus	Dump	Species					Campylobacter sp.					Humans		Cloacal swab, live and culled gulls	
Witteveen et al 2016	94	Larus dominicanus	Landfill	Colony											Debris in nests		
Wrege et al 2001	95	Larus californicus		Colony											Sex differences	Focal obs.	
Zelenskaya 2014	96	Larus schistisagus		Colony													Temporal patterns in diet
Yorio et al 1996	97	Larus dominicanus	Refuse dumps	Species												Obs.	Use of refuse dumps
Frere et al 2000	98	Larus dominicanus	Fishery tip	Species					Escherichia, Proteus, Citrobacter, Salmonella, Hafnia, Shigella, Enterobacter and Yersinia						Sex and age class differences	Culling, cloacal swab	
Marateo et al 2013	99	Larus dominicanus, L. maculipennis, L. cirrocephalus	Dumps and landfills	Species												Count	Spatial and temporal use of dumps and landfills
Raines 2010	100	Larus dominicanus		Species					Pasteurella multocida							Review	Diseases in Antarctica

Reference	#	Species	Source of refuse	Level of biological organization	Impacts on Fecundity (+, -, 0)	Impacts on rep success (+, -, 0)	Impacts on pop growth	Impacts on movement patterns	Pathogen Infection	Wat qual	Chick's diet	Habitat use	Airport security	Impacts on other species	Other impacts	Method	Obs.
Yorio & Giaccardi 2002	101	Larus dominicanus, L maculipennis	Dump	Species												Count	Spatial and temporal patterns in dump use
Harris 1965	102	Larus argentatus, L. marinus		Species												Regurgitations, stomach content	Spatial and temporal variation in diet
McClerry & Sibly 1986	103	Larus argentatus	Tip	Species												Obs.	Temporal use of foraging habitats
Smith & Carlile 1993	104	Larus novaehollandiae	Landfill	Colony											Movement patterns, chick growth	Regurgitates, obs, tagging	
Greig et al 1983	105	Larus argentatus	Landfill												Immature birds	Obs. Video recordings	
Girdwood et al 1985	106	Larus argentatus, L. fuscus	Landfill	Species					Salmonella	Yes							Spatial and temporal patterns of infection
Kihlman & Larson 1974	107	Larus argentatus	Landfill	Species												Counts	Temporal patterns in habitat use
Gotmark 1984	108	Larus marinus, L. argentatus, L. fuscus, L. ridibundus	Landfill	Species												Review	
Quessy & Messier 1992	109	Larus delawarensis	Dump	Species					Salmonella, Campylobacter, Listeria monocytogenes								

Reference	#	Species	Source of refuse	Level of biological organization	Impacts on Fecundity (+, -, 0)	Impacts on rep success (+, -, 0)	Impacts on pop growth	Impacts on movement patterns	Pathogen Infection	Wat qual	Chick's diet	Habitat use	Airport security	Impacts on other species	Other impacts	Method	Obs.
Bellebaum 2005	110	Larus ridibundus, L. argentatus	Dump	Species												Obs.	Effect of bulldozers on foraging
Smith et al 1993	111	Larus argentatus, L. ridibundus	Dump	Species					Cryptosporidium								Temporal and spatial variation in infection
Andersson 1970	112	Larus argentatus	Dump	Colony												Pellets	Temporal variation in diet
Mudge & Ferns 1982	113	Larus argentatus, L. canus, L. marinus, L. canus, L. ridibundus	Tips	Species							Yes						Temporal and spatial variation in diet
Skorka & Wojcik 2008	114	Larus cachinnans	Dump	Species										Larus ridibundus	Kleptoparasitism	Obs.	Foraging behavior
Horton et al 1983	115	Larus ridibundus, L. fuscus, L. canus, L. argentatus, L. marinus	Tip	Species												Obs., ringing.	Temporal variation in tip use
Blanco & Marchamalo 1999	116	Larus audouinii, L. ridibundus, L. cachinnans, L. fuscus	Dump	Species										Larus ridibundus	Kleptoparasitism		Spatial and temporal variation in dump use
Ceia et al 2014	117	Larus michahaellis		Colony												Pellet, SIA, GPS	Temporal variation in foraging behavior
Caron-Beaudoin et al 2013	118	Larus delawarensis		Colony												Gastrointestinal tracts, SIA, GPS	Feeding ecology
Maciusik et al 2010	119	Larus ridibundus	Urban habitat	Species												Surveys	Urban habitat use

Reference	#	Species	Source of refuse	Level of biological organization	Impacts on Fecundity (+, -, 0)	Impacts on rep success (+, -, 0)	Impacts on pop growth	Impacts on movement patterns	Pathogen Infection	Wat qual	Chick's diet	Habitat use	Airport security	Impacts on other species	Other impacts	Method	Obs.
Camphuysen et al 2011	120	Larus argentatus	Tips	Species				Yes								Capture-mark-recapture	Habitat use
Gould & Fletcher 1978	121	Larus marinus, L. fuscus, L. argentatus, L. canus, L. ridibundus							Total coliforms, faecal coliforms, faecal streptococci, Clostridium perfringens	Yes						Experiment	
Lisnizer et al 2011	122	Larus dominicanus	Tips	Species			+										
Fenlon 1981	123	Larus sp., humans, cattle, sheep	Dump	Species					Salmonella							Cloacal sampling	
Anza et al 2014	124	Laridae	Landfill	Species					E. coli, Salmonella, Clostridium,	Yes						Cloacal sampling	Also in other matrices, and correlated with environmental parameters.
Lloyd et al 1976	125	Laridae	Refuse tips	Species					Clostridium botulinum							Obs. and review	Outbreaks report
Macdonald et al (1978)	126	Larus argentatus, L. ridibundus, L. L. fuscus	Refuse tip	Species					Clostridium botulinum							Dead birds	Experiments with inoculation of mice
Neimanis et al 2007	127	Larus argentatus	Landfill	Species					Clostridium botulinum								Experiments with inoculation of mice
Ortiz & Smith 1994	128	Laridae	Landfill	Species					Clostridium botulinum							Sampling of soil, waste,	
Sutcliffe 1986	129	Larus argentatus, L. fuscus	Landfill	Species												No experimental or sampling approach	

Reference	#	Species	Source of refuse	Level of biological organization	Impacts on Fecundity (+, -, 0)	Impacts on rep success (+, -, 0)	Impacts on pop growth	Impacts on movement patterns	Pathogen Infection	Wat qual	Chick's diet	Habitat use	Airport security	Impacts on other species	Other impacts	Method	Obs.
Converse et al 2012	130	Larus argentatus, L. delawarensis		Species					Several pathogens								
Brown et al 2001	131	Larus atricilla	Landfill	Species									Yes				
Burger 1983	132	Laridae	Landfill	Species									Yes				
Burger 1985	133	Laridae	Landfill	Species									Yes				
Dolbeer et al 1989	134	Larus atricilla	Landfill	Species									Yes				
Giroux et al 2016	135	Larus delawarensis	Landfill	Colony			+										
Veitch et al 2016	136	Larus marinus	Landfill	Colony							Yes			Predation of Uria aalgae, Rissa tridactyla. Kleptoparasitism on Fretecula arctica			
Bond 2016	137	Larus argentatus															
Navarro et al 2017	138	Larus michahellis	Dump	Colony								Yes					
Frixione et al 2012	139	Larus dominicanus	Landfill	Colony													Freshwater colony
Roscales et	140	Larus audouinii, L. michahellis	Dump	Colony											POPs	SIA-POP analysis	POPs-refuse dumps

3. ANTHROPOGENIC FOOD SUBSIDIES AFFECT THE ISOTOPIC NICHE OF A GENERALIST SEABIRD DURING NESTLING GROWTH

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3.1 Abstract

Anthropogenic food subsidies, such as refuse, are an important driver of global change. Gulls are an important group of animals that forage on this subsidy. The increasing gull population is negatively affecting species with challenging conservation issues, their growing abundances are a known threat to airport security, and their prevalence in urban and agricultural landscapes make them a potential health problem for humans, cattle, and ultimately affect water quality in many areas. Foraging on refuse during the chick rearing period could affect the acquisition of resources and the ecological niche of the chicks with potential consequences for the increase of their populations. Using conventional diet analysis and stable isotopes of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of blood and feathers of Kelp Gull (*Larus dominicanus*) chicks, we studied the variation of the isotopic niche during chick growth in response to foraging on refuse on a reproductive colony in the Rio de la Plata Estuary in Uruguay. Using Bayesian mixing models on isotopic data, we estimated the proportion and variation of natural food and refuse in the diet of nestlings. Then, we modelled the isotopic niche (as mean posterior densities) and its width (measured as standard deviation) with nestling morphometric measurements of different ages. We found that

anthropogenic food subsidies in the form of refuse were ingested and assimilated by Kelp Gull nestlings during the chick rearing period. The amount of refuse incorporated into nestling tissues and the isotopic niche width increased with chick's age. We argue that parents use more isotopically unique food sources during chick growth thereby increasing isotopic diversity. This study points out the need to improve the current waste management system, which is being reviewed in Uruguay. We believe that decision makers should consider the results of this study, which show that animal populations are adversely affected by refuse.

Key words: refuse, human impacts, global change, seabirds, stable isotopes, forging ecology.

3.2. Introduction

One of the least studied drivers of global change is anthropogenic food subsidies -- those food sources derived from human activities that become available to animals (Leroux and Loreau, 2008; Polis et al., 1997). Anthropogenic food subsidies are exponentially growing (Hoornweg and Bhada-Tata, 2012) and are becoming available for coastal animals in the form of fishing discards and offal in marine ecosystems and refuse in terrestrial and coastal ecosystems (Oro et al., 2013; Plaza and Lambertucci, 2017). While fishing discards are used by many birds, such as Charadriiformes, Procellariiformes, Sphenisciformes, and Pelecaniformes, refuse is exploited by lesser seabird taxa, where gulls emerge as the most significant group (Oro et al., 2013; Plaza and Lambertucci, 2017). Unlike other seabirds, gulls are usually generalist feeders, able to exploit a wide variety of food sources while employing a range of foraging strategies (Annett and Pierotti, 1999; Burger, 1988; Hand et al., 1987; Pierotti and Annett, 2001).

Considering the gull's plasticity in the use of food resources, the availability of anthropogenic food subsidies might have numerous socio-ecological implications. It has been observed that some species tend to increase their population sizes as a consequence of feeding on refuse (Belant et al., 1993; Coulson and Coulson, 1998; Duhem et al., 2005; Pons, 1992; Pons and Migot, 1995; Weiser and Powell, 2010). This phenomenon is of environmental concern because some gull species are predators of eggs and chicks of other bird species, some of them with declining population sizes and with considerable conservation challenges (e.g., Emslie et al., 1995; Hernández and Ruiz, 2003; Lenzi et al., 2010; Martinez-Abraín et al., 2003; O'Connell

and Beck, 2003; Voorbergen et al., 2012; Yorio et al., 1998; Yorio and Quintana, 1997). Likewise, in South America, gulls feed on skin and blubber of southern right whales (*Eubalena australis*), particularly on mother-calf pairs, which reduce calf health and survivorship (Groch, 2001; Rowntree et al., 1998; Sironi et al., 2009). Additionally, potential health and sanitary conflicts have been discovered because gulls are vectors of bacteria toxins for humans and cattle; these include *Clostridium botulinum*, *Escherichia* sp., and *Salmonella* sp. (Brand et al., 1983; Frere et al., 2000; Ortiz and Smith, 1994; Yorio et al., 1996). The most common bird that strikes aircraft are gulls and so they also pose a safety hazard for people traveling in planes (Sodhi, 2002). Hence, rising gull populations as they are driven by increasing refuse has numerous implications for society.

One critical phase of the life cycle through which refuse can affect the ecology of gulls is chick growth (Davoren and Burger, 1999). Changes in body size of chicks during this period could affect the acquisition of resources because larger individuals are capable of foraging on large prey (or handle larger size food, like refuse) (Hone and Benton, 2005). If we acknowledge that refuse, for instance chicken bones, could be larger and harder to manipulate and ingest than a natural food, like small fishes, we could expect that nestlings will incorporate a larger proportion of refuse to their diets as they grow. Moreover, refuse can modify the ecological niche of the nestlings (Werner and Gilliam, 1984) between two scenarios: diversifying the niche by increasing alternative prey types (Ceia et al., 2014), or by reducing the diversity of prey types through an increase in individual specialization (Annett and Pierotti, 1989). If refuse contributes to the expansion of the ecological niche, then we should expect an increase in the variability of refuse in the diet. On the other hand, if refuse shrinks the niche we should expect a reduction in variability of refuse in the diet of individual nestlings (Bearhop et al., 2004). These potential niche shifts should be particularly prominent during nestlings growth, because parents should follow the feeding strategy that maximizes their survivorship to ensure reproductive success (Ricklefs, 1990).

A powerful approach to the study potential consequences of refuse on animal trophic ecology is the use of the “isotopic niche” concept. The isotopic niche is a subset of the hypervolume proposed by Hutchinson (1957) reflecting aspects of trophic niche of species, where the number of dimensions is determined by the number of stable isotopes employed (Bearhop et al., 2004; Newsome et al., 2007). The isotopic niche concept is based on the

assumption that stable isotopes in the proteins of the consumers reflect those in their prey in a predictable way (Peterson and Fry, 1987; Thompson et al., 1999). In recent years it became an effective instrument to investigate the ecological niche of animals (e.g., Cherel et al., 2010; Rader et al., 2017).

The most frequently employed stable isotope ratios in trophic ecology are nitrogen (^{15}N / ^{14}N) and carbon (^{13}C / ^{12}C), respectively. Nitrogen isotopic signature ($\delta^{15}\text{N}$) of the proteins of consumers is enriched in 0.4-4.3 ‰ relative to their dietary source and is a good indicator of trophic level of the species (Hobson et al., 1994; Kurle et al., 2014; Newsome et al., 2007; Vanderkluft and Ponsard, 2003). On the other hand, the carbon isotopic signature ($\delta^{13}\text{C}$) is fractionated from prey to consumer tissues at a rate from 0.1 to 4.1 ‰, but generally they are between 0-1 ‰ (Hertz et al., 2016; Kurle et al., 2014). Those species which discrimination factors are small, no large differences from basal energy sources to final consumers in $\delta^{13}\text{C}$ are expected. Hence, in this situation small values of discrimination factors make carbon ideal to assess the principal basal food sources of a trophic network (e.g., Fry, 2006; Hertz et al., 2016). Moreover, we can infer marine or terrestrial food diets because marine ecosystems are more enriched in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in relation to terrestrial ecosystems (Peterson and Fry, 1987). Additionally, based on the distribution of $\delta^{13}\text{C}$ in marine ecosystems, we can also determine latitudinal variation in diets, as well as pelagic or benthic sources of $\delta^{13}\text{C}$ (Goericke and Fry, 1994; Hobson et al., 1994; Hobson and Sealy, 1991).

During the past decade, the application of the isotopic niche concept has been applied to a variety of seabird species and research topics. For instance, analyzes of trophic segregation, and spatial and temporal variation in the isotopic niche has been carried out in more than 30 seabird species worldwide (e.g., Cherel, 2008; Cherel et al., 2013; Fort et al., 2010; Hinke et al., 2015; Navarro et al., 2013). In addition, niche segregation between age classes and different time scales has also been assessed (Campioni et al., 2015; Ceia et al., 2012; Votier et al., 2011; Weimerskirch et al., 2014). However, there is very limited evidence addressing the isotopic niche of generalist and opportunistic seabirds and its variation when foraging on anthropogenic food sources like refuse (Ceia et al., 2014).

One species with opportunistic and generalist trophic behavior is the Kelp Gull (*Larus dominicanus*) (Coulson and Coulson, 1993; Yorio et al., 2016). In Uruguay, it breeds on eight colonies along the coast, and almost half of its breeding population is located on the Río de la

Plata Estuary (Yorio et al., 2016). This estuary is formed by the discharge of the Paraná and Uruguay rivers into the Atlantic Ocean in South America between Argentina and Uruguay. Diverse economic activities are developed in this estuary where 12 million people live. Household and recreational activities, as well as industries (fisheries, slaughterhouses, tanneries, crop mills, among others), generate a great amount of refuse available for the Kelp Gull to forage. Previous evidence based on pellet analysis has shown that refuse is present on the diet of this species in this estuary (Burgues, 2015; Lenzi et al., 2016; Petracci et al., 2004; Silva et al., 2000). Nonetheless, conventional dietary analyses have limitations, like bias towards hard parts when pellet samples are used, which provides short term information on individual diets (Barrett et al., 2007; Karnovsky et al., 2012). Stable isotope analysis overcomes these limitations offering a larger window of information on feeding habits, and an estimation of assimilated nutrients, not only the ingested ones, allowing us to reconstruct the chemical structure of the trophic network (Fry, 1991; Hobson and Clark, 1992a; Minagawa and Wada, 1984). An important disadvantage of stable isotope analysis is the inability to resolve prey taxonomy, however when combined with conventional dietary methodologies, it becomes a powerful integrative approach to study trophic niche variation (Bearhop et al., 2004).

We combined conventional dietary analysis with measurements of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of gull tissues (blood and feathers) and those of their prey to study the variation in the isotopic niche of Kelp Gull chicks during their growth. Using Bayesian mixing models (Moore and Semmens, 2008; Newsome et al., 2012) we modelled the contribution of natural and refuse sources to the nestlings' cohort in general and to each nestling biomass. Additionally, we evaluated the variation of refuse consumption among nestlings of different ages comparing the mean posterior densities of refuse with nestling's morphometric measurements. We expected that larger nestlings could be able to incorporate more refuse to their diets because they were able to handle larger meals than smaller nestlings that can ingest smaller prey, like small fish. Additionally, we correlate individual isotopic niche width, measured as the standard deviation of posterior densities of refuse (see Bearhop et al., 2004; Yeakel et al., 2016), with a nestling's morphometric measurements to evaluate if along nestling's growth: *a*) diet variability increases because feeding on refuse allows chicks to expand their isotopic niche, *b*) diet variability is reduced because feeding on refuse shrinks the isotopic niche increasing individual specialization, or *c*) feeding on refuse does not affect isotopic niche width during chick growth. These analyses are performed

separately for each tissue, that in case of blood integrates the diet during the past 15-21 days (half-life of 11.4 days), and feathers integrates the diet during the feather formation (Hobson and Clark, 1992a; Hobson and Clark, 1992b). This approach allows us to study changes in isotopic niche from the past days until hatching.

3.3. Materials and methods

3.3.1 Study area

Diet and stable isotope samples for this study were obtained on a Kelp Gull colony on Isla de Flores (34°56'30"S - 55°55'29"W), a coastal island in the Río de la Plata Estuary in Uruguay during the 2017 reproductive season. This island is 1,700 m long by 320 m wide with a surface of 29.6 ha; it is located 12 km from the coastal border of Montevideo and Canelones Departments in the Río de la Plata Estuary (Figure 3.1). This island supports more than 30 species of birds where the Kelp Gull is the most abundant species, especially during the reproductive season (unpubl. data). The Kelp Gull colony of Isla de Flores has been estimated in approximately 5,000 breeding pairs (Yorio et al., 2016).

3.3.2 Nestling's morphometric measurements, diet sampling, and stable isotope analysis

During the chick rearing period, 35 chicks were captured individually by hand or with the aid of a fishing landing net. Each chick was placed in a fabric bag within a box for further processing. Later on, culmen and tarsus length of chicks were measured using a digital caliper to the nearest 0.1 mm. Wing length was also measured with a ruler to the nearest 1 mm. Then, chicks were weighed with a scale to ± 0.1 g. To obtain diet samples, we performed a stomach pump following Wilson (1984). With the aid of a syringe and a catheter we propelled water into the stomach of the chick. Then, the gastric system was emptied applying pressure gently on the abdomen with the bird inverted over a bucket (Wilson, 1984). After the lavage, we weighed each sample using a scale to the nearest 0.1 g, and stored it at -20 °C for stable isotopes analysis. Then, we cut one growing dorsal body feather and stored it in a plastic bag for further processing. Additionally, we collected 0.1 ml of blood from the brachial vein. Each blood sample was scattered on a glass microscope slide, dried in air at room temperature, wrapped in aluminum foil, and stored for laboratory processing, following Bugoni et al. (2008). Each chick was released in the same spot they were captured. Post-release behavior was monitored for three

minutes (every chick showed normal escape behavior, and no injury was recorded during fieldwork activities).

In the laboratory, feathers were rinsed using distilled water. Lipids were not removed because lipids attached to the feathers in general do not alter carbon and nitrogen signatures, and if C:N is greater than 3.5 they can be removed mathematically (English et al., 2018; Kojadinovic et al., 2008; Post, 2002). Blood, feathers, and diet samples were oven dried at 60 °C for 48 hours. Then, dried blood samples were scraped over clean sheets of aluminum foil. Feather tips were finely cut and a sample of prey muscle were also grinded. A sub-sample of 0.5-1.5 mg of each tissue (diet samples, blood, and feathers) was encapsulated for further stable isotope analysis. Nitrogen and carbon isotope ratios were measured by Elemental Analyzer Continuous Flow Isotope Ratio Mass Spectrometry at the Center for Stable Isotopes, University of New Mexico (<http://csi.unm.edu>) using a Costech ECS 4010 Elemental Analyzer coupled to a ThermoFisher Scientific Delta V Advantage Plus mass spectrometer via a CONFLO IV interface. Average analytical precision based on routine analysis of a laboratory standards was better than 0.1‰. Lipids generally are depleted in ^{13}C relative to proteins and carbohydrates that introduces potential biases to the results. Thus, isotopic signatures of consumers and prey with a C:N ratio greater than 3.5 were mathematically lipid-corrected using eq. 3 of Post et al. (2007).

Stable isotope ratios were expressed in conventional notation as parts per thousand according to the following equation:

$$\delta X = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] * 1000$$

where δX is $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ and R_{sample} is the corresponding ratio $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$. R_{standard} is based on the $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$ ratio of a given standard. In the case of $\delta^{13}\text{C}$ the standard is the isotopic signature of Vienna Pee Dee Belemnite (V-PDB). The standard for $\delta^{15}\text{N}$ is the $^{15}\text{N}/^{14}\text{N}$ ratio is air nitrogen.

3.3.3 Stable isotope Bayesian mixing models

Because the use of multiple different basal resources can favor the under-determination of contributions of each resource in mixing models (Fry, 2013), the isotopic signatures of the most important resources were selected when represented more than 30% of the diet. The one

defined as “natural diet” was composed entirely by fish (Sciaenidae), and the “refuse diet” was composed entirely by chicken remains, which together account for the 70% of nestling’s diet (Table 1). According to Burgues (2015) and the first author’s unpublished data, fish and chicken are by far the most important prey items of the Kelp Gull in the Rio de la Plata Estuary.

The modelling approach considered the entire set of blood and feather samples representing the colony level and the individual level, which were considered in different models. Because carbon isotopic signature of refuse was highly variable, probably because chicken is fed with an important variety of carbon sources, we decided to use nitrogen only for the Bayesian mixing models with the two food sources, natural food and refuse (see Results). Additionally, nitrogen separated in a good way both food sources, which is why we decided to use it in our mixing models to estimate proportions of both sources. Thus, we modelled nitrogen signatures of blood and feathers, based on mean and SD of nitrogen signatures of natural food and refuse. We estimated the contribution of natural and refuse diets from samples actually found in chick’s stomach, because selecting only the well-known diet items is essential for models accuracy (Parnell et al., 2010). Priors for the Bayesian mixing models were set as uninformative, following a Dirichlet distribution.

The models also require the trophic fractionation values for $\delta^{15}\text{N}$ of blood and feathers, i.e., the difference of the isotopic signature between predators and prey. Because there are no published fractionation factors of blood and feathers for the Kelp gull, we used discrimination factors from published controlled experiments of other gull species. Blood fractionation factors used for blood was $+3.1 \pm 0.2 \text{ ‰}$ for nitrogen from an experimental study on Ring-billed Gull (*Larus delawarensis*) (Hobson and Clark, 1992b). For feathers, we followed the approach of Ramos et al. (2009b) and Moreno et al. (2010) who averaged fractionation factors from experimental studies on Ring-billed Gull, Black-tailed Gull (*Larus crassirostris*) and Yellow-legged Gull (*Larus michahellis*). Thus, we used a nitrogen fractionation factor of $+3.3 \text{ ‰}$ (Hobson and Clark, 1992b; Mizutani et al., 1992; Ramos et al., 2009b). Standard deviation for feathers was set in 1.0 ‰ (Ceia et al., 2014). To perform the Bayesian mixing models, we used MixSIAR package (Stock and Semmens, 2016) of R v. 3.4.3 (R, Development Core Team 2018).

3.3.4 Variation of the isotopic niche with nestling size

To analyze the variation between natural food and refuse with chick size, we correlated the mean posterior density of refuse, estimated by Bayesian mixing models of blood and feathers of individual chicks with wing length, culmen length, and tarsus length, and weight of chicks using Spearman correlation. For those significant correlations, we fitted linear and non-linear models with the morphometric measurements as dependent variables and the posterior density of refuse as independent variables. This way we examined the shape of the relationship between refuse assimilation and nestling growth measurements. The models employed were the Linear, vonBertalanfy, Logistic, and Gompertz equations. To discern which model best fitted the data we used Akaike Information Criterion (AIC) (Akaike, 1973, 1974). The model with the lowest AIC value was selected as the candidate to best explain the data. If the difference in AIC values between the model with the lowest AIC and the following competing model were less or equal 2 units, both models were selected as candidate models (Johnson and Omland, 2004).

To evaluate diet variability of individual chicks with their body size, we calculated the standard deviation (SD) of one of the two most important food sources estimated from the Bayesian mixing models, in this case SD of refuse. This way we obtained a measure at the individual level of the variability in the isotopic niche in the dietary proportions (p) space (Newsome et al., 2007). Thus, we followed the procedure as with the mean posterior density, using the same model selection approach. To perform model fitting and selection we used PAST software v 3.14 (Hammer et al., 2001).

3.4. Results

Six food categories were found in 23 nestling stomachs represented mostly by fish and chicken that accounted for 70% of the diet. Additionally, insects, animal fat, animal guts, and shrimp were observed as well but in much lower proportion (Table 3.1). Although we only found two samples of animal fat, this was the heaviest item with a mean of $41.75 \text{ g} \pm 2.1$ followed by shrimp, which was collected once (19.60 g).

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures in the blood of nestling were $-19.11 \text{ ‰} \pm 0.47$ and $12.72 \text{ ‰} \pm 0.84$, respectively (Figure 3.2). Isotopic signatures of nestling feathers were $-17.93 \text{ ‰} \pm 0.56$ for $\delta^{13}\text{C}$ and $13.66 \text{ ‰} \pm 1.11$ for $\delta^{15}\text{N}$ (Figure 3.2). Additionally, signatures for natural

food were $-16.98 \text{ ‰} \pm 1.44$ for $\delta^{13}\text{C}$ and $13.91 \text{ ‰} \pm 2.65$ for $\delta^{15}\text{N}$, and for refuse was $-17.37 \text{ ‰} \pm 1.98$ for $\delta^{13}\text{C}$ and $3.44 \text{ ‰} \pm 0.84$ for $\delta^{15}\text{N}$ (Figure 3.2).

Posterior densities estimated from Bayesian mixed models at the colony level showed that in the short (blood) and long (feathers) term, nestlings assimilated more natural food than refuse. Mean posterior densities of blood were for natural food 0.54 ± 0.05 , Bayesian credible intervals - BCI [0.46 - 0.64], and for refuse 0.46 ± 0.05 - BCI [0.36 - 0.54]. In feather tissues, chicks also incorporated natural food with a mean posterior density of 0.55 ± 0.09 , BCI [0.33 - 0.71], while for refuse was 0.45 ± 0.09 , BCI [0.29 - 0.67].

Refuse predicted from Bayesian mixing models for blood, positively correlated with wing and culmen lengths (Table 3.2). On the other hand, refuse from feather tissues (longer time frame) did not correlate with morphometric measurements (Table 3.2). The model selection approach showed that the Logistic Equation was the model that best explained the co-variation between refuse and wing length (Table 3.3). Alternatively, Logistic, vonBertalanfy and Gompertz equations were the models that best described the variation between culmen length and refuse (Table 3, Figures 3.3).

As individuals grow, the standard deviation, estimated from Bayesian mixing models for refuse in blood tissue (short time frame), positively correlated with wing and culmen lengths, although statistically marginal with P-values of 0.06 (Table 3.2). We then proceed to select the best model that describes this variation. As with the mean posterior density, SD of refuse estimated from feathers did not significantly correlate with morphometric measurements (Table 3.2). The best model that described the relationship between SD of refuse, from Bayesian mixing models on blood tissues with wing length and culmen length, was the Logistic Equation (Table 3.4, Figure 3.4).

3.5. Discussion

Fish and chicken were the most important diet items recorded in the stomach of Kelp Gull chicks on Isla de Flores. We did not record beef in the diet which is another important source of refuse for adults in this and other colonies in the Rio de la Plata Estuary of Uruguay (Burgues, 2015; unpubl. data). Additionally, fish was the most important diet item recorded in the diet of Kelp Gull chicks in frequency and weight. Unfortunately, we cannot establish if fish came from fishing discards or from natural foraging.

Larger nestlings tended to be fed with a higher proportion of refuse than smaller nestlings probably because refuse, mostly chicken wings in our study, have larger bones and are more difficult to manipulate and ingest than small fish (Annett and Pierotti, 1989; Spaans, 1971). In addition, availability of fish (e.g., Sciaenidae) could increase during the chick rearing period due to migration and reproductive movements (Jaureguizar et al., 2004) allowing Kelp Gull parents to feed their chicks with this natural source. We tried to avoid this potential confounding factor sampling the chicks in different moments of the breeding season. However, we acknowledge that physiological condition might vary with chick size and potentially affect isotopic signatures. Considering this as another potential confounding factor, we find support to the hypothesis that changes in body size of chicks could affect resource acquisition because larger individuals may be capable to ingest larger prey (Hone and Benton, 2005). This has been observed in the Herring Gull in the Netherlands and Finland, the Yellow-legged Gull and Audouin's Gull in the Mediterranean, and the Ring-billed Gull in Canada, where several investigations have shown, using conventional diet analyses, that younger chicks are fed with small prey, like invertebrates and small fishes, and then when they grow up, larger prey were incorporated to their diets, like large fish or refuse (Brousseau et al., 1996; Hillström et al., 1994; Kirkham and Morris, 1979; Pedrocchi et al., 1996; Ramos et al., 2009a; Spaans, 1971). A similar pattern has been observed in Antarctica, where Kelp Gull chicks incorporated more carrion as they grew older, while fish and krill decreased based on a study using pellet analysis (Favero and Silva, 1998). Other studies in adults of the Kelp Gull have shown that during reproduction they forage on refuse, particularly when they are rearing their chicks (Bertellotti and Yorio, 1999; Burgues, 2015; Ludynia et al., 2005) and this could indicate that chicks from other reproductive colonies could also be ingesting refuse during their early development.

The incorporation of refuse into a nestling's diet might have implications for nestling survival and recruitment as well. Refuse could provide more energy to individual nestlings than natural food (Hunt, 1972) and also could increase the frequency of feeding bouts because it is highly spatially predictable and renewed daily (Plaza and Lambertucci, 2017). This might increase recruits to the breeding population, which has direct implications to population dynamics (Chabrzyk and Coulson, 1976). In Argentina, Kelp Gull meta-population dynamics have been evaluated by Lisnizer et al. (2015) who found that 29% of the Argentinean colonies are increasing by 10-20% annually. These authors found that individuals forage on

anthropogenic food subsidies in those regions where recruitment and intrinsic population growth are very large. Other studies, however, have found that foraging on refuse may have negative implications for chick survival and population dynamics. For instance, Pierotti and Annett (1987) and Pierotti and Annett (2001) suggested that energy provided by refuse might not be a proper currency to address this issue. They proposed that nutritional quality could better explain why they observed lower fitness in those individuals that forage on refuse than those that forage on natural food.

Considering that there is an industrial trawling fleet that operates in the Rio de la Plata Estuary that produce 2800-6700 tons of discards annually (Kelleher, 2005; Rey et al., 2000), and that adult Kelp Gulls forage on this type of resource in the estuary (Yorio et al., 2016), the question is why Kelp Gull breeders of Isla de Flores do not feed their chicks exclusively with fishing discards, and feed their chicks with refuse instead. According to Pierotti and Annett (2001), fish are an important source of nutrients that chicks will not find in refuse like chicken, so this should be the principal food source for these chicks. Maybe the availability of fishing discards might not be as predictable as refuse is, and/or refuse, when mixed with other foods, can support the nutritional needs of the chicks. Pierotti and Annett (2001) performed an experiment where they fed a group of chicks with chicken and another group with fish, and the first group experienced severe developmental problems, unlike the second group. However, in a natural situation, like this study, individuals feed on a variety of food sources that might complement a chicken-based diet. A possible explanation for our results is that essential nutrients could be incorporated in nestling diet from natural sources (e.g., fish), which might contribute to prevent developmental problems and decrease mortality, especially during the first weeks after hatching. Likewise, a more energetic source could be incorporated through refuse (e.g., chicken), which might increase the frequency of foraging bouts for chicks and which might improve chick survival during the fledging period.

The precedent idea is in part supported by the fact that larger Kelp Gull chicks showed more variability in resource acquisition evidenced by an increment of the standard deviation of refuse. This result supports the idea that diet variability increases because feeding on refuse allows chicks to expand their isotopic niche. According to Yeakel et al. (2016), this is possible because parents use more isotopically unique food sources during chick growth, like refuse from terrestrial ecosystems, which is depleted in ^{15}N composition in comparison with marine food

sources. Thus, if isotopic variability increases during chick growth, we might agree that older nestlings become more generalists as they grow (Yeakel et al., 2016).

As an anthropogenic food subsidy, refuse has the potential to alter not only many aspects of the life cycle of seabirds, but also the insular ecosystems where they reproduce. Because adults of Kelp Gull acquire resources for their chicks in terrestrial ecosystems, they are able to translocate energy and nutrients to insular ecosystems (González-Bergonzoni et al., 2017). Refuse could not be only subsidizing the Kelp Gull, but also the rest of the terrestrial communities that inhabit the islands where they establish their colonies. For example, in the Río de la Plata Estuary, some coastal islands are greatly inhabited with exotic vegetation (Guido et al., 2013), which could be receiving an extra supply of nutrients from human refuse digested and excreted by chicks and adults. In this context, a positive feedback within the community is expected, because it has been observed that the Kelp Gull improves reproductive success when reproduced in association with vegetation (Yorio et al., 1995). If vegetation cover increases as a product of nutrient translocation, we could expect an improvement in reproductive success of the Kelp Gull, as well. Moreover, this translocation from continental terrestrial refuses to insular ecosystems is probably substituting previous marine subsidies (natural diets) to these systems and may represent a new source of input of continental contaminants to insular systems to further consider. Hence, anthropogenic food subsidies and insular ecological processes, which might apparently be unrelated, could be connected by animal movements, and might have unknown effects that require study, especially in sensitive insular ecosystems.

3.6. Conclusions and final remarks

This study supports the idea that one of the consequences of an inadequate waste management system is that it makes available energetic and nutritional subsidies to generalist seabirds like gulls with potential individual- to ecosystem-level effects. Anthropogenic food subsidies in the form of refuse were ingested and assimilated by Kelp Gull chicks during the chick rearing period. We observed that the amount of refuse incorporated to chick's tissues increased with chick's age. Additionally, the isotopic niche of the chicks was broader when chicks got older because they could incorporate bigger meals, like chicken, which contributed to diet variability.

Ecological and environmental implications of foraging on refuse by Kelp Gulls can be linked to a potential increase in survival and population sizes of the species. This situation is of concern because this gull predated eggs and chicks of other Critically Endangered and Vulnerable seabird species like Royal Tern (*Thalasseus maximus*) and Cayenne Tern (*T. acutiflavidus*), respectively (Lenzi et al., 2010), it could be a potential pathogenic vector capable of polluting water bodies and cattle, and a threat to airport security (Yorio et al., 2016). On the other hand, other investigations have shown that when foraging on refuse, the Kelp Gull also ingests hazardous materials like plastics, which could be a threat to their own health and survivorship (Lenzi et al., 2016). Another potential environmental problem are changes in energy and nutrient cycling that might be occurring as a consequence of gulls foraging on refuse in the continent and mobilizing that refuse and associated pollutants to the coast, and particularly to coastal islands.

The government of Uruguay and private organizations are elaborating a law to regulate waste production and management, which intends to promote the circular economy to optimize the use of materials along their life cycle, encourage new business opportunities from waste, generation of employment, and ultimately to achieve environmental and economic sustainability. Therefore, this study could be useful for law makers, so they can consider a dimension that is generally underestimated within this type of domains, such as the consumption of refuse by animal populations and its environmental consequences. Additionally, managers and administrators might find the results of this investigation useful to improve their decisions on managing insular ecosystems, urban planning, and other activities related to the management of coastal zones.

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Table 3.1. Diet of Kelp Gull chicks (n=23) based on stomach lavage hatched on Isla de Flores during 2017 reproductive season.

Item	Occurrence in chick's stomach	Percentage of occurrence	Weight (Mean \pm SD)	Weight %
Fish (Sciaenidae)	9	39.1	33.99 \pm 24.1	26.0
Chicken	7	30.4	18.11 \pm 12.8	13.8
Insects	1	4.3	2.20 \pm 0.0	1.7
Animal fat	3	13.0	15.17 \pm 8.4	11.6
Animal guts	2	8.7	41.75 \pm 2.1	31.9
Shrimp	1	4.3	19.60 \pm 0.00	15.0

Table 3.2. Spearman correlation between chick measurements and mean refuse and standard deviation (SD) predicted by Bayesian stable isotope mixing models for blood and feathers of Kelp Gull chicks on Isla de Flores. * indicate correlations that were marginally statistically significant that were used in the model selection analysis.

	Chick measurement	Spearman ρ	P-value
Blood			
Mean / SD Refuse	Wing	+0.49 / +0.45	0.03 / 0.06*
	Culmen	+0.55 / +0.44	0.01 / 0.06*
	Tarsus	+0.33 / +0.34	0.15 / 0.12
	Weight	+0.32 / +0.33	0.16 / 0.15
Feathers			
Mean / SD Refuse	Wing	+0.04 / +0.01	0.85 / 0.99
	Culmen	-0.12 / -0.11	0.55 / 0.57
	Tarsus	+0.01 / +0.01	0.98 / 0.99
	Weight	+0.01 / +0.01	0.95 / 0.96

Table 3.3. Linear and non-linear models describing the variation between wing length (WL) and culmen length (CL) with the mean posterior density of refuse (R) predicted by the Bayesian mixing Models in blood samples of Kelp Gull nestlings from Isla de Flores. AIC values that designate the selected models are shown in bold.

	Wing (WL)	Culmen (CL)
Blood		
Linear model	$WL=480.4 \cdot R - 63.9$	$CL=42.2 \cdot R + 18.3$
AIC	80727	743
Logistic	$WL=137.8/1 + 1.5E09 \cdot \exp(-34.6 \cdot R)$	$CL=41.5/(1 + 8.7.5 \cdot \exp(-10.1 \cdot R))$
AIC	72821	729
vonBertalanfy	$WL=193.5 \cdot (1 - 15.7 \cdot \exp(-10.1 \cdot R))$	$CL=42.8 \cdot (1 - 2.4 \cdot \exp(-6.7 \cdot R))$
AIC	75810	731
Gompertz	$WL=178.8 \cdot \exp(-1010.8 \cdot \exp(-21.3 \cdot R))$	$CL=42.1 \cdot \exp(-4.6 \cdot \exp(-8.4 \cdot R))$
AIC	74075	729

Table 3.4. Linear and non-linear models describing the variation between wing length (WL) and culmen length (CL) with the standard deviation of the estimation of refuse (SD_R) from the Bayesian mixing Models in blood samples of the Kelp Gull chicks from Isla de Flores. AIC values that designate the selected models are shown in bold.

	Wing (WL)	Culmen (CL)
Blood		
Linear model	$WL=6263.4*SD_R-716.1$	$CL=516.6*SD_R+34.7$
AIC	79153	768
Logistic	$WL=178.2/1+1.4E20*\exp(-303.8*SD_R)$	$CL=41.7/(1+2.8E09*\exp(-106.3*SD_R))$
AIC	72756	729
vonBertalanfy	$WL=193.4*(1-4.5E10*\exp(-124.0*SD_R)$	$CL=43.5*(1-1489.6*\exp(-66.7*SD_R))$
AIC	75370	764
Gompertz	$WL=180.5*\exp(-8.8E16*\exp(-232.2*SD_R))$	$CL=42.4*\exp(-20121*\exp(-86.3*SD_R))$
AIC	73762	763

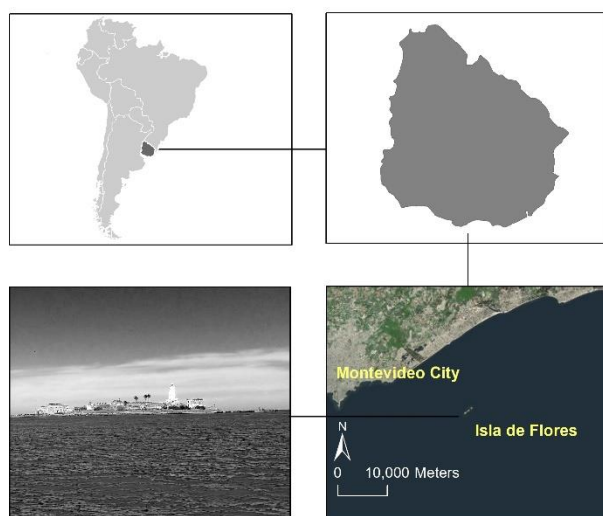


Figure 3.1. Geographical location of the study area.

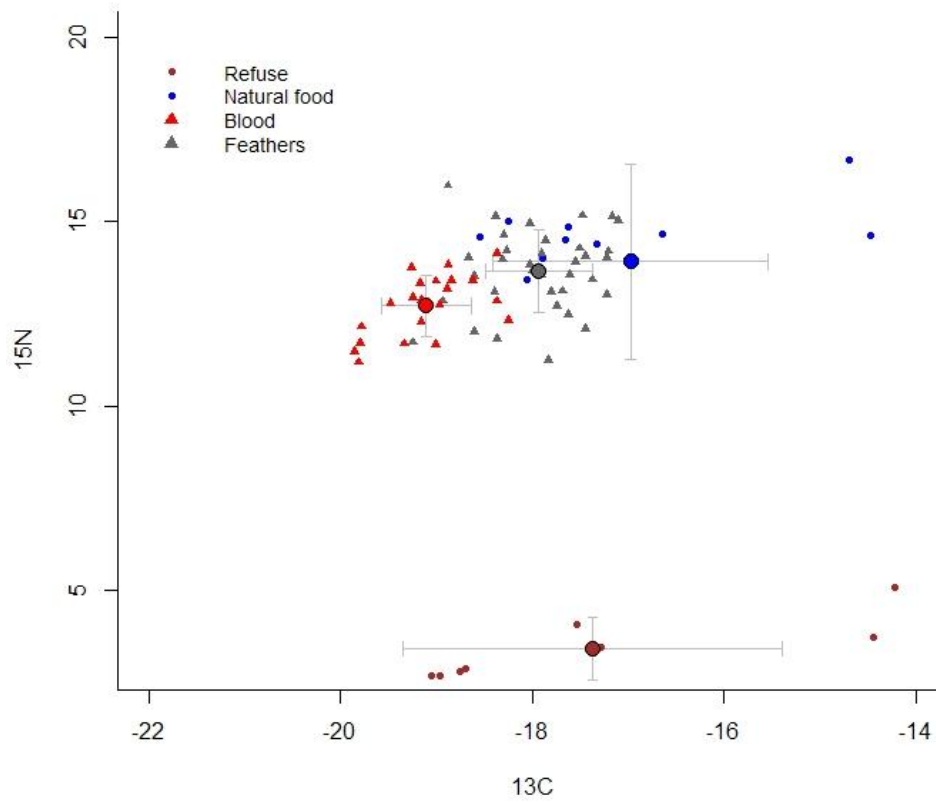


Figure 3.2. Biplot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of food sources (natural and refuse), chick's blood and feather tissues of Kelp Gull chicks from Isla de Flores. Lipid content was corrected in both consumers and prey following equation 3 of Post et al. (2007).

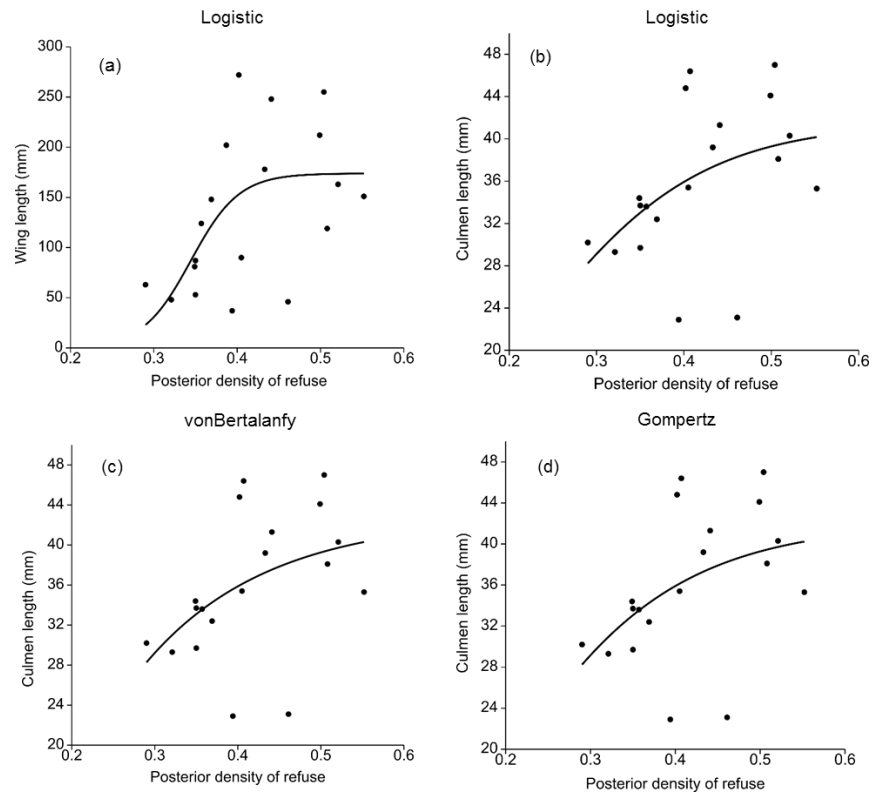


Figure 3.3. Non-linear equations selected from the model selection analysis describing wing (a) and culmen (b, c, d) lengths of chicks with the mean posterior density of refuse estimated by the Bayesian mixing models of chick's blood from the colony on Isla de las Gaviotas.

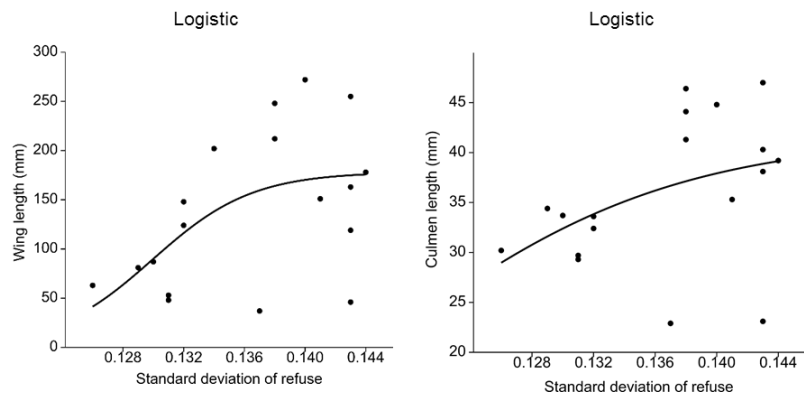


Figure 3.4. Equations selected from the model selection analysis describing wing length (a) and culmen length (b) of Kelp Gull chicks with the standard deviation of refuse estimated by the Bayesian mixing models of chick's blood from the reproductive colony on Isla de las Gaviotas.

4. ANTHROPOGENIC FOOD SUBSIDIES AFFECT FECUNDITY AND REPRODUCTIVE SUCCESS OF A GENERALIST SEABIRD

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4.1 Abstract

Resource acquisition and allocation significantly impacts individual fitness.

Anthropogenic food subsidies are known to be a significant part of seabirds' resource acquisition and allocation. Refuse is predictable and some seabirds, such as gulls, rely heavily on this food subsidy, which is likely to modify fecundity and reproductive success. Using pellet analysis and stable isotopes of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of down feathers of Kelp Gull (*Larus dominicanus*) chicks, we studied the fitness consequences of Kelp Gull females foraging on refuse during the pre-incubation period. We evaluated the correlation of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and the posterior densities of refuse (beef and chicken) and natural food (fish) from isotopic measures using Bayesian mixing models, with the fecundity and reproductive success of individual females. We found that $\delta^{15}\text{N}$ positively correlated with fecundity and reproductive success, and $\delta^{13}\text{C}$ correlated negatively with fecundity. Mean posterior density of refuse positively correlated with fecundity and negatively correlated with reproductive success. Moreover, mean posterior density of natural

food negatively correlated with fecundity and positively with reproductive success. We argue that refuse affects foraging decisions of individual females during the pre-incubation period, which could affect future fecundity and reproductive success. Additionally, available literature suggest that consumption of refuse affects fecundity and reproductive success of gulls at the individual and colony levels using conventional diet techniques. However, the use of stable isotopes has not much been employed to address these questions, making comparisons and conclusions difficult to generalize, so we encourage researchers to continue incorporating isotopic analysis into the study of food subsidies on gulls.

Key words: refuse, acquisition and allocation of resources, seabirds, stable isotopes, reproductive ecology.

4.2 Introduction

The acquisition and allocation of resources strongly affects the fitness of individuals (Pianka, 1981). Acquisition of materials and energy by animals is used for fitness dependent functions of predator avoidance, tissue maintenance, reproduction, storage, and growth, among others (Fox et al., 2001; Stearns, 1992; vanNoordwijk and deJong, 1986). A variety of methods have been employed to examine tradeoffs between resource acquisition and energetic expenditures, including the use of behavioral-energetic models some of which explore this in the context of optimality theory (e.g., optimal foraging theory; see Pyke 1984; Charnov 1976; Watanabe et al. 2014).

In recent decades, ecologists have been concerned about the role that anthropogenic food subsidies ultimately have on fitness components of certain animals (Howes and Montevecchi, 1993; Pierotti and Annet, 1991; Pierotti and Annett, 2001; Plaza and Lambertucci, 2017; Polis et al., 1997; Pons, 1992; Weiser and Powell, 2010). Many researchers have determined that refuse is often an important anthropogenic food subsidy for many animals because it is highly predictable in space and time. Animals most highly impacted by refuse include seabirds, and in particular, gulls, as they are generalist species that forage in a wide variety of habitats and are widely omnivorous (Oro et al., 2013; Plaza and Lambertucci, 2017). Foraging on refuse is thought to be advantageous for gulls for several reasons; refuse dumps provide a habitat free of

natural predators where food is always available and renewed, and these resources are in large supply along coastal environments (Plaza and Lambertucci, 2017; Sol et al., 1993).

There is considerable evidence that shows that anthropogenic food subsidies have significance impact on seabird populations and behavior although results vary greatly. For example, several researchers have shown that closure of refuse dumps reduced fecundity and reproductive success of several species of gulls (Kilpi and Öst, 1998; Pons, 1992; Pons and Migot, 1995; Ramos et al., 2009a; Steigerwald et al., 2015). Similarly, others have found that access to refuse improves fecundity and reproductive success (e.g., Hunt, 1972; Kadlec and Drury, 1968; Murphy et al., 1984; Weiser and Powell, 2010). However, other studies have shown that gulls breeding close to urban areas and foraging on refuse have lower reproductive success than those individuals breeding far from urban areas and forage on natural food (Pierotti and Annett, 2001). Reductions in reproductive success have been thought to be due to the fact that refuse lacks essential nutrients, which are necessary for egg and chick development (Pierotti and Annet, 1991; Pierotti and Annett, 1990). To explore relationships between food composition and individual health, most researchers have focused mainly on the analysis of feeding pellets to young during the reproductive period. Although informative, this approach provides a biased short term measure of the diet of individuals and its influence on fitness (Barrett et al., 2007; Karnovsky et al., 2012). Acquisition of resources during the pre-reproductive period could, for example, have long-term effects on allocation of resources to future fecundity and reproductive success as many elements could persist in body tissue for weeks to years (Gill et al., 2001; Sorensen et al., 2009).

Stable isotope analysis has been one approach used by researchers to evaluate long-term diet selection and resource use by animals (Kelly, 2000; Peterson and Fry, 1987; Rubenstein and Hobson, 2004). The isotopic signature of ^{13}C in body tissues of an animal can provide information about the source of carbon used. Stable isotope analysis is possible because isotopic forms react at different, and predictable rates, in different tissues, and the ratio between the isotopes reflects the relative abundance of the heaviest atom, which reflects dietary sources (Hobson and Sealy, 1991; Mizutani et al., 1990). These tissue-specific reactions are mediated by the physiology of the consumer and is called discrimination (Post, 2002; Zanden and Rasmussen, 2001). Using stable isotopes to estimate individual diets of consumers has allowed many researchers to reconstruct the chemical structure of trophic networks because one can estimate

the concentration of nutrients that have been assimilated, not only those that were ingested, which is unlike conventional diet studies (Fry, 1991; Hobson and Clark, 1992a; Minagawa and Wada, 1984). Moreover, by employing the latest statistical tools such as Bayesian mixing models, one can transform the delta space (δ), i.e., the ratio of the heavy and light isotope relative to internationally accepted standards, to proportions (p) of the different isotopic sources (Newsome et al., 2007; Phillips and Gregg, 2001) so that fitness components can be examined with isotopic composition. Investigating certain stable isotopes, for example of carbon and nitrogen, can allow one to separate benthic and pelagic or marine and continental food sources (Mizutani et al., 1990; Peterson and Fry, 1987). It is known that isotopic signatures of ^{15}N increase along the food chain, allowing for estimates of the trophic position of a consumer (Hobson et al., 1993; Thompson et al., 1999). In systems where gulls forage on refuse, the signature of ^{15}N in gull tissues is lower where the diet is primarily comprised of refuse than when the gulls are consuming their natural prey, marine fish (e.g., Abdennadher et al., 2014; Moreno et al., 2010; Weiser and Powell, 2011). Thus, the proportion of ^{15}N entering the food chain can be used to assess the level of diet from refuse as opposed to natural fish.

One gull species that exploits a wide variety of natural and anthropogenic food subsidies is the Kelp Gull (*Larus dominicanus*) (Burgues, 2015). This generalist and opportunistic species is distributed along the Southern hemisphere (Yorio et al., 2016). In the Rio de la Plata Estuary in South America, Kelp Gulls forage on anthropogenic food subsidies (Lenzi et al., 2016; Silva et al., 2000). This estuary, one of the largest in America at 35,000 km², supports the largest human settlements of Argentina and Uruguay with a population exceeding 12 million people the coast (Boschi, 1988). A variety of food for these gulls, like natural fish and those of anthropogenic sources, such as fishing discards, are available in this ecosystem. Refuse dumps and remains of animal tissues from meat industries contribute greatly to anthropogenic food subsidies of Kelp Gulls (Lenzi et al., 2016)

Using conventional diet techniques coupled with stable isotope analysis, we studied the fitness consequences of foraging on refuse during the pre-incubation period of Kelp Gull for individual females that reproduce on a coastal island of the Rio de la Plata Estuary, Uruguay. The pre-incubation period is an important phase of the life cycle of seabirds because acquisition of resources by the reproductive adults will affect their subsequent fecundity and reproductive success (Hiom et al., 1991). Particularly, we evaluated the relative importance of food items for

the breeding pairs during the incubation period using analysis of pellets (note: it is extremely difficult to trace individual female diets during the pre-incubation period using pellet analysis). In addition, using stable isotope analysis and Bayesian mixing models of down feathers of nestlings, we estimated the diet composition of breeding females during egg formation at the pre-incubation period (Hobson et al., 2000). Moreover, we evaluated the relationship between the fecundity and reproductive success of these breeding females and their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures. Finally, we correlated and modeled the variation in fecundity and reproductive success with the posterior densities of the most important natural food and refuse sources estimated using our Bayesian mixing models.

4.3 Materials and Methods

4.3.1 Study area

The Rio de la Plata Estuary contains several coastal islands including our study island, Isla de Flores (34°56'30"S - 55°55'29"W). The island is 54.4 ha in size with dimensions of 1,700 x 320 m. It is located 11 km from the coastal border of Montevideo and Canelones Departments along the Uruguayan coast (Figure 4.1). This island is elongated and aligned from NE to SW and is composed by three sub-islands that are separated by isthmuses that flood during high tide (Figure 1). Isla de Flores is a National Park within the national system of protected areas (SNAP) because it has natural and cultural value. This island is also part of the National Park of Coastal Islands of the Rio de la Plata and Atlantic Ocean (Decree N° 447/996).

4.3.2 Conventional diet and stable isotope analysis

To overcome the limitations that conventional diet studies and stable isotopes have, we used both approaches. Stable isotope analyses do not provide taxonomic resolution, however, they integrate medium- and long-term dietary information, as well as reflect the assimilation of nutrients, rather than only ingestion of diet items, as in conventional diet techniques (Post, 2002). Thus, both techniques provide rich information on the trophic ecology of individuals for short- and long-term consumption (Bearhop et al., 2004).

It is extremely difficult to study the diet of individual seabirds using conventional techniques during the pre-breeding period (Sorensen et al., 2009). Thus, we analyzed the diet of the Kelp Gull in the incubation period using pellet analysis (Barrett et al., 2007; Duffy and

Jackson, 1986). During the 2017 incubation period, a 100 m² quadrant was placed within the colony and all the pellets were collected in or near nesting territories during seven surveys. Pellets were stored in plastic bags until they were analyzed in the laboratory. During the analysis, each item was categorized as garbage or natural food, and was later identified to the lower taxonomic level if possible or assigned to a lower debris category.

To analyze the diet of the Kelp Gull using stable isotopes, we gathered samples from nestlings and potential food sources. To gather gull samples, we captured nestlings by hand < 48 hrs. after hatching, collected a sample of down feathers, and stored the feathers in individual polyethylene bags for further processing. Down feathers of nestlings reflect their nutrient acquisition during embryo development (Hobson and Clark, 1992a; Pérez et al., 2008; Sanpera et al., 2007) that ultimately reflects the diet of the mother around the breeding colony during egg formation (Hobson et al., 2000). In the laboratory, down feathers were oven dried at 60 °C for 48 hours, then finely cut, and a sub-sample of 0.1 - 0.15 mg was encapsulated for further stable isotope analysis. Lipids were not removed because feathers had a very low lipid content with C:N ratios < 3.5 (English et al., 2018; Post et al., 2007) and it has been reported that lipids potentially attached to the feathers do not alter isotopic signatures (Kojadinovic et al., 2008).

To gather samples of food sources for stable isotope analysis, we used two methods. First, we gathered samples of natural food sources from another study of the same colony during the same period, where we used a stomach pump on chicks, following the technique of Wilson (1984). Secondly, because most of the diet of breeding adults was composed of refuse (cooked beef and chicken bones with no muscular tissue, see Results), samples were obtained from local kitchen scraps including barbequed beef and chicken muscle. All samples were oven dried at 60 °C for 48 hours and a subsample of 0.1 mg was weighed into a miniature tin capsule for further stable isotope analysis. To account for biases in $\delta^{13}\text{C}$ from the ingestion of lipids by predators and its presence in muscular tissues of prey, we followed equation 3 of Post et al. (2007) for those samples with C:N > 3.5. Nitrogen and carbon isotope ratios were measured by Elemental Analyzer Continuous Flow Isotope Ratio Mass Spectrometry at the Center for Stable Isotopes, University of New Mexico (<http://csi.unm.edu>) employing a Costech ECS 4010 Elemental Analyzer coupled to a ThermoFisher Scientific Delta V Advantage Plus mass spectrometer via a CONFLO IV interface. Average analytical precision based on routine analysis of a laboratory standards was better than 0.1‰.

4.3.3 Fecundity and reproductive success

During the reproductive season, we conducted 11 surveys to gather information on fecundity and reproductive success. We assessed fecundity from clutch size and the mean egg dimension measurements and weight for each nest. To measure egg width and length, we used calipers (Carrera Precision CP8806-T) and recorded to the nearest 0.1 mm; and, to measure their weight, we used a scale to the nearest 0.1 g (Ohaus CL201). From egg measurements we calculated egg volume (V) as: $V = \text{length} * \text{width}^2 * 0.476$ (Harris, 1964). Once the eggs hatched, we marked each chick with a code using a fiber-tape tag in the tarsus and monitored every two-four days, depending on weather conditions. We measured reproductive success as *a*) hatching success, i.e., the number of chicks hatched per nest, and *b*) fledging success, the number of chicks of each nest that survived for a period of nine days (after that period chicks became to be difficult to follow because mark loss was high, and chicks started to hide more efficiently).

4.3.4 Data analysis

To compare the isotopic signatures of carbon and nitrogen of the principal food sources we used MANOVA with the stable isotopes as the dependent variables and beef, fish, and chicken as the independent variables. Then, we used ANOVAs and *post hoc* Tukey tests to analyze differences in stable isotope means of each food source. To estimate the relative contribution of the main food sources of adult females during the pre-breeding period, we used Bayesian mixing models on carbon and nitrogen isotopic signatures of down feathers of chicks (Parnell et al., 2010; Parnell et al., 2013; Phillips, 2012). Using the MixSIAR package (Stock and Semmens, 2016) of R v. 3.4.3 (R, Development Core Team 2018) we modelled the posterior probabilities of the food sources for the entire sample (the colony level) and for each nest individually. We averaged isotopic signatures for each nest when we had more than one chick per nest sampled, to avoid potential pseudo-replication. To improve the accuracy of the models, we selected the basal food sources that best reflected the diet of the adult females. These sources were fish, beef, and chicken, and were used as the basal sources for fitting the models.

Fractionation factors for the mixing models were obtained from controlled experiments published for Ring-billed Gull, Black-tailed Gull (*Larus crassirostris*) and Yellow-legged Gull (*Larus michahellis*) because there are no published factors for the Kelp Gull. Thus, we used fractionation factors of +1.6‰ for carbon and +3.3‰ for nitrogen (Hobson and Clark, 1992b;

Mizutani et al., 1992; Ramos et al., 2009b). Standard deviation was set in 1.0‰ (Ceia et al., 2014). We fit the Bayesian mixing models using informative and uninformative priors and selected the models with the lowest standard deviation. Thus, we used informative priors from the conventional data analysis (Table 1) and uninformative priors using a Dirichlet distribution, and then we compared their standard deviation using two-tailed t tests. Because the standard deviation of uninformative priors was lower in the models for the entire sample and for each individual nest (Supplementary Material, Table S1), we used uninformative priors to fit the Bayesian mixing models. From these models, we compared the posterior densities of the Bayesian mixing models of beef, fish, and chicken for each individual nest using ANOVAs and *post hoc* Tukey tests.

To evaluate variations in the isotopic niche with fecundity and reproductive success of the Kelp Gull, we fit General Linear Models with the fecundity and reproductive success metrics of individual nests as the dependent variables and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures as the independent variables. Additionally, we correlated fecundity and reproductive success metrics with the posterior densities of the Bayesian mixing models of beef, fish, and chicken using Spearman correlation. From those significant correlations, we fit linear and non-linear models to examine the relationship between fecundity and reproductive success with the posterior densities of the Bayesian mixing models of beef, fish, and chicken. We selected the models that best fit the data using Akaike Information Criterion (AIC; Akaike, 1973, 1974). The models with the lowest AIC values were those that best fit the data. If the difference in AIC values of the best and second (or *i*th) best models were equal or lower than 2 units, then both models were selected (Johnson and Omland, 2004). To perform model fitting and selection we used PAST software v3.0 (Hammer et al., 2001).

4.4 Results

The analysis of the pellets collected in the colony showed that diet of breeding adults was based on chicken (29.7%), beef (14.2%), and fish (5.4%) (Table 4.1). Although vegetables occurred in high frequency (10.4%), we consider them a very small proportion in biomass because they represented 2% of the total weight (unpubl. data) (Table 4.1).

General differences were detected in carbon and nitrogen among beef, chicken, and fish (MANOVA Pillai's trace = 1.19, $F_{approx} = 14.1$, $df = 2, 19$, $P < 0.01$, Table 4.2). Isotopic signature

of carbon was smallest in beef samples and largest in chicken, although did not statistically differ (ANOVA: $F_{2,19} = 2.6$, $P = 0.09$; Tables 4.2, 4.3). Isotopic signature of nitrogen, on the other hand, varied statistically between chicken, beef, and fish (ANOVA: $F_{1,19} = 464.1$, $P < 0.01$; Table 4.2). Nitrogen isotopic signature of fish was the largest and the signature of chicken was the smallest (Tables 4.2, 4.3).

Considering the entire sample (colony level), Bayesian mixing models predicted that down feathers of chicks contained a large proportion of beef (posterior density mean = 0.49 ± 0.19), followed by fish (posterior density mean = 0.35 ± 0.16), and chicken (posterior density mean = 0.16 ± 0.11). When posterior density of food sources were estimated for each nest, we detected that their means differed significantly (ANOVA: $F_{2,48} = 107.9$, $P < 0.01$) following the same pattern like the entire sample (Table 4).

Measurements of fecundity and reproductive success varied with stable isotope signatures. Clutch size was associated positively with $\delta^{15}\text{N}$ and negatively with $\delta^{13}\text{C}$ (Table 4.5). In addition, fledging success varied positively with $\delta^{15}\text{N}$ (Table 4.5). Additionally, posterior probabilities of food sources from Bayesian mixing models showed that beef and chicken positively correlated with egg weight (Table 4.6, Figure 4.2). On the other hand, posterior probability of fish negatively correlated with egg weight (Table 4.6, Figure 4.2). Additionally, fledgling success negatively correlated with posterior probability of beef and chicken, and positively correlated with the posterior probability of fish (Table 4.6, Figure 4.2).

Models that best described the variation in egg weight with Bayesian posterior probabilities of the principal food sources were: the linear and the logistic model for beef, the linear, logistic and Gompertz models for fish, and the linear model for chicken (Table 4.7, Figure 4.2). Additionally, the model that best described the variation between fledgling success and the Bayesian posterior probabilities of beef, fish and chicken was the linear model (Table 4.7, Figure 4.2).

4.5 Discussion

Reproductive adults of Kelp Gull during the incubation period on Isla de Flores foraged on a large proportion of refuse, evidenced by chicken and beef remains, and on a much less proportion of natural food, like fish. Additionally, the large presence of refuse, and other food remains, support the generalist and opportunistic feeding behavior of this species, which has

been evidenced by other studies (Coulson and Coulson, 1993; Ludynia et al., 2005; Petracci et al., 2004; Silva et al., 2000).

Pellet and stable isotope analyses differed in the estimated proportion of chicken and beef (although both indicated a higher proportion of refuse in general), maybe because the basis of both methods is different and/or the foraging strategies of individuals change between the pre-incubation and the incubation periods (pellets were collected during the incubation period, see Material and Methods). Pellet analysis showed a larger proportion of chicken, than beef and fish, in the diet of the individuals analyzed. On the other hand, by employing Bayesian mixing models on isotopic data we observed that chick's feathers showed an isotopic signature that reflected a higher proportion of beef than fish and chicken ingested by individual females during the pre-incubation period. The potential causes for the difference in diet are not mutually exclusive.

Pellet and stable isotope analyses have differences in their estimation and quantification of diet sources. Pellets integrate the diet of adults over short periods of time; stable isotopes, on the other hand, integrate longer periods of time of individual diets from one week to several months, depending on the tissue. Additionally, through our stable isotope analysis we found that the proportion of the food sources that were assimilated in the tissues could be estimated using Bayesian mixing models. Hence, an individual might ingest more chicken than beef evidenced by pellet analysis, however the digestible biomass of beef could be more important than chicken. Conversely, other studies have found that both conventional diet and stable isotope analyses provide similar outputs (Weiser and Powell, 2011). This observation supports the idea that foraging preferences might change between the pre-incubation and incubation periods, although it needs further study.

Temporal and spatial variability in foraging strategies could be influenced by the stage of the life cycle and/or the energetic quality of the resources. For example, chicken remains could have been obtained in other places, other than refuse dumps where Kelp Gulls generally forage on refuse (Lenzi et al., 2016; Yorio et al., 2016). In an ongoing investigation, using GPS trackers, we were able to determine that adults, during the incubation period, foraged on an area of poultry processing plants (unpubl. data) that could be an important source of chicken remains. This idea is partially supported by another ongoing study, which using ^{15}N and ^{13}C stable isotopes showed that during the chick rearing period chicks are fed by an increasing proportion of chicken as they grow (unpubl. data). Other studies, using conventional diet techniques, have

found that Western Gulls (*L. occidentalis*) change their foraging preferences from refuse (chicken) during the incubation period, to natural food (fish) during the chick rearing period (Annett and Pierotti, 1989). Using stable isotopes, changes in foraging strategies in Cassin Auklets (*Ptychoramphus aleuticus*) suggest that females foraging on highly energetic diets during the pre-incubation period are able to allocate more resources to fecundity than females that foraged on lower energetically food sources (Sorensen et al., 2009). Additionally, Ramos et al. (2011) which analyzed stable isotopes in feathers molted in different stages of the life cycle of the Yellow-legged Gull, have found an important range of feeding strategies in several colonies in the Mediterranean coast. Some colonies changed their dietary preferences and foraging habitats between the reproductive and non-reproductive periods, while others remained unchanged. Interestingly, another study of the same species at the individual level found short-term consistency in the foraging strategies between the pre-reproductive and incubation periods (Ceia et al., 2014). Additionally, these authors found that the foraging strategies between the reproductive and the non-reproductive periods were similar. Thus, spatial and temporal changes in the foraging strategies might have consequences on acquisition of resources along the life cycle of individual seabirds, in addition to the potential differences in food quality.

Refuse might modify foraging strategies of individual females during the pre-incubation period, which could affect future fecundity and reproductive success. According to our results, Kelp Gull females that foraged more on refuse during the pre-incubation period could be able to allocate more resources to fecundity, than those females that foraged more on natural food sources. On the other hand, consumption of refuse during the pre-incubation period negatively correlated with reproductive success of the individuals analyzed. Our results appear to be intermediate between studies that found positive associations between gulls foraging on refuse with fecundity and reproductive success (Pons, 1992; Steigerwald et al., 2015; Weiser and Powell, 2010) and those that found negative associations with both traits (Annett and Pierotti, 1999; Pierotti and Annett, 1987, 2001; Pierotti and Annett, 1990). The former studies have analyzed gull's feeding ecology during the incubation or chick rearing periods, at the colony level, and using conventional diet techniques. In contrast, the second set of studies address trophic ecology at the individual level comparing individuals of the same reproductive colony, and using conventional diet techniques.

According to the available literature, refuse might affect fecundity and reproductive success of gulls depending on the level of analysis employed (individual versus colony), which indicates that the comparative axis of the studies might be different. As we introduced earlier, distance from colonies to sources of refuse could positively correlate with fecundity and reproductive success at the colony level. At the individual level, however, the reproductive outcome might depend more on intra-colony differences in foraging behaviors. When studies combine individual parameters into colony averages, they assume that reproductive colonies are a collection of identical individuals. On the contrary, individual properties, such as foraging decisions, nutritional state, age, or genotype, may disappear at the colony level (McCauley et al., 1993). For instance, our study shows that, on average, Kelp Gull females foraged more on refuse than natural food. However, at the level of individual females using stable isotopes, we could observe that there is a great variation in foraging preferences. Thus, we could observe a correlation between food sources and fecundity and reproductive success of the different individual females. These results evidence the importance to consider the individual level to study the effect of anthropogenic food subsidies on the foraging ecology and fitness components of seabirds.

Experimental food manipulation studies might offer insights about the individual responses of females to changes in food availability induced by the presence of refuse near the colonies. This type of experiments have been conducted using fish and hen eggs in order to evaluate the effects of food supply during the pre-incubation period on reproductive parameters, such as fecundity and reproductive success. Not many experiments have been conducted for gulls. However, they generally show a positive relationship between supplemental feeding with fecundity and reproductive success, like egg mass, clutch size, and the number fledglings per nest (Bolton et al., 1992; Bukacinski et al., 1998; Hiom et al., 1991). Refuse could play a role analogous as the supplemental feeding on fecundity and reproductive success. Nevertheless, our results showed a reduction in reproductive success of those females that foraged more on refuse during the pre-incubation period. According to Bolton et al. (1992), who did not find a positive relationship between supplemental feeding and reproductive success in Black-backed Gulls, individuals might lay as many eggs as they can and reduce their brood later in case food supply is scarce in the future. This is in accordance with the life history of long-live animals, which increase their future survivorship decreasing their current reproductive effort when

environmental conditions are adverse (Weimerskirch, 2002). However, refuse is available and renewed permanently and is predictable spatially and temporally (Plaza and Lambertucci, 2017). This suggests that individuals might not adjust their reproductive effort to food availability, but foraging on refuse could have produced eggs of larger size and quantity, but of bad quality, which later on produced bad quality chicks with low survivorship (Bolton, 1991). Therefore, we consider that it could be beneficial to perform experimental approaches to study the effect of garbage on individual traits of gulls, and considering different levels of biological organization.

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Table 4.1. Diet of breeding adults on Isla de Flores during the 2017 reproductive season.

Category	No. of Pellets	FO%	Description
<i>Natural origin</i>			
Vegetables	33	10.4	Leafs, grass and seeds
Fish	17	5.4	Bones, vertebrates, and otoliths
Mammals	2	0.6	Bones
Birds (gulls)	9	2.8	Long bones, skulls, gull's foot.
Mussels	6	1.9	Valve remains
Insects	2	0.6	Beetles, exoskeletons
Stones	3	0.9	
<i>Garbage</i>			
Chicken	94	29.7	Long bones, fat, vertebrates, skulls.
Plastic	81	25.6	Film, packaging, pieces of polystyrene, undetermined hard pieces of plastic.
Beef	45	14.2	Bones
Metal	7	2.2	Aluminum foil, coper and bottle caps
Glass	7	2.2	Small and big pieces green and transparent
Animal fat	5	1.6	
Lamb and pork	3	0.9	Bones
Threads	3	0.9	Pieces of cotton threads
Paper	1	0.3	Pieces of paper

Table 4.2. Isotopic signature of carbon and nitrogen (mean \pm SD) of the principal prey types registered in pellets breeding adults and stomach pump of chicks of the Kelp Gull on Isla de Flores during 2017 reproductive season.

Prey types	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	n
Fish	14.36 ± 0.83	-17.12 ± 1.43	10
Beef	7.95 ± 0.39	-19.31 ± 1.41	4
Chicken	3.43 ± 0.84	-17.37 ± 1.98	8

Table 4.3. Tukey tests between stable isotope signatures and food sources of Kelp Gull chicks on Isla de Flores during 2017 reproductive season.

	Difference in means	<i>P</i>
$\delta^{13}\text{C}$		
Chicken-Beef	1.94	0.16
Fish-Beef	2.19	0.09
Fish-Chicken	0.26	0.94
$\delta^{15}\text{N}$		
Chicken-Beef	-4.52	<0.01
Fish-Beef	6.72	<0.01
Fish-Chicken	11.24	<0.01

Table 4.4. Tukey test between posterior densities of food sources predicted by Bayesian mixing models for each nest of Kelp Gull at Isla de Flores during 2017 reproductive season.

Food source	Difference in means	<i>P</i>
Chicken-Beef	-0.48	<0.01
Fish-Beef	-0.19	<0.01
Fish-Chicken	0.29	<0.01

Table 4.5. Estimation of the parameters, standard deviation (SE) and P-value of the models with the fecundity and reproductive success metrics versus $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of Kelp Gull chicks on Isla de Flores during 2017 reproductive season.

	Estimate	SE	P-value
Eggs per nest			
Intercept	-14.55	6.81	0.05
d15N	0.30	0.12	0.02
d13C	-0.72	0.04	0.04
Egg length			
Intercept	101.77	43.57	0.04
d15N	-0.38	0.77	0.63
d13C	1.37	2.03	0.51
Egg width			
Intercept	44.11	22.36	0.07
d15N	-0.20	0.39	0.61
d13C	-0.51	1.04	0.63
Egg weight			
Intercept	191.21	179.25	0.31
d15N	-1.39	3.18	0.67
d13C	4.78	8.36	0.58
Egg volume			
Intercept	92035.50	111810.20	0.43
d15N	1112.50	1981.00	0.58
d13C	-594.90	5211.70	0.91
Hatching success			
Intercept	-566.22	265.53	0.06
d15N	-7.66	4.62	0.13
d13C	21.21	12.46	0.12
Fledging success			
Intercept	-2.21	8.86	0.81
d15N	0.36	0.15	0.04
d13C	0.06	0.41	0.88

Table 4.6. Spearman correlation coefficients of fecundity and reproductive success versus estimated beef, fish and chicken by the Bayesian mixing models of the Kelp Gull on Isla de Flores during 2017 reproductive season.

	Beef		Fish		Chicken	
Trait	Rho	P-value	Rho	P-value	Rho	P-value
Clutch size	-0.20	0.45	0.20	0.45	-0.28	0.29
Egg length	0.16	0.56	-0.16	0.55	0.06	0.82
Egg width	0.24	0.37	-0.22	0.40	0.19	0.49
Egg volume	0.32	0.23	-0.31	0.24	0.22	0.41
Egg weight	0.61	0.02	-0.59	0.02	0.57	0.03
Hatching	0.07	0.81	-0.07	0.81	0.16	0.59
success						
Fledgling	-0.77	<0.01	0.77	<0.01	-0.69	<0.05
success						

Table 4.7. Selection of linear and non-linear models between fecundity and reproductive success versus food sources exploited by the Kelp Gull during the pre-breeding period on Isla de Flores. Bold AIC vales are those that selected the corresponding mod

	Beef	Fish	Chicken
Egg weight			
Linear model	EW=31.6*Beef+74.0	EW=-30.0*Beef+102.9	EW=414.9*Chicken+62.8
AIC	448	448	497
Logistic	EW=5.6/(1+7.4*exp(-0.4*Beef))	EW=1.5/E-11/(1+1.4*exp(0.3*Fish))	EW=178.1/(1+1.8*exp(-9.3*Chicken))
AIC	450	449	500
vonBertalanfy	EW=513.4*(1-0.9*exp(-0.07*Beef))	EW=479.3*(1-0.8*exp(0.08*Fish))	EW=302.6*(1-0.79*exp(-1.9*Chicken))
AIC	452	451	500
Gompertz	EW=607.6*exp(-2.1*exp(-0.2*Beef))	EW=1232.7*exp(-2.5exp(0.1*Fish))	EW=275.4*exp(-1.5*exp(-4.1*Chicken))
AIC	451	450	500
Fledgling success			
Linear model	FS=-3.9*Beef+3.9	FS=3.8*Fish+0.3	FS=-50.9*chicken+5.6
AIC	7	7	8
Logistic	FS=2.8/(1+0.1*exp(6.7*Beef))	FS=2.8/(1+6.1*exp(-6.3*Fish))	FS=4.3/(1+0.1*exp(52.7*Chicken))
AIC	10	11	11
vonBertalanfy	FS=5.8*(1-0.4*exp(0.9*Beef))	FS=7.2*(1-0.9*exp(-0.7*Fish))	FS=70.2*(1-0.9*exp(0.7*Chicken))
AIC	11	11	11
Gompertz	FS=3.3*exp(-0.1*exp(3.7*Beef))	FS=3.4*exp(-2.4*exp(-3.4*Fish))	FS=10.5*exp(-0.5*exp(17.0*Chicken))
AIC	10	11	11

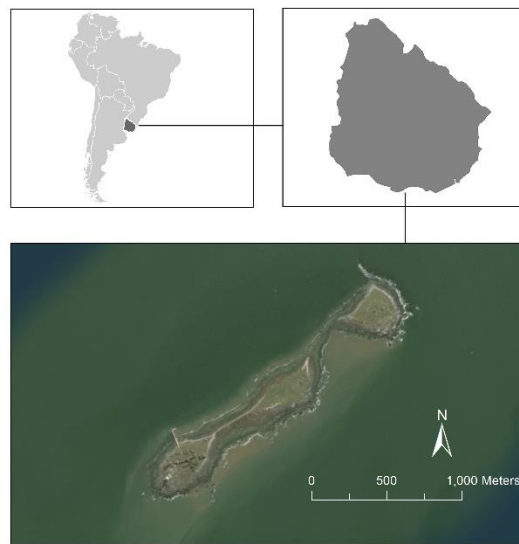


Figure 4.1. Geographical location of Isla de Flores.

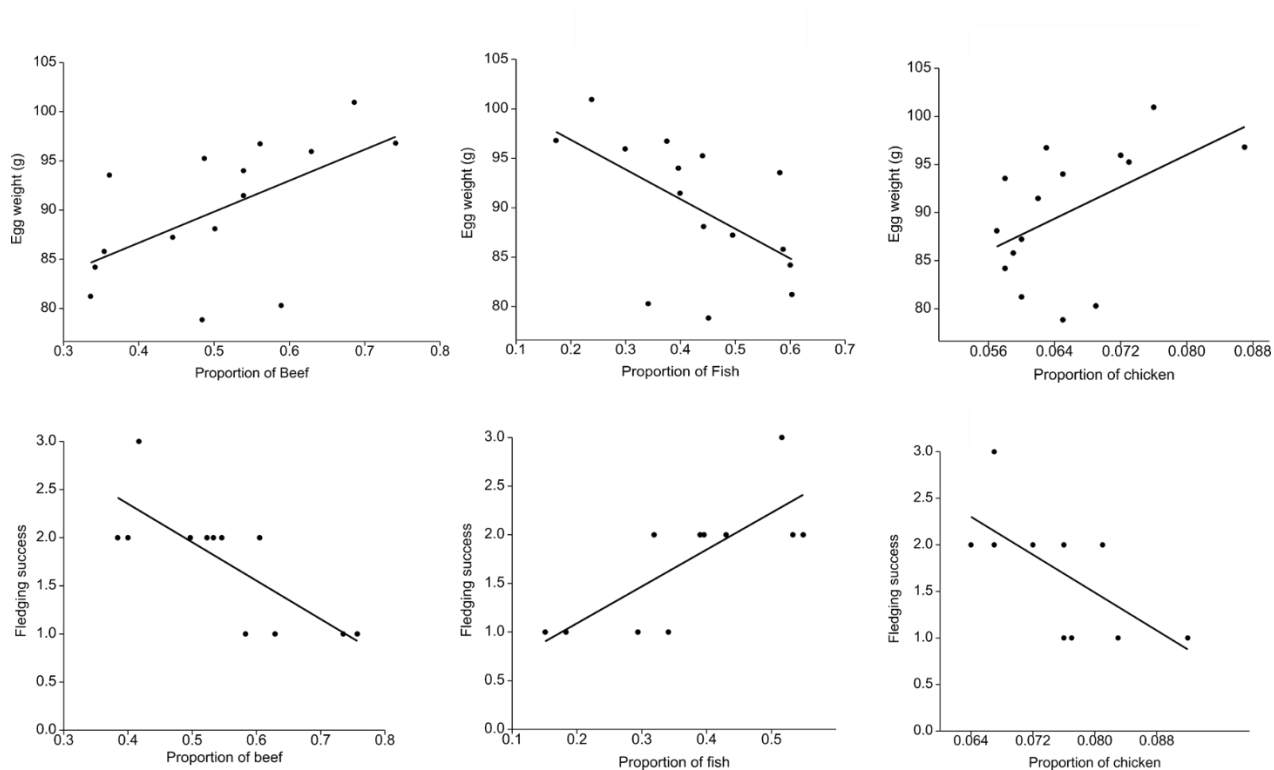


Figure 4.2. Relationship between egg weight and fledging success with proportion of beef, fish and chicken of Kelp Gull chicks on Isla de Flores during 2017 reproductive season. Plots and models are those selected using Akaike Information Criterion (Akaike, 1973).

4.8 Supplementary Material

We analyzed the goodness of fit of the Bayesian mixing models examining the standard deviation of the fitted models with informative and uninformative Dirichlet prior distributions. Informative priors were derived from the conventional diet analysis performed during the incubation period (fish = 17, beef = 45, chicken = 94) and comparisons were made using two tailed t-tests.

When food sources were estimated for the entire population, we detected that standard deviation of beef (mean of $SD_{\text{informative priors}} = 0.55 \pm 0.24$; mean of $SD_{\text{uninformative priors}} = 0.51 \pm 0.21$) and chicken (mean $SD_{\text{informative priors}} = 0.24 \pm 0.15$; mean $SD_{\text{uninformative priors}} = 0.15 \pm 0.12$) were lower than using informative priors. On the other hand, standard deviation of fish (mean $SD_{\text{informative priors}} = 0.21 \pm 0.18$; mean $SD_{\text{uninformative priors}} = 0.34 \pm 0.18$) was lower or equal when estimated with uninformative priors than with informative priors (Table S1). However, when basal food sources were estimated for each nest, standard deviation using informative priors was smaller than using uninformative priors for the three basal food sources (Table S1).

The discrepancy between the estimations with informative and uninformative priors might be due to the different timing between conventional diet analysis (incubation period) and stable isotope analysis (egg formation during pre-breeding period). Priors obtained during the incubation period might not reflect the diet choice patterns during egg formation at the pre-incubation period. In fact, occurrence of beef in the Kelp Gull diet during the incubation period was almost half of the occurrence of chicken. However, the opposite pattern was recorded during the pre-incubation period, that possibly explains why informative priors might not have improved the output of the Bayesian mixed models as other studies have shown (Franco-Trecu et al., 2013).

In addition, changes in diet choice along the life cycle of this species have been recorded. Although we did not have information about the diet of the Kelp Gull during egg formation, we have detected diet changes between the breeding and non-breeding periods at a nearby colony (Burgues, 2015). Moreover, we have recorded that during the chick rearing period on Isla de Flores, chicks were fed just with chicken and with no beef (unpubl. data). Thus, changes in diet choice might occur in the short and long term, as other studies have also shown (Burgues, 2015; Giaccardi and Yorio, 2004; Ludynia et al., 2005). This

situation highlights the need to count with improved estimations of priors for Bayesian mixing models.

Table S1. Comparison of standard deviation (SD) of Bayesian mixing models, estimated for each nest of Kelp Gull on Isla de Flores, using informative and uninformative priors. (d.f. = degrees of freedom).

	Informative priors	Uninformative priors		
	Mean of SD (\pm SD)	Mean of SD (\pm SD)	<i>t</i> -value (d.f.)	P-value
Beef	0.24 \pm 0.02	0.19 \pm 0.02	6.09 (30)	<0.01
Chicken	0.15 \pm 0.01	0.09 \pm 0.01	42.26 (16)	<0.01
Fish	0.22 \pm 0.05	0.18 \pm 0.04	2.74 (30)	0.01

Supplementary References

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5. PLASTIC INGESTION BY A GENERALIST SEABIRD ON THE COAST OF URUGUAY

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5.1 Abstract

We analyzed plastic ingestion by Kelp Gull (*Larus dominicanus*) from 806 pellets collected between 2011 and 2013. Employing a Raman spectroscopy, we characterized those polymers used to produce the plastics ingested. Debris was recorded in 143 pellets (%FO= 17.7%, n= 202, 92.58 g). Plastic was found in 119 pellets (%FO= 83%) and non-plastic occurred in 56 pellets (%FO= 39%). The most important debris category was plastic film with 55.3% (n= 79). Plastic bags were observed in 19 pellets (%FO= 2.4%, weight= 25.02 g). Glass was the second most important component (%FO= 18.9%) followed by plastic fragments (%FO= 17.8 %). Plastic debris represented the 65.3% of the debris fragments (n= 132, weight= 58.84 g), and were composed by polyethylene (52%), polypropylene (26%), polyamide (12%), polystyrene (6%), polyvinyl chloride (2%), and polyethylene terephthalate (2%). How plastics were obtained by gulls and the effects on individuals are discussed, as well as environmental considerations to reduce plastic pollution on coastal environments.

Keywords: Plastic pollution, seabirds, synthetic polymers, plastic film, Uruguay.

5.2 Introduction

Semi-synthetic products, resulting from a combination of natural tissues and chemicals, started to appear in our daily life in 19th century as a cheap substitute for increasingly scarce natural materials like ivory, wood, or hard-working manufacturing products such as glass or metal (Moore and Phillips, 2011). But it was not until after the second world-war that synthetic plastics became to be used massively changing humanity lifestyle, for instance in transport, packaging, clothing, food, health care, construction, and telecommunications (Thompson et al., 2009). Since then, plastics rapidly increased their presence in our modern debris (Barnes et al., 2009). Due their massive use and persistence, plastics have been accumulating in aquatic ecosystems such as beaches, waterways, estuaries, lakes, the open as well as the deep sea (Free et al., 2014; Lima et al., 2014; Moore et al., 2011; Moore and Phillips, 2011; Van Cauwenberghe et al., 2013). For instance, Moore et al. (2011) showed that only two rivers in California drained to the Pacific Ocean 30 metric tons of plastic debris every 72 hours. The global ubiquity of this material entails the need to fully understand the magnitude of plastic pollution and create measures to mitigate it.

Ecological consequences of plastic-biodiversity interactions are one of the most important environmental problems globally. For instance, marine organisms that are entangled with plastic objects (e.g., packaging bands, ropes, fishing lines, or drift nets) suffer from drowning, strangulation, reduction of foraging efficiency and reproduction (Derraik, 2002; Moore, 2008). Consequently, effects are generally related with death, or a reduction in fitness. In Uruguay plastic pollution has been recently reviewed in aquatic ecosystems and has been detected that fresh water fishes, marine turtles, and seabirds ingest plastic objects, and that rafting plastic pieces can transport and improve dispersal of invasive marine invertebrates (Lozoya et al., 2015).

Plastic ingestion by seabirds have received increasing attention globally over the last years (e.g., Acampora et al., 2014; Avery-Gomm et al., 2013; Blight and Burger, 1997; Codina-García et al., 2013; Jiménez et al., 2015; Lavers et al., 2013; Ryan and Fraser, 1988; van Franeker and Law, 2015). Evidence has rapidly increased on pelagic seabirds

such as Shearwaters, Albatrosses, and Petrels that are not able to produce pellets and often die because of obstruction of their digestive tract. However, fewer studies have explicitly assessed plastic pollution on coastal seabirds like gulls (Lindborg et al., 2012; Yorio et al., 2014), but see Camphuysen et al. (2008), Ceccarelli (2009), Thiel et al. (2011), and Kühn et al. (2015) for further information. This species is generally easier to study because a great amount of information in a short period of time could be obtained by pellet analysis.

Moreover, Kelp Gull (*Larus dominicanus*) is a suitable species to assess plastic ingestion because it is widely distributed throughout its range. This species breeds in the Southern Hemisphere: South America, South Africa, New Zealand, Sub Antarctic Islands and in the Antarctic Peninsula (Harrison, 1983), and in Uruguay reproduce on eight coastal islands and group of islands (Yorio et al., 2016). This species is perceived by scientists and managers as an environmental concern. For instance, a) it is a potential vector of pathogens (e.g. *Enterobacteriaceae*), b) predate eggs and chicks of sympatric breeding species, affecting in some cases, the reproductive performance of threatened populations, c) feeds on skin and fat of Southern Right Whales (*Eubalaena australis*) modifying the behavior of mothers and calves during their breeding season, and d) they are a threat to airport security as they are a risk for aircraft collisions (Frere et al., 2000; Lenzi et al., 2010; Rowntree et al., 2001; Yorio et al., 2016; Yorio and Quintana, 1997). On the other hand, Kelp Gulls have been reported to be killed and injured by marine debris such as fishing lines in Argentina (Yorio et al., 2014).

In addition, Kelp Gull is a generalist seabird that learned how to exploit energy subsidies such as garbage (Yorio and Giaccardi, 2002) and fishing discards (González-Zevallos and Yorio, 2006) that are easily acquired. As with other *Larus* sp., several researchers proposed that these food supplies could have a significant effect in the increase of their populations along its distributional range (Coulson and Coulson, 1998; Giaccardi et al., 1997; Yorio et al., 1998). Therefore, this trait of its natural history could potentially intensify the negative effects described above.

In Uruguay, there has been detected that Kelp Gull feeds on a great variety of natural prey, including debris. One of the most important anthropogenic food items identified in its diet were plastic objects (Lozoya et al., 2015). As Kelp Gull frequently forage in landfills (Bertellotti et al., 2001; Giaccardi et al., 1997; Yorio and Giaccardi,

2002), they can find a large amount of organic food but also synthetic products that they can ingest. This figure may be occurring in Uruguay because Kelp Gull breeding colonies are close to urban areas, and subsequently to garbage dumps.

In this paper we made an assessment of plastic ingestion by Kelp Gull in a breeding colony of Uruguay from pellets collected between 2011 and 2013. In addition, using Raman spectroscopy we characterized the polymers employed to produce the plastic objects ingested, and further track the potential commercial products where they come from (e.g. plastic bags, bottles, etc.).

5.3 Materials and Methods

5.3.1 Study area

Isla de las Gaviotas (34°54'10" S, 56°06'16" W) is located 400 m off Montevideo city. Is a small island with a surface of 1.7 ha. (Figure 5.1). Guido et al. (2013) analyzed the vegetation of the island and found that is dominated by herbaceous plants and some woody species such as canary palm (*Phoenix canariensis*), castor oil plant (*Ricinus communis*) and saltcedar (*Tamarix ramosissima*). Despite its small size this island is habitat of more than 40 bird species (Unpubl. data) some of them of national conservation concern (Soutullo et al., 2009). Breeding population size of the Kelp Gull is small, which was estimated in 115 breeding pairs (Yorio et al., 2016), however, non-breeding population size is about five times higher. Kelp Gull breeds on the island in sympatry with American Oystercatcher (*Haematopus palliatus*), Cattle Egret (*Bubulcus ibis*), and Snowy Egret (*Egretta thula*).

5.3.2 Pellet analysis

Pellet samples were collected on Isla de las Gaviotas during 31 surveys between 2011 and 2013 (Figure 5.1). Pellets are those structures regurgitated by several bird species containing hard parts that are not digestible (Barrett et al., 2007). This technique has the advantage of being non-invasive, simple, and can provide large amount of information in a short period of time (Karnovsky et al., 2012). As pellets were collected in the same sites of the island, we can assume that pellets integrate the diet of the population between surveys. A total of 806 pellets were collected and analyzed in the laboratory where particles of debris were separated, weighed (to the nearest 0.001 g.), and stored for further analysis,

considering the pellet from which they came. Debris was categorized as plastic and non-plastic materials. Then, we sub-divided both categories as follows. Plastic: plastic film, user plastic, threadlike user plastics, laminated paper, styrofoam, and rubber; non-plastic: glass, threads, paper, metal, ceramic, and cotton. Although resin pellets were not present in the diet of the Kelp Gull (see Results), the category user plastic was created in order to differentiate both sources of plastic and facilitate comparison with other studies (see van Franeker and Law, 2015).

5.3.3 Plastic analysis

A sub-set of samples were selected randomly for polymer identification considering that we were able to analyze a limited amount of 50 samples. In order to determine sub-sample size per category, we took the sub-samples considering the size of the categories into account: Plastic film (24 of 82: 30%), User plastics (15 of 26: 57%), Threadlike user plastics (9 of 16: 56%), and foam (2 of 3: 66%). A less proportion of sub-samples was analyzed for Plastic film as we expected to have less diversity in the polymer composition, and we wanted to explore more deeply the nature of User plastic materials.

In order to characterize polymer composition, the subsample was analyzed using Raman spectroscopy with a Raman imaging microscope (Thermo Scientific™ DXR™xi). Cross-sectioned samples for Raman analysis were prepared using the Thermo Scientific™ Polymer Slicing Tool for DXR Raman microscopes.

5.3.4 Data analysis

Data analysis was carried out considering that our primary interest was to analyze the composition of the debris found in the diet of the Kelp Gull. Therefore, we estimated those indices commonly used in the literature (see Silva-Costa and Bugoni, 2013) considering the total amount of debris as our population: number of pellets (i.e. number of pellets where each category was present), frequency of occurrence (%FO as percentage of each category relative to the amount of pellets that contained debris), numeric percentage (%N as percentage of the number of debris fragments of each category relative to the total number of fragments), weight (sum of the weights of each category), and percentage of weight (percentage relative to the total debris weight).

5.4 Results

5.4.1 Debris composition

From the 806 analyzed pellets, 143 had debris (%FO= 17.7 %), represented by 202 debris fragments and 92.58g (Figure 5.2). Debris weight did not show variation among years (ANOVA: $F_{1,203}= 0.008$, $P > 0.05$). Within the array of pellets containing debris, plastic was found in 119 pellets (%FO= 83%) while non-plastic debris occurred in 56 pellets (%FO= 39%; we have to consider that in one pellet we will find plastic and non-plastic debris, so the sum of these percentages will be more than 100). Weight of plastic debris was 61.33 g. (66%) and weight of non-plastic debris summed 31.25 g. (34%).

The most important debris category was plastic film, found in 79 pellets that corresponded to 55.2% of pellets containing debris (Table 5.1, Figure 5.2a). Also its weight was the most important in terms of total weight (28.82 g.), percentage (31.1% of all the debris) and %N (40.6%) (Table 5.1). When a plastic film occurred in a pellet, sometimes it occupied 100% of it, because it frequently corresponded to an entire plastic bag or a big piece of it (Figure 5.3a). We detected plastic bags in 19 pellets (24% of those pellets containing plastic films), which corresponded to 13.0% of all the pellets containing debris. Their average weight was 1.32 g. per pellet and the total weight was 25.02 g. Surprisingly, glass was the second most important component of the debris (Table 5.1, Figure 5.2). Plastic fragments were the third most important category followed by threads and plastic paper (Table 5.1, Figure 5.3). Paper, metal and styrofoam were among the less important categories, while ceramic, cotton, and rubber, were of less importance (Table 5.1, Figure 5.3).

5.4.2 Polymer composition

Of the 202 debris fragments that were found in pellets, 132 (65%) were plastic debris, which weighted 58.84 g (65.3% of total debris). The sub-set of 50 sub-samples analyzed to determine polymer composition showed that polyethylene (PE) was the most important polymer found in the diet of *Larus dominicanus* with 52% (n= 26). Then, polypropylene (PP) was the second most important with 26% (n= 13). Polyamide (PA) was also high with 12% (n= 6). Other polymers were found in lower frequency: polystyrene

(PS, 6%, n=3), polyvinyl chloride (PVC, 2%, n=1), and polyethylene terephthalate (PET, 2%, n=1).

If we consider polymer composition within each sub-sample, Plastic film was composed by 22 fragments of PE (92%), 1 of PP (4%), and 1 of PET (4%). User plastics were mostly composed by PP (80%) followed by PE, PS, and PVC with one fragment (7%) each. Threadlike user plastics were mainly PA with six fragments (67%) and PE with three fragments (33%). Finally, the two fragments of styrofoam were composed entirely by PS.

5.5 Discussion

Plastic film was the most important debris category, while polyethylene and polyethylene were the most important polymers found in pellets. Based on that, we can suggest that plastic bags and nylon films may be a primary plastic contaminant for the Kelp Gull. These particular products are commonly known by poly bags and are widely used for instance in the food industry and as liners for an extensive array of products. As there are no restrictions for its use in Uruguay or in the region, this product is widely used and widespread in open dumps and in the inner zone of the Rio de la Plata estuary, as well (Acha et al., 2003; Lozoya et al., 2015).

The nature of the debris found in the pellet samples suggests that a high proportion, if not all of it, comes from landfills. For instance, we found threads used in food manufacturing, medication wrappers, clothing labels, parts of food wrappers and containers. Furthermore, organic matter was found in the samples (e.g., chicken bones, terrestrial invertebrates, small rodents, Unpubl. data) supporting the idea that gulls use landfills to forage at a great extent. Moreover, in studies where stomach contents are analyzed, such as in pelagic seabirds, plastic pellets are frequently recorded suggesting that they do not come from landfills, but from oceanic or coastal environments. Future investigations should quantify how much plastic and garbage comes from landfills, shoreline or the ocean to delineate management strategies for coastal species.

In addition, those species or individuals that use landfills to forage may be considered dispersal sources of plastics from inland to coastal regions and the oceans. This pathway of plastic transportation may be added to the list of already known ways, such as waterways carrying debris of human activities (landfills included), recreational activities on

the coast, marine traffic, failure in cleaning systems of municipalities, among others (Derraik, 2002; Lozoya et al., 2015). In addition, an assessment of this potential new connection between landfills and the ocean should be conducted in the future. Particularly, a quantification of the amount of debris carried by gulls from the inland to the coast would be necessary.

Identification of polymers may be a powerful tool to suggest what kind of products may be observed in the diet of the Kelp Gull. For example, polyethylene was the most important polymer found in pellets and is used to produce plastic bags and plastic films. These particular products are commonly known as poly bags and are widely used, for instance in the food industry and as liners for an extensive array of products. As there are no restrictions for its use in Uruguay or in the region, this product is commonly found in open dumps and in the inner zone of the Rio de la Plata estuary, as well (Acha et al., 2003; Lozoya et al., 2015).

Surprisingly, glass was the second most important debris component in the diet of the Kelp Gull. Other studies such as Coulson and Coulson (1993) also found glass with a lot of refuse in the Kelp and Pacific (*Larus pacificus*) gulls in southern Tasmania. In Argentina glass was recorded in the diet of the Kelp Gull, although its occurrence was very low and not quantified (Bertellotti and Yorio, 1999) or classified as garbage (Petracci et al., 2004). In addition, glass was also recorded in other species of gulls like Herring Gull (*Larus argentatus*) and Lesser Black-backed Gull (*Larus fuscus*), Yellow-legged Gull (*Larus michahellis*), Pacific Gull (*Larus pacificus*), Great Black-backed Gulls (*Larus marinus*), and Glaucous-winged Gull (*Larus glaucescens*) (Camphuysen et al., 2008; Coulson and Coulson, 2008; Ewins et al., 1994; Gilliland et al., 2004; Lindsay and Meathrel, 2008; Neves et al., 2006; Nogales et al., 1995; Trapp, 1979). On the other hand, glass was not recorded in the diet of a Kelp Gull population in Peru as it nested on an island far from anthropogenic food sources (Flores, 2005).

To explain the important amount of glass as a debris component, three possible non-mutually exclusive explanations could be drawn. First, glass is made from attractive and bright colors that may be interesting for the Kelp Gull to feed on. However, to our knowledge there is no antecedent about the incidence of coloration in food selection in gulls. Additionally, feeding on glass could also play the same role as feeding on stones in

the digestive process (Nogales et al., 1995) by helping in crushing those hard items in the gizzard (Goutner, 1994). Finally, its ingestion could also be accidental while trying to feed on other items. We can add to this figure the fact that glass availability may be very important in landfills because recycling has completely stopped since 90's, despite that since 2008 glass started to be recycled again but in a very limited amount.

Digestive tract of Kelp Gulls may allow them to regurgitate plastic fragments without dying of starvation as occurs in Procellariiform species. Charadriiformes, like gulls, do not have the constriction between the gizzard and proventriculus as Procellariiforms do, so gulls are able to regurgitate plastic fragments in pellets along with other indigestible materials (Azzarello and Van Vleet, 1987; Bergmann et al., 2015; Furness, 1985b; Lindborg et al., 2012). Although Gull's direct mortality resulting from plastic ingestion may not be common, it has not been thoroughly evaluated yet, as well as those indirect and sub-lethal effects. Nevertheless, gull mortality has been recorded by entangling with monofilament (polyamide) lines and fishing nets (Berón and Favero, 2009; Gregory, 2009; Moore et al., 2009; Taylor, 1996; Yorio et al., 2014). Yorio et al. (2014) found that, during a survey carried out in four Kelp Gull breeding colonies along nine days, 27 individuals were tangled and 22 of them were freshly dead. This indicates that lethal effects of plastics on gulls can be related with entanglement more than by direct ingestion.

Even though gulls may not die by plastic ingestion, they face several challenges that could affect their fitness. Seabirds can suffer a reduction in hunger and satiety, or a reduction in the stomach volume preventing them to assimilate food correctly (Ryan, 1989). Moreover, plastic "compete" with food in the gizzard reducing the amount of preys that could be digested. This situation may lead to a decrease in foraging efficiency as individuals have to allocate more time and energy to forage (Ryan, 1989). This situation may limit the amount of energy that could be allocated to life history traits like body weight, reproduction, development, or survival (Ryan, 1989; Stearns, 1992). Although our knowledge of these effects in seabirds' life histories is limited, evidence that body weight and condition are negatively affected by plastic ingestion is available for other seabird species, including pellet producers like Charadriiformes (Furness, 1985a; Spear et al., 1995). Knowledge from other animal taxa such as lugworms and barnacles (Besseling et al., 2012; Hentschel, 2015; Wright et al., 2013) support the claim that plastic ingestion

affects individuals life histories and that more studies are necessary to quantify the effect size of this animal-plastic interaction.

Another important aspect of plastic debris ingestion by seabirds is the exposure to organic contaminants (e.g., persistent organic pollutants, POPs) associated with plastics. It is well known that plastic debris accumulate contaminants due its hydrophobic nature. Persistent organic pollutants include industrial chemicals such as polychlorinated biphenyls (PCBs), and chlorinated pesticides such as dichlorodiphenyltrichloroethane (DDT), hexachlorobenzene (HCB) or hexachlorocyclohexanes (HCHs). Several reports (Ogata et al., 2009; Rios et al., 2007) have found the occurrence of POPs on marine plastic debris and state that these plastics are important sources of this contaminants into the marine environment. Moreover, several studies have found POPs in the tissues of seabirds around the world, with a similar contamination profile than the plastic debris associated with the animals analyzed (Colabuono et al., 2010). Thus, there is a concern regarding the possible transfer and deleterious effects of these contaminants from plastics to the marine organisms.

Unmanaged open sky landfills may be the main source for plastics ingested by gulls, as well as for plastic pollution on the coast and oceans. When landfills are managed food availability is reduced, because landfills are less attractive to them (Giaccardi et al., 1997). These authors found that abundance of Kelp Gull decreased in a landfill in Argentina after management practices were implemented. In addition, (Lozoya et al. (2015)) found that waterways can be an important way of plastic transportation from landfills to the coast of Uruguay. These authors estimated that 15 landfills were less than 300 m from the nearest waterway, and two of them were placed directly on waterways or discharging their leakages directly into a waterway. Accordingly, proper management of landfills needs to be a priority to reduce plastic ingestion by gulls as well as pollution on the coast.

The high proportion of plastic debris in the diet of the Kelp Gull on Isla de las Gaviotas reinforces the general idea that production and use of plastics need to be regulated, as it is known how harmful they are for the environment (e.g., Gregory, 2009; Moore, 2008). Fortunately, there are countries and local governments that has been increasingly taking actions to reduce their use, for instance India or Bangladesh (Ritch et

al., 2009), and recently the State of California in the United States. Unfortunately, although legislation in Uruguay seems to be modern and “inspired in European Directives” (Lozoya et al., 2015), it is not enforced by the government at all.

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Table 5.1. Types of debris found in Kelp Gull pellets on Isla de las Gaviotas. * synthetic polymers that were later analyzed by using Raman spectroscopy.

Polymer	No. Pellets	FO	%N	Weight	%weight
Plastic film *	79	55.2	40.6	28.82	31.1
Threadlike user plastics *	16	11.2	7.9	0.33	0.4
Glass	34	23.8	21.3	16.15	17.4
Rubber	1	0.7	0.5	0.02	0.0
Threads	13	9.1	6.4	2.22	2.4
User plastic *	24	16.8	12.9	26.8	29.0
Paper	5	3.5	2.5	5.19	5.6
Foam	3	2.1	1.5	0.31	0.3
Metal	5	3.5	2.5	2.69	2.9
Laminated paper	6	4.2	3.0	4.97	5.4
Ceramic	1	0.7	0.5	3.96	4.3
Cotton	1	0.7	0.5	1.05	1.1



Figure 5.1. Location of Isla de las Gaviotas on the coast of Montevideo city.

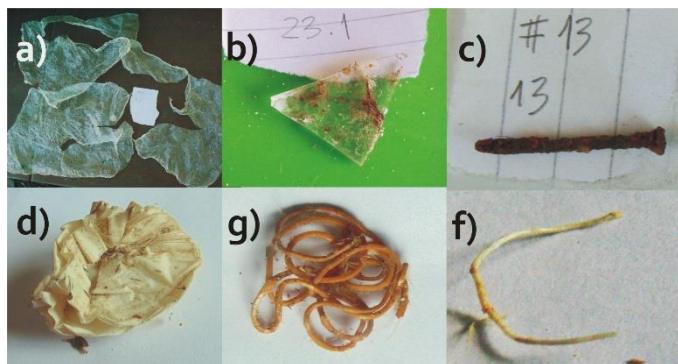


Figure 5.2. Some samples of debris categories found in the diet of the Kelp Gull on Isla de las Gaviotas. a) plastic film, b) glass, c) metal, d) paper, e) rubber, and f) thread.

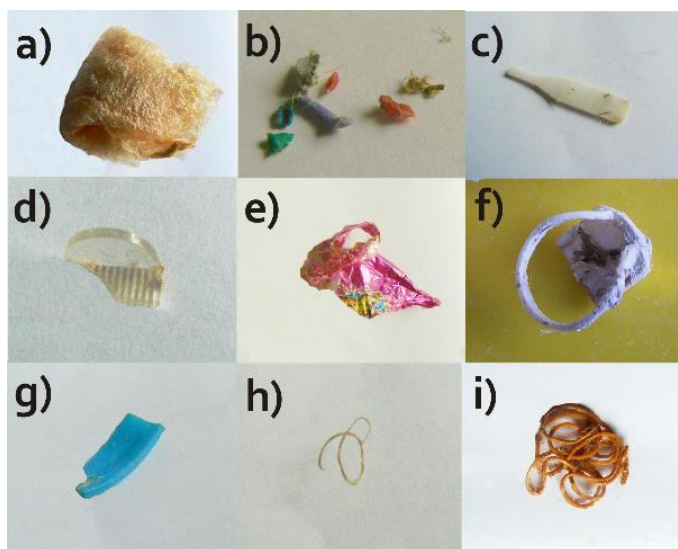


Figure 5.3. Fragments of synthetic polymers found in the diet of the Kelp Gull on Isla de las Gaviotas. a) plastic bag, b) plastic fragments, c) tip of a disposable coffee spoon, d) fragment of a compact disc case, e) candy envelope, f) container security cover, g) plastic fragment, h) plastic thread, and i) rubber band.

6. CONCLUSIONS

The central aim of this research was to assess the impacts of anthropogenic food subsidies on the acquisition and allocation of resources of gulls with a particular focus on their impacts on fitness components, i.e., fecundity and reproductive success. In the literature review (Chapter 2), we assessed the impact of refuse on gulls in broad context. In addition to fitness components, we incorporated those direct and indirect ecological factors impacted by refuse, such as movement patterns, habitat use, interspecific interactions, pathogen infections, and water quality. In Chapters 3-5, we studied the consequences of foraging on refuse on the Kelp Gull and produced three research papers. The first analyzed the impacts of refuse on chick's isotopic niche; the second studied the impact of refuse on fitness components of the Kelp Gull; and the third addressed the ingestion of plastic polymers by this species associated to foraging on sources of refuse.

At least 23 species of gulls use refuse as an anthropogenic food source. The impacts of refuse are diverse, affecting resource acquisition and allocation processes, in many positive and negative ways. Most of the reviewed evidence concludes that refuse has more energy than natural food positively impacting gull's fitness components and population dynamics. However, some evidence also show that refuse negatively impacts fitness components and population dynamics. Some authors like Raymond Pierotti and Cynthia Annet have argued that energy might not be a proper currency, but nutritional quality of food might be better to analyze the impacts of this subsidy on resource acquisition and allocation. Based on our results, we argue that the mix of levels of biological organization (i.e., individuals, colonies, populations, and species), as well as the methodological tools (e.g., incorporation of stable isotopes to traditional diet studies), might have prevented suitable comparisons among studies, reducing our ability to propose general mechanisms to explain our observations. We conclude that there is a need for research that incorporates the individual level of biological organization, which is largely underrepresented in the literature, and stable isotope approaches that reflect actual absorption of nutrients into body tissues.

This dissertation work brings new insights to the existing knowledge about the impacts of anthropogenic food subsidies on gulls. We found that changes in resource

acquisition and allocation processes of the Kelp Gull in response to anthropogenic food subsidies evidenced the variable impacts on the ecology and evolution for this group of species. A proportion of Kelp Gull parents modified their foraging decisions in response to the presence of refuse near the reproductive colony, with behavioral and fitness consequences. We conclude that nestling's size might determine what foraging habitats parents will use during the nestling rearing period. Individual nestlings foraged more on natural prey after hatching and incorporated refuse later in the rearing period when they are big enough to incorporate bigger meals. Moreover, they expanded their isotopic niche width as they grew, incorporating new isotopic signatures of carbon and nitrogen, reflecting a potential expansion in at least two dimensions of the ecological niche: the foraging habitat and the identity of basal trophic sources.

Impacts of refuse on fitness components of Kelp Gull parents might have occurred as well because individual females, which during the pre-laying period foraged more on refuse, tended to lay heavier eggs and produce fewer fledglings, while those females that foraged on natural prey tended to lay lighter eggs, but produced more fledglings. These impacts are expected to have consequences at the population level. Importantly is to study the Kelp Gull ecology along a broader part of the life cycle of individuals. Additionally, we need to know the distribution of individual reproductive success within the population, in order to predict population trajectories according to individual foraging strategies (see Section 7 below).

The results of this dissertation reinforces the notion that inadequate waste management systems not only make available energetic and nutritional subsidies to gulls, but also that these systems make available harmful materials, like plastic polymers. The large proportion of plastic polymers waste found in Kelp Gull diets supports the idea that plastic production and use need to be regulated. Fortunately, a recent law (No. 19,655) was issued by the senate that only allows the production, commercialization, and use of compostable or biodegradable bags in Uruguay. Additionally, the parliament is discussing an additional law to comprehensively improve the waste management system, which promotes the circular economy concept through a variety of instruments for each economic sector. This dissertation offers the opportunity to generate a baseline to address the effectiveness of these new legislative efforts to reduce environmental pollution.

7. FURTHER RESEARCH

It is widely accepted that populations of generalist species are composed by ecologically heterogeneous individuals that differ in their foraging behavior (Bolnick et al., 2002). Thus, a population could be composed by generalist individuals that take the same wide range of resources (Type A generalists), or by individuals that specialize on different specific resources (Type B generalists) (Bearhop et al., 2004). Acknowledging this type of configuration on the foraging behavior of a population, we can formulate better mechanistic models from the individual traits, and be able to incorporate individual variation (Araújo et al., 2011). Additionally, individuals could experience different kind of selective pressures based on the exploitation of specific resources (Bolnick et al., 2002) and anthropogenic food subsidies could play a major role in relaxing or tensing these pressures.

Trying to integrate these ecologically and evolutionary implications of individual specialization in gulls, we could propose that generalization type A or B could arise at different stages of the life cycle, depending on the environmental and physiological constraints. For instance, we could predict type B generalization in those extremes of Kelp Gull females that during the pre-incubation period foraged mostly on fish or refuse. In addition, those type A individual females, i.e., females with true mixed diets, could also be predicted similarly to the observations of Pierotti and Annet (1991). Type B generalization is plausible in our dataset because we observed a large variability in the proportion of fish and garbage among individuals. In a scenario where time and spatial constraints imposed by reproduction are not acting, we could expect type A generalization become predominant, because individuals are dispersed and could be able to exploit foraging habitats more errantly than during the reproductive period.

Although we have available literature showing that population sizes varies over time, we unfortunately lack of studies that address population fluctuations coupled with changes in the extent of human settlements, and how this might have historically impacted on gull's foraging behavior. An interesting set of tools to reconstruct the historical ecology of gulls and the impacts of humans on their population dynamics and foraging behavior are the use of stable isotopes on museum specimens and the coalescent theory approach. The benefits of stable isotope analysis have already been explained in this dissertation.

Regarding the coalescent theory, we could say that it is a molecular approach that allows us to extract information about the relationships between individuals and populations, population sizes (present and historical), and estimate divergence times from molecular markers (Friesen, 2007; Taylor and Friesen, 2007). Thus, applying the proper spatial scale, we could be able to study how this species have become synanthropic and might have changed its foraging patterns according to the presence or absence of human settlements along its range.

Negative impacts of ingestion of refuse, like plastics, are amplified by the toxic compounds (e.g., pesticides, agrochemicals, fire retardants, additives) present in refuse sources, like landfills and sewage offal. Leachates, wastewater effluents, and landfill air contain high levels of these toxic compounds (Gorga et al., 2013; Kim et al., 2013; St-Amand et al., 2008), which could be adsorbed not only by foraging, but also by contact, inhalation, and ingestion of dust or soil (Mineau, 2011). Thus, considering that gulls use these habitats, it is important to analyze what is the use of the landscape by individuals in order to trace the exposure pathways of gulls to chemical contamination. Moreover, considering the existence of individual specialization within generalist populations, it might be interesting to introduce inter-individual foraging strategies to examine pollutant sources and pathways, and its ecological and evolutionary implications.

As with chemical pollutants, pathogens like *Salmonella* and *Clostridium*, are present in refuse sources. For instance, they have been found in food but also in the soil and water of different foraging habitats, e.g., waste water treatment plants, landfill, sewage offal, coastal waters, among others. Thus, a strong spatial dimension also arises when we want to establish the sources and pathways for pathogen infection, its link with the foraging behavior of individual gulls, and consequently the implications at ecological and evolutionary levels.

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