

### UNIVERSIDADE D COIMBRA

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### **URBAN GULLS: ADVANTAGES AND DISADVANTAGES OF LIVING IN THE CITY**

Dissertação no âmbito do Mestrado em Ecologia, orientada pelo Professor Doutor Jaime Albino Ramos e pela Doutora Ana Claúdia do Souto Gonçalves Norte e apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia da Universidade de Coimbra.

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob a orientação científica do Professor Doutor Jaime Albino Ramos (Universidade de Coimbra) e da Doutora Ana Claúdia do Souto Gonçalves Norte (Universidade de Coimbra).

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#### Abstract

Human population has been growing exponentially over the past decades and the majority now occupies large urbanized areas. Urban landscapes are continuously growing and taking place of once natural habitats for many animals. Wildlife must either adapt to the new conditions or flee with great consequences on ecosystems. Gulls seem to adapt easily to the urban environment and are, therefore, a good model species to evaluate its impacts on wildlife. Urban gull colonies have been growing around the world, their adaptability is attributed to the generalist and opportunistic feeding behaviour allowing gulls to feed on different areas from aquatic to terrestrial. Urban gull populations are growing rapidly and dominate urban communities leading to several conflicts with humans.

Our main goal was to assess and understand the main differences between natural and urban nesting yellow-legged gulls *Larus michahellis*. Four colonies were studied, two natural (Deserta and Berlenga) and two urban (Peniche and Porto), across two breeding seasons (2018 and 2019). Samples were collected in each colony regarding reproductive (clutch size, egg volume, hatching success, chick growth), behavioural (intraspecific aggression) and physiological parameters (erythrocyte sedimentation rate, heterophile/lymphocyte general stress index, white blood cell count, haemoglobin concentration, oxidative status, general immune system response, body condition).

Results show that clutch size and egg volume were lower in urban colonies however no difference was found on chick growth. Intraspecific aggression was also lower in urban colonies because urban colonies present lower nest density. Adults from urban colonies exhibited lower erythrocyte sedimentation rate and denoted higher stress levels. Chicks from natural colonies also showed high stress levels with higher heterophil/lymphocyte index and white blood cell counts and lower haemoglobin concentration. However, chicks from Porto had higher concentration of reactive oxygen metabolites showing higher oxidative stress.

Overall, it is not possible to determine the best nesting habitat for gulls. Natural colonies seem to enjoy greater reproductive success but showed higher stress levels. There are advantages and disadvantages to breeding in each habitat, however it seems that urban colonies are growing at a much faster rate. This study enables us to understand some of the advantages that urban habitats may have for breeding gulls, which is important to manage the antagonistic relationship between urban gulls and humans.

**Keywords:** urbanization, yellow-legged gulls, reproductive output, adaptation, physiological stress

#### Resumo

A população humana tem crescido exponencialmente nas últimas décadas e a maioria agora ocupa grandes áreas urbanizadas. As paisagens urbanas estão constantemente a crescer e ocupar o lugar de outrora habitats naturais para muitos animais. A vida selvagem tem de adaptar-se às novas condições ou "fugir", com grandes consequências para os ecossistemas. As gaivotas parecem adaptar-se facilmente ao ambiente urbano e, portanto, são uma boa espécie para avaliar os seus impactos na vida selvagem. As colónias de gaivotas urbanas têm crescido em todo o mundo e a sua adaptabilidade é atribuída ao comportamento alimentar generalista e oportunista, permitindo que as gaivotas se alimentem em diferentes áreas desde aquáticas até terrestres. As populações de gaivotas urbanas crescem rapidamente e dominam as comunidades urbanas levando a vários conflitos com humanos.

O objetivo principal foi avaliar e compreender as principais diferenças entre as gaivotas-de-patas-amarelas *Larus michahellis* nidificantes em colónias naturais e urbanas. Foram estudadas quatro colónias, duas naturais (Deserta e Berlenga) e duas urbanas (Peniche e Porto), ao longo de duas épocas de reprodução (2018 e 2019). Em cada uma das colónias foram avaliados parâmetros reprodutivos (tamanho da postura, volume dos ovos, sucesso de eclosão, crescimento das crias), comportamentais (agressão intraespecífica) e fisiológicos (taxa de sedimentação de eritrócitos, índice geral de stress heterófilos/linfócitos, contagem de leucócitos totais, concentração de hemoglobina, estado oxidativo, resposta geral do sistema imunitário, condição corporal).

Os resultados mostram que o tamanho da postura e o volume dos ovos foi menor nas colónias urbanas, porém nenhuma diferença foi encontrada no crescimento das crias. A agressão intraespecífica também foi menor nas colónias urbanas, pois estas apresentam menor densidade de ninhos. Adultos de colónias urbanas exibiram menor taxa de sedimentação de eritrócitos denotando maiores níveis de stress. Crias de colónias naturais também apresentaram altos níveis de stress com maior índice de heterófilos / linfócitos e contagem de leucócitos e menor concentração de hemoglobina. No entanto, crias do Porto apresentaram maiores concentrações de espécies reativas de oxigénio, apresentando maior stress oxidativo.

Não é possível determinar qual o melhor habitat de nidificação para as gaivotas. As colónias naturais parecem ter maior sucesso reprodutivo, mas apresentam níveis mais altos de stress. Existem vantagens e desvantagens na reprodução em cada habitat, no entanto, parece que as colónias urbanas crescem a um ritmo muito mais rápido. Este trabalho permitiu compreender melhor algumas vantagens que o ambiente urbano pode proporcionar às gaivotas nidificantes e, assim, propor medidas que ajudem a mitigar a sua relação antagonista com os humanos.

**Palavras-chave:** urbanização, gaivotas-de-patas-amarelas, adaptação, output reprodutivo, stress fisiológico

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### **1. Introduction**



#### 1.1. Urbanization – a shift in ecosystem functioning

Over the past decades human population has grown exponentially and continues to grow to this day. Predictions aim to a population of 11 000 000 000 (11 billion) people by 2100 (Roser et al. 2019). As the human population grew, so did it's impacts on wildlife and ecosystem functioning (Chamberlein et al. 2009). In a developed and more technologically advanced world the tendency is for population to converge in large urban centers, in a process known as urbanization. As a result of this, the world's urban population has multiplied tenfold in the last century (Marzluff et al. 2001) and by 2008, already more than half of the world's human population was living in urban areas (United nations 2014; Reynolds et al. 2019). Space became a constraint; people tend to redistribute themselves through suburban or exurban areas causing cities to grow much larger and faster in area than in population (Alberti et al. 2001; Marzluff et al. 2001). This originated modifications of the landscape, artificial infrastructures replaced natural habitats (Hamer and McDonnell 2010; Lowry et al. 2012), and the structure and functioning of ecosystems was modified (Vitousek et al. 1997).

Urbanization refers to the population shift from rural areas to urban areas by gradually transforming uninhabited areas into land including some degree of relatively permanent human presence (Marzluff et al. 2001). It is characterized by land-use changes and transformations such as increased residential housing, business development and transport infrastructure, resulting in destruction and fragmentation of habitats (Reynolds et al. 2019; Sol et al. 2020). Between one-third and one-half of the land surface has been transformed by human action and many ecosystems are dominated by man (Vitousek et al. 1997), urban expansion is the major driver of land modification, with a projected increase in urban land cover of 1.2 million km<sup>2</sup> by 2030 (Seto et al. 2012). This poses a tremendous threat to wildlife as urbanization is driving rapid declines in species richness and diversity worldwide (Beissinger and Osborne 1982; Para-Torres et al. 2020; Sol et al. 2020), and it was identified as the single most important driver of extinction during the last century (Marzluff et al. 2001). Extinction is a natural process, but is occurring at unprecedented rates, for instance, about one-quarter of the bird species on Earth have been driven to extinction over the past two millennia (Vitousek et al. 1997).

Urban areas pose new challenges to wildlife: human disturbance, non-native predators, noise, traffic, light pollution and air pollution, among other stress factors (French et al. 2008; Partecke et al. 2006; Reynolds et al. 2019). Only a few generalist species can thrive under these urban conditions leading to an overall loss of biodiversity and biotic homogenization (Mckinney and Lockwood 1999). As these generalist species

grow exponentially in urban areas, species richness in cities is often lower than in surrounding natural habitats (Sol et al. 2020). To prosper in urban conditions requires great adaptability and behavioural flexibility/plasticity (Lowry et al. 2012). This process is called synurbization, which denotes the adjustment of wildlife to specific urban conditions (Luniak 2004). Such adaptations can include reduced stress responses in european blackbirds Turdus merula born in cities when compared to those born in natural habitats, an effect of possible downregulation of their physiological stress response to allow them to endure the stressful urban environment (Partecke et al. 2006). Other evidence shows that urban artic terns Sterna paradisaea take only a few seconds to get back to the nest after human disturbance while natural Artic terns take several minutes. This shows that urban populations of many species developed a habituation to human presence (Syrová et al. 2020). Adaptations can come in many other forms for different animals such as: reduced migratory behavior caused by animals wintering in the city instead of migrating to wintering natural areas due to warmer microclimate conditions in the cities (Partecke et al. 2006), prolonged breeding season, prolonged circadian activity, changes in nesting habits, tameness towards humans, etc. (Luniak 1996).

However, the main key factor for animal populations to thrive in urban conditions is availability and accessibility to food resources. Food from human origin such as refuse dumps, discards from fisheries, livestock middens, or crop leftovers are readily available, abundant and predictable (Ramírez et al. 2020) favouring generalist and opportunistic animals that alter their distribution and activity patterns in response to such changes in food resources (Para-Torres et al. 2020). Several species take advantage of human derived food, for instance predators such as red foxes *Vulpes vulpes* and coyotes *Canis latrans* have expanded their foraging activities to urban areas to profit from anthropogenic resources (Donk et al. 2019).

A group that successfully thrived and adapted to the urban environment are avian species. In some cases, residential and urban communities of birds have higher densities than those in outlying natural areas (Graber and Graber 1963, Emlen 1974). Among the most thriving birds is the pigeon *Columba livia*. In every city one can see them in great numbers, prospering by taking advantage of human litter and food. Another group that as seen a dramatic increase of their populations worldwide are gulls (Auman et al. 2008).

Gulls (*Larus spp.*) are very well adapted to urban life. Gull adaptability is mainly explained by their generalist and opportunistic behaviour (Ceia et al. 2014; Duhem et al. 2005) allowing them to forage in various areas, from aquatic to terrestrial habitats (Washburn et al. 2013). The rapid growth of their populations and relative dominance in urban communities, in a short time period, led to several conflicts with humans, therefore urban populations are often considered undesirable or pests (Beissinger and Osborne

1982; Pedro et al. 2013; Ramos et al. 2009b). Such conflicts derive from noise, aggression to people, food-snatching (Goumas et al. 2019), corrosion of buildings or other structures by faeces, clogging of buildings' outflow, occurrence of parasitic larvae as a result of dead chicks on roofs (Raven and Coulson 1997; Oro and Martínez-Abraín 2007; Soldatini et al. 2008; Coulson and Coulson 2009). Gulls can also cause problems disrupting air traffic in airports. There is evidence that gulls act as vectors for disease transmission (Belant 1997), including those caused by *Salmonella sp., Bacillus sp., Clostridium sp., Cryptosporidium sp.*, and *Escherichia coli* (Belant 1997; Anderson and Devlin 1999; Bosch et al. 2000; Ramos et al. 2009a; Coulson and Coulson 2009). Also, gulls have been held responsible for negative effects on species of conservation, when sensitive species can be disturbed, predated and displaced from their breeding areas by gulls (Vidal et al. 1998; Oro et al. 2005; Matias and Catry 2010).

#### 1.2. Ecology of urban gulls

Several reasons may explain the rise of gull populations in urban areas such as protection from hunting and egg harvesting in the city (Ackerman et al. 2018). However, the most important reason is attributed to the increased availability of human-derived food resources present in landfills, refuse dumps, fishing offal and discards or irrigated agriculture (Auman et al. 2008, Duhem et al. 2003, Matias and Catry 2010, Washburn et al. 2013). Duhem et al. (2008) found a strong positive correlation between the exponential growth of yellow-legged gull (*Larus michahellis*) populations in 3 archipelagos along 80 km of the French Mediterranean coastline and the increased availability of anthropogenic food resources near the colonies.

Gull species have been shown to feed both on fisheries discards and refuse tips (Bosch et al. 1994; Sol et al. 1995) and gull's movements and space use are largely dictated by the location of such landfills and refuse dumps. It has been reported that gulls will reduce their consumption of marine prey when their colony is situated farther from a fishing harbour in comparison to a landfill or other anthropogenic food sources (Zorrozua et al. 2019). As a consequence, urban gull colonies show higher rates of foraging site fidelity, because of the predictable and consistent anthropogenic food sources, and decreased search effort and energy expenditure (Fuirst et al. 2018). The high trophic plasticity exhibited by gulls allows them to alter their overall foraging ecology (Ramírez et al. 2020) in relation to the food sources available. California gulls (*Larus californicus*) have been shown to alter the timing of their foraging pattern by adjusting it with laboring hours of nearby landfills. They foraged from 6 a.m., throughout the day while the garbage

was being delivered, and finished around 6 p.m. when garbage deliveries ended, and the exposed refuse was covered (Ackerman et al. 2018).

There are costs and benefits from the ingestion of urban-derived food. These foods are often of lower nutritional quality (Derryberry and Coomes 2020) when compared to marine resources that are generally recognized as a more profitable resource (Duhem et al. 2005). But the overall effects on individual health are dependent on location and some studies report greater body condition for specific urban gull populations (Auman et al. 2008). Pierotti and Annett (1990, 1991) found that a diet of human refuse was of poorer quality for Herring Gulls (Larus argentatus) but had the highest caloric value and the highest fat and protein content per gram when compared to a range of natural dietary items, easily explained by the availability of specific food items in refuse dumps such as chicken, pork and cow meat remains (Romero et al. 2019). However, feeding in these locations may come at a cost. Regurgitates from a pacific gulls (Larus pacificus) population in Tasmania were found to contain large debris such as plastic, food wrappers, dental floss, plastic toothpicks, personal-hygiene products and even glass debris (Stewart et al. 2020). Overall, the abundance and nature of anthropogenic food resources play an important role in determining gull diet (Belant et al. 1993; Bosch et al. 1994; Oro et al. 1995), the spatial distribution of gulls during both the breeding season and winter (Fasola and Canova 1991; Sol et al. 1995), the choice of nesting sites (Scarton and Valle 1996) and reproductive parameters (Bukacínska et al. 1996; Oro et al. 2004).

Gull diet can affect growth and survival of chicks both in terms of the food and its energetic and nutritive value, but also because of the time that adults take to forage for food leaving the chicks unsupervised (Watanuki 1992; Alonso et al. 2015). In this matter, evidence is contradictory, with some studies finding a positive correlation between anthropogenic food diet and reproductive success (Kadlec and Drury 1968; Hunt 1972; Sibly and McCleery 1983; Pons 1992; Belant et al. 1993) and other studies finding the opposite (Pierotti and Annett 1991; Belant et al. 1998; Weiser and Powell 2010). Pierotti and Annett (1991) also found that adults that foraged mainly for mussels produced larger and heavier clutches, hatched more eggs and had more fledging chicks in comparison to adults that fed on refuse. An increased accessibility to large amounts of urban food may not compensate for the lack of specific nutrients, which are important for breeding adults and their chicks (Weiser and Powell 2010). Once again, the effect of urban-derived food in gull's diet on the reproductive success is highly dependent on location because different locations have different types of food available with higher or lower quality.

However, diet is not the only factor affecting reproductive success, which depends on an interaction between several factors (Watanuki 1992).

Nesting location is an important factor to consider. As previously mentioned, the abundance and availability of anthropogenic food has a role in determining appropriate nesting sites leading to an increase of the recruitment rate of colonies and consequently increasing urban gull populations (Duhem et al. 2008; Egunez et al. 2017). Landfills, refuse dumps, parking lots and shopping centers provide fixed and predictable food sources that may be advantageous in the breeding season when adults have limited energy to forage for food and time to spend away from their nest (Egunez et al. 2017). Such locations have also been shown to provide a place for social interaction between gulls (Belant et al. 1993), which is another reason for gulls to nest closer to these areas. In the beginning of 20<sup>th</sup> century, urban gull colonies were small and scarce and, although cities are often considered sub-optimal nesting habitats, the numbers of urban nesting individuals have increased continuously, particularly nesting in building's rooftops (Soldatini et al. 2008). One of the hypothesis to explain why gulls choose to nest on rooftops is that population growth rates are higher than territory vacancy rates in traditional natural habitats, and therefore adults have to use a non-preferred nesting site because of lack of natural sites (Dolbeer et al. 1989). On the other hand, other studies suggest that rooftops may be of equal or higher quality than natural habitats (Perlut et al. 2016). Most nests are built on flat roofs, usually from industrial, commercial, office or uninhabited buildings (Zelenskaya 2018). This is determined by the low accessibility of people to the rooftops, because such buildings have a low number of visitors to the roofs. Gulls appear to differentiate between buildings, by avoiding one-story buildings and preferring higher buildings to build their nests (Zelenskaya 2018). The nest is generally not built in an open area but is usually located next to a barrier (behind pipes, next to walls, or any larger objects or structures) (Hooper 1988; Zelenskaya 2018). Gulls can also nest in other man-made structures such as bridges, pipelines, shipyards or even oil rigs (Raven and Coulson 1997).

Many reasons can explain why urban colonies may enjoy greater reproductive success compared to non-urban nesting conspecifics. Most obviously, mammalian predators are often deterred from urban environments because of the use of tall buildings (Kroc 2018). Therefore, rooftops can be beneficial for breeding gulls because: a) it protects eggs from ground predators and provides a structural territorial boundary that cannot be physically breached by any chicks that have not yet learned to fly (Kroc 2018), and b) the size of one rooftop often allows only a single nest or a small group diminishing any contact and intraspecific predation from other adults (Ellis and Good 2006). However, several studies showed that rooftop nesting populations present a lower reproductive success, indicating that this may not be the best nesting habitat (Dolbeer et al. 1989; Soldatini et al. 2008). On the contrary, other studies show that rooftop nesting

gull populations have, in fact, lower clutch sizes than natural breeding conspecifics, but they enjoy a considerably greater post-hatch fledging success and thus a greater breading success (Monaghan 1979; Perlut et al. 2016; Kroc 2018).

Overall, urban living has its advantages and disadvantages. In urban areas animals are exposed to several stressful factors (Partecke et al. 2006; French et al. 2008; Bailly et al. 2017; Bauerová et al. 2017; Phillips et al. 2018; Injaian et al. 2018, 2019; Reynolds et al. 2019) that can affect animals in many ways and have physiological repercussions on their immune system, oxidative stress, heart rate, body condition, reproductive output and behaviour, among others (Blanco et al. 2004; Belskii et al. 2017). The presence and impacts of such stress factors can be assessed, in great part, using blood analyses: heterophil/lymphocyte index, erythrocyte sedimentation rate, white blood cell count, haematocrit, haemoglobin, corticosterone levels, oxidative balance, among others (Blanco et al. 2004; Partecke et al. 2006; Norte et al. 2008; Heylen and Matthysen 2008; Cīrule et al. 2011; Constantini et al. 2014b; Mallory et al. 2015; Injaian et al. 2018). These parameters can give an overall picture of the individuals health and the stress they endure whilst living in cities and might help explaining their reproductive success and population trends.

#### 1.3. Study and Objectives

In this study we aim to compare reproductive, behavioural and physiological parameters of nesting yellow-legged gulls (*Larus michaellis*) in four locations with an increasing urbanization gradient across two breeding seasons. This species is widely distributed and presents a great degree of adaptability making it a good model to evaluate the impacts of urbanization and assess the differences between natural and urban nesting gulls. The results should provide a better understanding of the positive and negative effects of breeding in urban areas and how well this gull species has adapted to the urban environment. Aiming to answer the question - Is it better to breed in the city? – we hypothesize that reproduction should be negatively impacted by urban life resulting in lower clutch sizes, hatching success and chick growth. Physiological parameters evidencing nutritional quality and stress are also expected to be negatively affected in urban areas enable less intraspecific aggression due to the decrease in nest density. However, our prediction of urban nesting costs for gull populations can be disrupted by their adaptative behaviour. Long-term coexistence with such conditions may develop

habituation in the individuals and factors that previously had a bigger effect on gulls can now be suppressed.

With increasing urban-nesting gull populations there is also an increased conflict between humans and gulls. Gulls can be very noisy and messy neighbours and many species seem to be very tolerant to human deterrent efforts (Kroc 2018). In response, humans can assume a destructive role towards gull reproduction by destroying eggs and nests. Thus, it is very important to comprehend the effects of urbanization on gulls in order to understand ecological implications of urban-adapted living and reproduction and how to mediate this difficult relationship with humans. As many species adapt to living in the city this can prove to be an opportunity for species conservation and therefore is important to understand the adaptative behaviour of wildlife and its relationship with different degrees of urbanization.

## 2. Materials and methods



#### 2.1. Study Areas

This study was conducted at four different locations with four distinct colonies: two natural (Deserta and Berlenga) and two urban colonies (Porto and Peniche). The field work was carried out during the gull's breeding season between the months of May and June of 2018 and 2019. Deserta Island (Figure 1d) (36°58'N, 7°52'W), also known as Barreta, is one of five barrier islands that are part of the Ria Formosa National Park, Algarve. This Natural Park is composed by the five barrier islands (Barreta, Culatra, Armona, Tavira and Cabanas) and by two peninsulas (Ancão and Cacela) (Ceia et al. 2010). The park also includes a complex system of channels, marshlands, saltpans and fish farms alongside the main lagoon. It extends for 60km and covers up to 18400 ha (Catry et al. 2004, 2006; Paiva et al. 2008). It was designated as a Natural Reserve in 1978, Natural Park in 1987 and is now part of the Natura 2000 network and it is protected by the Ramsar and Bern conventions (Ceia et al. 2010). Deserta island has large areas of dunes and sandy beaches and it holds a large population of breeding yellow-legged gulls: in 2014 there were about 900 breeding pairs (Calado et al. 2018), 1200 in 2018 (Matos et al. 2018) and 900 in 2019 (unpublished data). There is a high fishing activity in the area with the main fishing port, in Olhão, just 8 Km from the colonies (Matos et al. 2018).

Berlenga (Figure 1c) (39°24′55″N, 9°30′34″W) is located on a continental shelf about 11 km of the coast of Peniche, Portugal. It is characterized by shallow waters and high marine productivity due to costal upwelling (Sousa et al. 2008). With 78.8 ha, it is the largest island of a group of three (Berlenga, Farilhões and Estelas), which make an archipelago. This island has the largest population of yellow-legged gulls in Portugal (Catry et al. 2010). In 1994 there were about 45000 individuals in the island (Alonso et al. 2015). Since then, control measures have been taken and in the last years a stable population of about 8500 pairs is estimated to breed in the island (Ceia et al. 2014; Mendes et al. 2018). Similarly to Deserta Island, Berlenga is also part of the Natura 2000 network, it is classified by UNESCO as a World Biosphere Reserve since 2011 and it is also a Special Protection Area for Wild Birds and a Natural Reserve.

Peniche (Figure 1b) (39°21'20"N, 9°22'52"W) is a town on the west coast of Portugal with an area of 77.55 Km<sup>2</sup> and about 27 000 inhabitants. It is surrounded by the Atlantic Ocean at the north and west, making it a peninsula. There are large sandy beaches on the north and south of the peninsula. It has one the largest traditional fishing harbors of the country and it has a large fishing activity thus attracting seagull populations.

Porto (Figure 1a) (41°08'58"N, 8°36'39"W), on the other hand, is a large metropolitan area with about 230 000 people with an area of 41.42 Km<sup>2</sup>. It is also bathed by the Atlantic Ocean and it is crossed by the Douro river. Data from 2010/2011 estimated about 2500 up to 6300 gull individuals during the breeding season (CIIMAR 2011). In recent years, the number of individuals in these two coastal cities has increased.



**Figure 1**: Study areas geographical location: (a) Porto and (b) Peniche (urban colonies outlined in orange), (c) Berlenga and (d) Deserta or Barreta (natural colonies outlined in blue). Satellite images from Google Earth.

#### 2.2. Study Species

Our study focused on the yellow-legged gull (*Larus michahellis*). The yellow-legged gull is commonly found in Europe, North Africa and in the Middle East. It is a rather large gull, about 800 to 1200 g of body mass (Calado et al. 2018), and it is mainly characterized by the yellow legs and a red spot on the bill (as adults). This species has a high foraging strategy plasticity and can exhibit both generalist and opportunistic feeding behaviours depending on the availability of resources (Ceia et al. 2014). This wide trophic niche (Duhem et al. 2003; Soldatini et al. 2005) allows them to rely on food

from different origins such as from natural habitats where they can find vegetable and animal prey (Bosch et al. 1994), and also from refuse tips and fishing discards from anthropogenic activities (Soldatini et al. 2005; Pedro et al. 2013; Duhem et al. 2003; Bosch et al. 1994). Yellow-legged gulls are colonial and long-lived (Possenti et al. 2018), and the breeding season goes from April to June, females lay 1-3 eggs, usually 3, with 27-31 days of incubation (Sanz-Aguilar et al. 2009; Possenti et al. 2018). Gull populations have undergone a large increase in numbers throughout the years and the main explanation for this is the rapid increase in the availability of highly renewable and predictable anthropogenic food resources (Duhem et al. 2003, Pedro et al. 2013), either from fisheries or waste (Bosch et al. 1994; Ramos et al. 2009b). The easy adaptability to different food resources and habitats make it a good model species to analyze the effects of living in urban environments and what sort of benefits and/or downsides such populations may experience.

#### 2.3. Reproductive parameters

In both years, nests were randomly selected in the natural nesting areas in Deserta and Berlenga islands. In Porto and Peniche nests were selected from building's rooftops. The nests were marked (Figure 2a) and characterized according to the percentage of vegetation cover and distance to the nearest nest. Also, the usage of foreign materials (Figure 2b), more specifically materials from anthropogenic origin, by adults to build their nests was included in the nest characterization. Data regarding clutch size, egg volume, hatching success and early chick growth was obtained from each nest, whenever possible. Not all measures were obtained for all nests for several reasons, including: difficult access in urban areas where nests could be seen but not necessarily reached, sometimes the eggs would disappear from unknown causes or the chicks would hide away from the nest due to their nidifugous behaviour and could not be found.

Clutch size was defined as the number of eggs laid per nest and the hatching success was the number of eggs that successfully hatched in relation to the number of eggs laid per nest (Figure 2c) (in some cases chicks would die during the hatching process). Egg length (L) and width (W) were measured (in mm) using calipers and egg volume (cm<sup>3</sup>) was then calculated from the formula L x W<sup>2</sup> x 0.476 (Harris 1964). As chicks would hatch, they were designated as chick A, B or C (in hatching order) and were marked with non-toxic paint in three different colours (Figure 2d) (red, green and blue, respectively) (Paiva et al. 2006). Chicks were then weighted daily (in grams) during their first five days of life (day 0 to 5) with a Pesola scale. The chick growth parameters were

obtained fitting a quadratic regression model to the data:  $M_d = M_0 + ad + bd^2$  ( $M_d = chick$  weight on day d,  $M_0 = chick$  weight on day 0, d = age in days, a = linear growth, b = quadratic growth) (Ramos 2002).

To assess the nearest nest distance the linear distance from the center of each nest to the nearest nest was measured (in meters) (Hooper 1988).



Figure 2: (a) Marked gull nest, (b) fishing cords found in a nest, (c) chick hatching, (d) marked gull chick.

#### 2.4. Behavioural parameters

Intraspecific aggression was registered based on observational data. Observations were made every day, if possible, through a portable hide for one hour in the morning (between 10 a.m. and 1 p.m.) and one hour in the afternoon (between 3 p.m. and 6 p.m.). During the one-hour period all interactions between individuals were registered either between adults or between adults and chicks (Ramos 2003). Aggressions were classified according to a scale ranging from 1 to 3: 1 – intimidation with no contact between individuals, 2 – attempt to beak/beak or bite, 3 – physical contact/fight.

#### 2.5. Physiological parameters

Blood samples were collected from both adults and chicks. Adults were captured with the help off cage traps during incubation and chicks were caught by hand at the nest's vicinity. Upon capture, adults were also weighted, and measures of the wing and tarsus length were taken to assess their body condition as the residuals of a linear regression of adult body mass on wing length. For chicks, blood was taken from the brachial vein (Bennett 1970; Norte et al. 2009; Pollet et al. 2014) when they were preferably close to 5 days old, but in a few cases, samples were taken from older individuals or chicks of unknown age. In order to minimize disparities, a regression was performed between each parameter and chicks' age upon sample collection, followed by a residual analysis. Standard residuals were used for the statistical analysis in this case. For unknown age chicks, age was extrapolated using growth curves previously published for *Larus* spp. (Harris 1964; Burger and Gochfeld 1988).

The physiological parameters obtained from the blood samples were erythrocyte sedimentation rate (ESR), heterophil/lymphocyte index (H/L) and white blood cell count (WBC). In 2019, we additionally measured blood haemoglobin (Hb; g/L), oxidative stress levels, and cell-mediated immunity index (in chicks only). Blood was taken by puncture of the tarsal (adults) and brachial (chicks) veins with a 25-gauge needle and collected into heparinized tubes to avoid coagulation.

ESR is the rate at which erythrocytes fall through a column of blood (Heylen and Matthysen 2008). The pace of red blood cells through plasma is enhanced by increased levels of one of the major acute-phase proteins (fibrinogen) and immunoglobulins (Masello and Quillfeldt 2004, Heylen and Matthysen 2008). High ESR values may be indicative of many acute and chronic diseases and other stress factors as well, and it may be negatively correlated with body condition (Masello and Quillfeldt 2004). The

heparinized capillary tubes containing the blood sample were sealed and kept vertically in a cool icebox for 4 to 5 hours (Bailly et al. 2017). After that, ESR was calculated from the ratio between plasma and total volume of blood (Heylen and Matthysen 2008) (Figure 3a).

A small portion of blood was saved for haemoglobin concentration analysis. The rest of the blood was centrifuged for 10 minutes at 6200rpm and kept at -20°C until further analysis (Image 3d).

The differential and total white blood cells count was determined examining whole blood smears (Figure 3b) made in the field following standard procedures (Bennett 1970). The smear was air dried and stored until fixation. They were dipped in methanol for 2 minutes for cell fixation, four to six hours after sample collection, and air dried again (Bustnes et al. 2004; Bobby Fokidis et al. 2008). In the laboratory, blood smears were stained using Giemsa method (Figure 3c) (Bennett 1970; Cīrule et al. 2011; Bobby Fokidis et al. 2008) and examined at the microscope to obtain counts of white blood cells (granulocytes) (Totzke et al. 1999; Bobby Fokidis et al. 2008; Mallory et al. 2015) using 1000x magnification and immersion oil (Norte et al. 2008; Mallory et al. 2015). WBC is a general, non-specific indicator of health and immune system status (Bustnes et al. 2004) and high levels usually indicate either infection or inflammation (Norte et al. 2009b; Sakas 2002). WBC is considered a reliable indicator of physiological stress (Cīrule et al. 2011). Heterophil to lymphocyte (H/L) ratio has been considered an indicator of longterm exposure to multiple stressors (Bauerová et al. 2017; Blanco et al. 2004). In birds, innate immunity is provided by heterophils and the number of heterophils may increase in the presence of infection or inflammation (Sakas 2002). Periods of stress lead to an increased H/L ratio (Mallory et al. 2015). Such stress can have different origins such as fear and social disruption, starvation, temperature stress, noise, injuries, among others (Ruiz et al. 2002; Norte et al. 2009b). WBC included lymphocytes (L), heterophils (H), monocytes, basophils and eosinophils (Ruiz et al. 2002; Bauerová et al. 2017) that were identified based on their morphology (Julian et al. 1962; Mallory et al. 2015). H/L ratio was estimated by identifying 100 of the previously mentioned white blood cells (Blanco et al. 2004; Mallory et al. 2015) in an evenly distributed monolayer. For WBC the total number of leukocytes in 10000 erythrocytes was registered (Norte et al. 2008; Cīrule et al. 2011).

Haemoglobin (Hb) reflects the oxygen-carrying capacity of the blood, and high Hb values are usually associated with good health and nutrition as this molecule is costly to produce (Norte et al. 2008). It is very attractive as a condition index because it requires minimal amounts of blood (Johnstone et al. 2015). Parasitism can lead to a decrease in Hb levels leading to anemia (Fair et al. 2007; Norte et al. 2009b). The Hb was measured using a Hemoglobin Assay Kit (Sigma-Aldrich) according to manufacturer's instructions. Using this method hemoglobin is converted to a colorimetric product and measured at a wavelength of 400nm in a microplate reader (Multiskan FC, Thermo Scientifc). Blood samples were diluted 100-fold in water.

Oxidative stress is the rate at which oxidative damage is generated to biomolecules (Constantini et al. 2014b), it arises when there is an imbalance between antioxidant defenses and reactive oxygen species (ROS) production, which originate from normal metabolism or inflammation processes and phagocytic activity towards bacteria and parasites, as well as from fatty-acid metabolism (Norte el al. 2008). As a consequence of this imbalance, ROS oxidize biomolecules and thus creating damage (Monaghan et al. 2009). Oxidative stress can be induced by various environmental, social and internal conditions (Constantini et al 2014c) with potential to be severely affected by urban stressors (Isaksson 2015). Long-term oxidative damage can lead to cell senescence, loss in organ and organism performance, and may influence life-history strategies (Constantini et al 2014a; Norte et al. 2009b). Reactive Oxygen Metabolites (ROMs), mostly hydroperoxides were measured in plasma using the d-ROMs assay (Diacron International, Grosseto, Italy), photometrically at a wavelength of 550nm and expressed as mg H<sub>2</sub>O<sub>2</sub>/dL. To account for the cell hemolysis rate, d-rom values were corrected using a regression of d-rom values on plasma reading at 450nm. Residuals of this regression were used for statistical analysis. The non-enzymatic antioxidant capacity was measured in plasma using the OXY-adsorbent assay (Diacron International, Grosseto, Italy). This will quantify the in vitro plasma antioxidant barrier to cope with the oxidant action of hypochlorous acid (HCIO) using a photometric reading at a wavelength of 550nm in a microplate reader and is expressed in µMol of HCIO neutralized/mL of sample.

The Phytohemagglutinin (PHA) test is commonly used to assess immune function in birds (Grasman et al. 2012). It is a non-lethal, minimally invasive method to measure T-lymphocyte mediated immunity (Grasman 2009). The test consisted on the sub-dermal injection of two different solutions in the chick's tarsus. On one side we injected 50  $\mu$ L of the PHA solution (4 mg/mL), on the other side we injected 50  $\mu$ L of sterile phosphate buffer saline (PBS). The PHA will stimulate T-cells to release cytokines that cause an inflammatory influx of leukocytes and fluid, creating a swelling effect. The tarsus was measured, at the injection spot, before and 24h after the injection of PHA (Grasman 2009). We used two methods to measure the perimeter of the tarsus: 1) we used dental floss placed around the tarsus and measured it with a caliper, 2) we measured the tarsus front to back and side to side with a caliper. We calculated an average diameter based on those two measures and then calculated the tarsus' perimeter (in mm). A stimulation index was calculated as the change of thickness of the PHA-injected tarsus minus the PBS-injected one.



Figure 3: (a) Capillary tube used for ESR assessment, (b) blood smear, (c) blood smear staining with Giemsa, (d) plasma and red blood cell fractions after centrifugation of the sample.

#### 2.6. Data analysis

Prior to the statistical analysis, data was checked for normality, with a Komolgorov-Smirnov test, and homogeneity of variance, using Levene's test. When the previous assumptions were verified, data was subjected to parametric tests, ANOVA (One-way ANOVA or Factorial ANOVA) or GLM analysis. In case the assumptions were not met, data was transformed, using logarithm (Log) or derivates (e.g. Log+1). If after transformation assumptions were still not verified a non-parametric Wald test was used to analyse the data. This method was used in conjunction with an ANOVA test to compare and corroborate any significant findings as ANOVA is generally robust to violations of the assumptions.

Factorial ANOVAs were used to test if mean values differed between colonies (Deserta, Berlenga, Peniche and Porto), years (2018 and 2019) and the interaction between these two (independent variables) for clutch size, egg volume, hatching success, erythrocyte sedimentation rate, H/L index, body condition (dependent variables).

One-way ANOVAs were used to test if mean WBC, Hb concentration, nearest nest distance, vegetation cover, intraspecific aggression, chick growth rate, cellmediated immunity and oxidative status values varied between different colonies in 2019 or separately by year (independent variable). ANOVAs were followed by a post-hoc multiple comparison Tukey-test.

For WBC, nearest nest distance and nest vegetation cover both years were analysed separately because of some disparities in the data presumably arising from the fact that different people collected the data in different years/locations. On the contrary, early chick growth data was analysed with the two years together as sample size for each year was small. To analyse hatching success, only data from Deserta and Porto was used due to lack of data and small sample size from the other locations. Data from adults and chicks were analysed separately. Residuals of a multiple regression of chick's parameters on age upon sample collection was made to uniformize data.

All data were analysed using STATISTICA 7 (StatSoft Inc. 2004).

### 3. Results



The results obtained for each parameter are shown on Table 1 as mean values (±standard deviation). To better understand the statistical difference between years, colonies and interactions year\* colony we present on Table 2 the F and p values for each of variable.

#### 3.1. Reproductive parameters

Clutch size and egg volume both presented significant differences between colonies ( $F_{3,324} = 17.286$ ,  $p \le 0.001$ ;  $F_{3,315} = 17.69$ ,  $p \le 0.001$ , respectively) (Figure 4; Table 1, 2) and no differences between years and no interaction year\*colony. No significant difference was found for linear chick growth between colonies as both years were analysed together. For clutch size, Peniche presented significantly lower values than both natural colonies (Tukey test: both  $p \le 0.001$ ) and Porto was significantly lower than Berlenga (Tukey test:  $p \le 0.001$ ) (Figure 4a). Egg volume was significantly lower in Porto than at all other colonies (Tukey test:  $p \le 0.001$ ) and there was a significant difference between Deserta and Berlenga (Tukey test: p = 0.026)(Figure 4b).



**Figure 4**: Mean (± 95% CI) of (a) clutch size and (b) egg volume (cm<sup>3</sup>) in natural (Deserta and Berlenga) and urban (Peniche and Porto) colonies.

**Table 1:** Comparison of reproductive, behavioural and physiological parameters and nest characteristics between urban and natural colonies. Values are Mean ±SD and the number of samples is indicated in parenthesis.

VARIABLES	YEAR	DESERTA	BERLENGA	PENICHE	PORTO
CLUTCH SIZE	2018	2.80 ± 0.41 (30)	2.86 ± 0.41 (86)	2.19 ± 0.83 (34)	2.64 ± 0.64 (25)
	2019	2.72 ± 0.46 (39)	2.97 ± 0.18 (63)	2.48 ± 0.70 (27)	2.50 ± 0.69 (20)
EGG LENGH (MM)	2018	71.2 ± 2.23 (30)	70.0 ± 2.60 (86)	69.7 ± 1.90 (34)	67.1 ± 2.63 (25)
	2019	70.24 ± 2.57 (39)	70.1 ± 3.16 (63)	69.1 ± 2.40 (27)	68.7 ± 1.62 (20)
EGG WIDTH (MM)	2018	48.3 ± 1.16 (30)	48.2 ± 1.49 (86)	48.1 ± 1.49 (34)	46.2 ± 1.89 (25)
	2019	48.82 ± 1.91 (39)	47.8 ± 1.43 (63)	48.3 ± 1.37 (27)	47.2 ± 0.93 (20)
EGG VOLUME (CM <sup>3</sup> )	2018	79.3 ± 4.79 (30)	77.5 ± 6.51 (86)	76.8 ± 5.54 (34)	68.6 ± 7.11 (25)
	2019	79.9 ± 7.53 (39)	76.4 ± 6.82 (63)	77 ± 5.89 (27)	73 ± 3.38 (20)
HATCHING SUCESS	2018	0.67 ± 0.39 (22)	-	1 ± 0.14 (6)	0.57 ± 0.41 (15)
	2019	0.59 ± 0.44 (39)	0.94 ± 0.14 (6)	-	0.88 ± 0.20 (14)
CHICK A GROWTH					
MO	2018	60.5 ± 4.17 (15)	59.6 ± 4.10 (2)	60.2 ± 2.75 (2)	54.2 ± 5.99 (4)
	2019	54.28 ± 9.60 (24)	55.88 ± 9.36 (4)	-	52.69 ± 5.44 (7)
LINEAR GROWTH	2018	- 0.66 ± 7.47 (15)	4.19 ± 0.51 (2)	5.24 ± 0.20 (2)	11.06 ± 4.30 (4)
	2019	3.55 ± 8.86 (24)	2.07 ± 2.13 (4)	-	5.07 ± 6.63 (7)
QUADRATIC GROWTH	2018	2.18 ± 1.35 (15)	2.51 ± 0.37 (2)	1.64 ± 0.40 (2)	0.48 ± 0.76 (4)
	2019	1.63 ± 1.82 (24)	1.92 ± 0.73 (4)	-	1.46 ± 1.12 (7)
CHICK B GROWTH					
MO	2018	59.4 ± 5.59 (16)	-	57.5 ± 2.09 (2)	62.4 ± 8.26 (4)
	2019	51.92 ± 13.41 (20)	53.1 ± 6.57 (5)	-	56.74 ± 2.96 (6)
LINEAR GROWTH	2018	1.72 ± 7.57 (16)	-	5.08 ± 8.45 (2)	4.05 ± 7.31 (4)
	2019	3.91 ± 8.89 (20)	-0.29 ± 1.24 (5)	-	1.7 ± 1.60 (6)
QUADRATIC GROWTH	2018	2.03 ± 1.11 (16)	-	2.13 ± 1.94 (2)	1.15 ± 0.75 (4)
	2019	1.87 ± 1.76 (20)	2.07 ± 1.24 (5)	-	1.97 ± 0.41 (6)

	0040				
MU	2018	$58.3 \pm 5.07(7)$	-	-	-
	2019	55.95 ± 8.23 (10)	52.5 ± 4.95 (2)	-	53.32 ± 4.16 (3)
LINEAR GROWTH	2018	3.08 ± 5.63 (7)	-	-	-
	2019	5.72 ± 14.39 (10)	-10.25 ± 3.89 (2)	-	3.98 ± 0.99 (3)
QUADRATIC GROWTH	2018	1.70 ± 0. 84 (7)	-	-	-
	2019	0.06 ± 5.73 (10)	4.25 ± 1.06 (2)	-	0.7 ± 0.64 (3)
ESR CHICKS	2018	0.43 ± 0.11 (18)	0.34 ± 0.19 (9)	0.22 ± 0.06 (3)	0.46 ± 0.12 (10)
	2019	0.36 ± 0.098 (21)	0.40 ± 0.14 (5)	0.29 ± 0.13 (9)	0.27 ± 0.17 (17)
ESR ADULTS	2018	0.15 ± 0.11 (8)	0.32 ± 0.08 (17)	0.22 ± 0.07 (7)	0.12 ± 0.10 (7)
	2019	0.17 ± 0.08 (13)	0.11 ± 0.06 (16)	0.09 ± 0.05 (10)	0.096 ± 0.06 (9)
H/L INDEX CHICKS	2018	0.73 ± 0.59 (18)	0.35 ± 0.34 (9)	0.38 ± 0.37 (3)	0.28 ± 0.22 (9)
	2019	0.64 ± 0.27 (21)	0.37 ± 0.46 (12)	0.58 ± 0.19 (9)	0.50 ± 0.41 (15)
H/L INDEX ADULTS	2018	0.38 ± 0.30 (8)	0.36 ± 0.17 (16)	0.17 ± 0.10 (7)	0.44 ± 0.25 (7)
	2019	1.07 ± 0.80 (13)	0.60 ± 0.32 (16)	0.57 ± 0.35 (10)	0.77 ± 0.36 (9)
WBC CHICKS	2018	119 ± 75.5 (18)	90 ± 52.86 (9)	59 ± 27.46 (3)	92 ± 64.93 (9)
	2019	20.81 ± 10.1 (21)	24.67 ± 23.80 (12)	17.78 ± 13.93 (9)	13 ± 10.08 (15)
WBC ADULTS	2018	69 ± 20.85 (8)	100 ± 66.48 (16)	79 ± 43.28 (7)	77 ± 45.90 (7)
	2019	23.46 ± 10.0 (13)	19.79 ± 11.72 (16)	17.9 ± 8.86 (10)	21.89 ± 11.78 (9)
HB (G/L) CHICKS	2019	32.83 ± 3.95 (13)	-	42.07 ± 8.15 (8)	38.04 ± 7.37 (11)
HB (G/L) ADULTS	2019	-	74.46 ± 12.74 (11)	67.65 ± 6.94 (8)	66.49 ± 8.80 (8)
	2019	191.11 ± 30.95 (12)	-	188.19 ± 39.79	206.27 ± 41.30 (9)
	0040		000.07 . 00.00	(/)	
OXY ADULIS (µMOL OF HCIO NEUTRALIZED / ML OF SAMPLE)	2019	231.58 ± 45.87 (12)	220.27 ± 38.86 (15)	236.89 ± 43.46 (10)	229.56 ± 33.55 (9)
DROM CHICKS (MG H <sub>2</sub> O <sub>2</sub> /DL)	2019	3.67 ± 4.01 (10)	-	1.65 ± 2.03 (7)	7.39 ± 3.41 (8)
DROM ADULTS (MG H <sub>2</sub> O <sub>2</sub> /DL)	2019	13.25 ± 6.33 (12)	13.17 ± 6.39 (15)	$23.42 \pm 14.45$	13.62 ± 11.95 (9)
				(10)	

	2019	$0.25 \pm 1.10(0)$	0.29 + 0.07 (16)	$0.47 \pm 0.04 (7)$	0.06 + 0.55 (7)
BODT CONDITION	2016	$0.25 \pm 1.19(9)$	$-0.36 \pm 0.97$ (16)	$0.47 \pm 0.94(7)$	$0.06 \pm 0.55(7)$
	2019	-0.50 ± 1.14 (14)	0.43 ± 0.76 (18)	0.54 ± 0.68 (10)	-0.51 ± 0.78 (9)
TYPE 1	2018/2019	0.14 ± 0.13 (27)	-	0.22 ± 0.34 (23)	0.07 ± 0.09 (62)
INTERACTION/NEST/HOUR					
TYPE 2	2018/2019	0.03 ± 0.03 (27)	-	0.03 ± 0.08 (23)	0.01 ± 0.02 (62)
INTERACTION/NEST/HOUR					
TYPE 3	2018/2019	0.02 ± 0.03 (27)	-	0.00 ± 0.00 (23)	0.01 ± 0.03 (62)
INTERACTION/NEST/HOUR					
NEAREST NEST DISTANCE	2018	6.40 ± 1.70 (30)	7.40 ± 7.05 (25)	14.44 ± 14.66	30.26 ± 27.07 (69)
(M)				(45)	
	2019	5.60 ± 2.49 (39)	-	9.54 ± 7.97 (27)	14.03 ± 7.50 (38)
<b>VEGETATION COVER (%)</b>	2018	5.3 ± 11.8 (30)	-	42.73 ± 40.37	3.67 ± 16.16 (67)
				(44)	
	2019	37.41 ± 38.17 (39)	96.1 ± 17.3 (41)	43.44 ± 34.21	0.23 ± 1.05 (44)
				(48)	
CELL-MEDIATED IMMUNITY	2019	0.23 ± 1.38 (11)	2.01 ± 2.39 (4)	-0.34 ± 1.88 (6)	1.62 ± 4.00 (8)
INDEX (DENTAL FLOSS )(MM)					
CELL-MEDIATED IMMUNITY	2019	-	0.55 ± 0.47 (4)	1.13 ± 1.38 (6)	0.81 ± 1.43 (6)
INDEX (CALLIPER) (MM)					

M0 = estimated weight on day 0, LG = linear growth phase and, QG = quadratic growth phase, obtained from Md = M0 + ad + bd2. ESR = erythrocyte sedimentation rate; H/L = heterophil/lymphocyte; OXY = non-enzymatic antioxidant capacity; d-rom = reactive oxygen metabolites; Type 1 = intimidation acts, Type 2 = attempt to beak/beak or bite, Type 3 = fight/physical contact; WBC = white blood cell count; Hb = haemoglobin

**Table 2:** Statistical analysis (F and p values) for each of the variables studied. Significant p values are highlighted in red.

	Year	Location	Interaction year*location
Clutch size	$F_{1,324} = 0.142,$ p=0.7064	F <sub>3,324</sub> = 17.286, p≤0.0001	F <sub>3,324</sub> = 1.284, p=0.2798
Egg Volume	F <sub>1,315</sub> = 1.88, p=0.1713	F <sub>3,315</sub> = 17.69, p≤0.0001	F <sub>3,315</sub> = 2.13, p=0.0967
Egg Lenght	F <sub>1,316</sub> = 0.1, p=0.8175	F <sub>3,316</sub> = 11.6, <u>p≤0.0001</u>	F <sub>3,316</sub> = 2.4, p=0.0695
Egg Width	F <sub>1,315</sub> = 3.0, p=0.0821	F <sub>3,315</sub> = 14.3, p≤0.0001	F <sub>3,315</sub> = 2.8, p=0.0418
Hatching sucess	F <sub>1,86</sub> = 1.5580, p=0.2153	F <sub>1,86</sub> = 1.1097, p=0.2951	F <sub>1,86</sub> = 4.782, p=0.0315
Linear growth (Chick A)		F <sub>3,55</sub> = 1.762, p=0.165	
Linear growth (Chick B)		F <sub>3,48</sub> = 0.3397, p=0.797	
Linear growth (Chick C)		F <sub>3,20</sub> = 1.286, p=0.306	
ESR Adults	F <sub>1,79</sub> = 15.926, p=0.0001	F <sub>3,79</sub> = 6.132, <mark>p≤0.0001</mark>	F <sub>3,79</sub> = 8.750, <mark>p≤0.0001</mark>
ESR Chicks	F <sub>1,81</sub> = 0.008, p=0.928	F <sub>3,81</sub> = 1.035, p=0.3818	F <sub>3,81</sub> = 3.348, p=0.023
H/L Adults	F <sub>1,78</sub> = 29.343, p≤0.0001	$F_{3,78} = 3.216,$ p=0.0273	F <sub>3,78</sub> = 1.307, p=0.2781
H/L Chicks	F <sub>1,76</sub> = 1.295, p=0.2587	F <sub>3,76</sub> = 2.943, p=0.0383	F <sub>3,76</sub> = 0.92, p=0.4346
WBC Adults		<b>2018:</b> F <sub>3,34</sub> = 0.803, p=0.5012	
		<b>2019</b> : F <sub>3,44</sub> = 0.597, p=0.6207	
WBC Chicks		<b>2018:</b> F <sub>3,34</sub> = 0.917, p=0.443	
		<b>2019:</b> F <sub>3,42</sub> = 6.673, p=0.0009	
Hb Adults		F <sub>2,24</sub> = 1.735, p=0.1979	
Hb Chicks		F <sub>2,29</sub> = 3.62, p=0.0395	
OXY Adults		F <sub>3,42</sub> = 0.367, p=0.7774	
OXY Chicks		F <sub>2,24</sub> = 1.132, p=0.3391	
DROM Adults		F <sub>3,42</sub> = 0.3355, p=0.7997	
DROM Chicks		$F_{2,22} = 8.027,$ p=0.0024	

Body conditon	F <sub>1,82</sub> = 0.3173, p=0.5748	F <sub>3,82</sub> = 2.121, p=0.1039	F <sub>3,82</sub> = 4.0104, p=0.0102
Type 1 interactions		F <sub>2,109</sub> = 6.145, p=0.00296	
Type 2 interactions		F <sub>2,109</sub> = 1.987, p=0.142	
Type 3 interactions		F <sub>2,108</sub> = 3.523, p=0.033	
Nearest nest distance		<b>2018</b> : F <sub>3,165</sub> = 16.13, p≤0.001	
		<b>2019:</b> F <sub>2,100</sub> = 17.51, p≤0.001	
Vegetation cover		<b>2018:</b> F <sub>2,138</sub> = 34.03, p≤0.0001	
		<b>2019</b> : F <sub>3,168</sub> = 90.25, p≤0.0001	
Cell-mediated immunity (dental floss)		F <sub>3,25</sub> = 1.133, p=0.355	
Cell-mediated immunity (calliper)		F <sub>2,13</sub> = 0.261, p=0.774	

In the case of the hatching success there was a significant difference only in the interaction year\*location ( $F_{1,86}$  = 4.782, p = 0.0315) motivated by an increase of hatching success in Porto for 2019 when compared to Deserta for 2019 and Porto for 2018 (Tukey test: p = 0.037 and p = 0.02, respectively) (Table 1, 2).

For nearest nest distance and percentage of vegetation cover both years were analysed separately. There were significant differences in these two parameters in both years ( $F_{3,165} = 16.13$ ,  $p \le 0.001$  (nest distance 2018);  $F_{2,100} = 17.51$ ,  $p \le 0.001$  (nest distance 2019);  $F_{2,138} = 34.026$ ,  $p \le 0.001$  (vegetation cover 2018);  $F_{3,168} = 90.25$ ,  $p \le 0.001$  (vegetation cover 2019)) (Figure 5; Table 1, 2). In 2018, nests in Porto were significantly farther away from each other than at all the other colonies (Tukey test: all  $p \le 0.001$ ), and in 2019 both urban colonies differed from Deserta (Tukey test: p = 0.038 (Peniche),  $p \le 0.001$  (Porto)) and differed from each other (Tukey test: p = 0.016) (Figure 5a). Peniche also presented a higher nest vegetation cover than other colonies in 2018 (Tukey test: both  $p \le 0.001$ ), and in 2019 Porto had significantly lower vegetation cover than all other colonies (Tukey test: all  $p \le 0.001$ ) and Berlenga had a significantly higher vegetation cover than all other colonies (Tukey test: all  $p \le 0.001$ ) (Figure 5b).



**Figure 5**: Mean  $(\pm 95\% \text{ CI})$  of (a) nearest nest distance (meters) and (b) nest vegetation cover (%) in natural (Deserta and Berlenga) and urban (Peniche and Porto) colonies.

To assess the usage of foreign materials in gulls' nests we focused on the number of nests that contain materials derived from human activity. Both urban colonies, Porto and Peniche, presented higher prevalence of foreign materials in the nests compared to the natural colonies (Figure 6).



Figure 6: Percentage of nests with foreign materials in natural (Deserta and Berlenga) and urban (Peniche and Porto) colonies.

#### 3.2. Behavioural parameters

Type 1 and type 3 gull interactions presented significant differences between colonies ( $F_{2,109} = 6.145$ , p = 0.003;  $F_{2,108} = 3.523$ , p = 0.033 (Table 1, 2). Birds from Peniche exhibited significantly higher number of intimidation acts (type 1 aggression) than those from Porto (Tukey-test:  $p \le 0.001$ ) (Figure 7a), and individuals from Deserta were significantly more aggressive and had more fights (type 3 aggression) than birds from Peniche (Tukey-test: p = 0.02) (Figure 7b).



**Figure 7:** Mean (± 95% CI) of (a) type 1 and (b) type 3 interactions (per hour per nest) in natural (Deserta and Berlenga) and urban (Peniche and Porto) colonies.

#### 3.3. Physiological parameters

Erythrocyte sedimentation rate showed significant differences between years and colonies for adults ( $F_{1,79} = 15.926 \text{ p} \le 0.001$ ;  $F_{3,79} = 6.132$ ,  $p \le 0.001$  respectively) and there was a significant interaction year\*colony for both adults and chicks ( $F_{3,79} = 8.750$ ,  $p \le 0.001$ ;  $F_{3.81} = 3.348$ , p = 0.023 respectively) (Table 1, 2). Adults from Porto had lower ESR values than both natural colonies. Deserta and Berlenga (Tukey-test: p = 0.044;  $p \le$ 0.001 respectively) (Figure 8a). Also. adults from Berlenga in 2018 presented exceptionally higher ESR values than in other colonies causing a significant interaction year\*colony (Figure 8a), more specifically adults from Berlenga in 2018 differed from adults from Deserta and Porto in 2018 and Deserta, Berlega, Peniche and Porto in 2019 (Tukey-test: p = 0.002;  $p \le 0.001$ ; p = 0.007;  $p \le 0.001$ ;  $p \le 0.001$ ;  $p \le 0.001$  respectively). Also, adults from Peniche in 2018 presented differences from adults from Peniche and Porto in 2019 (Tukey-test: p = 0.02; p = 0.03 respectively). For chicks, Porto was the place of divergence: in 2018 Porto differed from Berlenga and Peniche in the same year (Tukey test: both p = 0.01) and in 2019 Porto differed from Deserta in 2018 (Tukey-test: p = 0.03). Chicks from Porto in 2018 also differed from those from Porto in 2019 (Tukeytest: p = 0.008) (Figure 8b).



Figure 8: Mean (± 95% CI) of erythrocyte sedimentation rate (ESR) of (a) Adults and (b) Chicks in natural (Deserta and Berlenga) and urban (Peniche and Porto) colonies.

The H/L index presented significant differences between years for adults ( $F_{1,78}$  = 29.34, p ≤ 0.001) (Figure 10a) and among colonies for both adults and chicks ( $F_{3,78}$  = 3.22, p = 0.027;  $F_{3,76}$  = 2.94, p = 0.038) (Table 1, 2). For adults, Deserta's birds differed from Berlenga's and Peniche's (Tukey-test: p = 0.039; p = 0.01 respectively) (Figure 9a). As for chicks, birds from Deserta presented differences from Berlenga's and Porto's chicks (Tukey-test: p = 0.02 respectively) (Figure 9b).



**Figure 9:** Mean (± 95% CI) of heterophil/lymphocyte (H/L) index of (a) Adults and (b) Chicks in natural (Deserta and Berlenga) and urban (Peniche and Porto) colonies.

There was no difference between colonies in either year regarding WBC for adults. In chicks there was no difference in 2018, however in 2019 significant differences were found among colonies ( $F_{3,42} = 6.673$ ,  $p \le 0.001$ ) (Table 1, 2): chicks from Peniche displayed differences in relation to every other colony, more specifically being lower than Deserta and Berlenga (Tukey-test: p = 0.001, p = 0.003 respectively) and higher than chicks from Porto (Tukey-test: p = 0.04) (Figure 10a). Haemoglobin also did not show any differences between colonies for adults but did for chicks ( $F_{2,29} = 3.62$ , p = 0.0395) (Table 1, 2). Chicks from Peniche showed a higher Hb concentration than chicks from Deserta (Tukey-test: p = 0.03)(Figure 10b). Note that for this analysis (Hb) there were samples only from 2019.



**Figure 10:** Mean (± 95% CI) of (a) White Blood Cell count and (b) haemoglobin concentration in chicks of natural (Deserta and Berlenga) and urban (Peniche and Porto) colonies in the year of 2019.

Regarding cell oxidative status, no differences were found in the non-enzymatic antioxidant capacity of either adults or chicks. As for the Reactive Oxygen Metabolites (d-ROMs), significant differences were found only in chicks ( $F_{2,22} = 8.027$ , p = 0.0024) (Table 1, 2). Chicks from Porto showed significantly higher d-ROMs than chicks from Deserta and Peniche (Tukey-test: p = 0.006, p = 0.005 respectively) (Figure 11).



**Figure 11**: Mean ( $\pm$  95% CI) of ROMs (mg H<sub>2</sub>O<sub>2</sub>/dL) in chicks of natural (Deserta and Berlenga) and urban (Peniche and Porto) colonies in the year of 2019.

Adult gull's body condition did not show any differences between years or colonies, however there was an interaction year\*colony ( $F_{3,82} = 4.0104$ , p = 0.01). Adults from Berlenga in 2018 presented significantly lower body condition than those from Peniche in 2018 (Tukey-test: p = 0.04) and from adults from Berlenga and Peniche in 2019 (both p = 0.01). Adults from Peniche in 2018 had higher body condition than Deserta's and Porto's in 2019 (p = 0.02, p = 0.03 respectively). Adult's body condition for Deserta in 2019 was also lower than the body condition for adults from Berlenga and Peniche in 2019 (p = 0.005, p = 0.007 respectively). Adults from Porto 2019 had significantly lower body condition than those from Berlenga and Peniche in 2019 (p = 0.01) (Figure 12. Table 1, 2).



**Figure 12:** Mean (± 95% CI) of adult's body condition from natural (Deserta and Berlenga) and urban (Peniche and Porto) colonies.

Linear chick growth, type 2 interactions, cell-mediated immunity index and enzymatic antioxidant capacity did not show significant differences among colonies and/or years (Table 2).

# 4. Discussion



This work was developed with the intention of assessing and quantifying the differences in reproduction, behaviour and physiology between natural and urban nesting gulls in order to understand possible costs of urbanization on breeding wildlife. For this purpose, a wide range of different techniques and tests were used to collect data from four gull colonies (natural: Deserta and Berlenga; urban: Peniche and Porto) across two breeding seasons, which enabled us to discuss differences between natural and urban colonies and further understand the advantages and/or disadvantages of reproducing in urban environments.

#### 4.1. Urban living – effects on reproduction

Clutch size was generally lower for urban colonies in relation to natural colonies. Several studies have confirmed that urban nesting gulls can exhibit lower clutch sizes (Perlut et al. 2016: Bailly et al. 2017). The cause may be attributed to the lack of highquality food items in urban environments thus resulting in less energy breeding gulls are willing to spend on reproduction (Blight 2011; Bailly et al. 2017). Although urban gulls should reduce foraging movement and effort to obtain food, anthropogenic food resources are likely to have poor nutritional value (Steigerwald et al. 2015). Egg volume was considerably lower in Porto, the most urbanized habitat in our urbanization gradient, compared to the other 3 colonies. This evidence is in accordance to the fact that newly formed urban colonies are predominantly composed by a younger generation of individuals (Raven and Coulson 1997; Soldatini et al. 2008). These younger breeding pairs are unexperienced and the oviduct of young females is not yet fully developed thus producing smaller eggs (Robertson et al. 1994; Forslund and Pärt 1995; González-Solís et al. 2004). Both clutch size and egg volume are positively correlated with age (Fowler 1995) and also the acquisition of high food quality (Christians 2002). Taken altogether, this suggests that the urban colony of Porto is composed mostly by young breeding individuals.

The body mass of chicks on day zero, i.e. upon hatch, was lower in urban habitats consistent with a lower egg volume. However, chick linear growth rate did not show any differences between colonies. As the urban gulls' nest in coastal cities, there is high availability of fisheries discards and refuse tips. Although a fish-based diet is generally considered as the preferable food supply for young chicks (Murphy et al. 1984; Belant et al. 1993; Duhem et al. 2005), high quality refuse items may have high energy and protein content (Romero et al. 2019). On the contrary, adults from natural habitats may spend hours at sea foraging for food and leaving their chicks hungry for long periods of time

(Navarro and González-Solís 2007) and unprotected (Watanuki 1992; Alonso et al. 2015), which should affect chick growth.

Also contributing to an equal linear chick growth in urban colonies when compared to natural colonies is the higher nearest nest distance for urban colonies and consequently lower nest density. As nests are more disperse, intraspecific aggression which should have a negative contribution for chick growth (Pierotti 1982; Ellis and Good 2006) should be much lower in urban colonies. Moreover, rooftop nesting offers protection from mammalian predators that would normally be on the ground (Monaghan 1979). Therefore, the possible negative effects of unexperienced parents and lower food quality in urban areas may be traded against positive effects of reduced competition and predation.

Another factor to affect reproductive success is the usage of anthropogenic origin materials in nest building. Urban gulls are prone to use human-derived materials in their nest construction (Battisti 2019). Materials such as plastic, glass, paper, metal, fabric, fishing equipment, cigarette butts, among others are frequently used (Yaghmoura and Al Marashdab 2019; Lopes et al. 2020; Thompson et al. 2020). Close to 95% of nests in Porto, the most urban colony. exhibited presence of foreign materials which can modify nest natural characteristics and cause entanglement of chicks and/or adults (Votier et al. 2011; Lopes et al. 2020) with possible consequences on reproductive success and chick survival. These factors should be assessed in further studies.

#### 4.2. Urban living – effects on behaviour

Intraspecific aggressive behaviours with physical contact and fights were lower in urban colonies, which is directly correlated with the higher nearest nest distance and lower nest density (Ellis and Good 2006; Perlut et al. 2016). A higher nest density in natural habitat increases the potential to trespass a nest's territory encouraging fights between adults to protect eggs and/or chicks or aggression from adults towards chicks. Although intraspecific aggression was lower in the urban environment, one can argue that interspecific aggression contacts, particularly with humans, should be higher. It has been reported that gull can use human cues to locate food sources (Goumas et al. 2019 and 2020) reducing energy expenditure but they are also known to frequently attack humans to steal food and to protect themselves from human entering their territory. Rooftop nesting, where many times only one nest fits on the roof, seems to be preferable in avoiding territory trespassing, physical aggression and more suited for incubating individuals (Ellis and Good 2006).

#### 4.3. Urban living – effects on physiology

Adults from Porto presented lower ESR values than adults from natural colonies and this was consistent in both years. This evidence, points to higher physiological stress for adults in natural colonies and is corroborated by a higher H/L index, a general stress indicator, in adults from Deserta. Stress in natural colonies may be explained by the high density of individuals which can affect stress levels, especially in the breeding period, where nest and chick defense is high a priority for adults and can lead to high aggression levels (Siegel 1980). On the other hand, in 2019, breeding individuals from Deserta were apparently subjected to a novel threat. The colony was a victim of some sort of infection, presumably originated in a contaminated water supply where birds would normally visit and spread throughout the colony leading to the death of many individuals (personal observations). This can explain the higher values of H/L index and lower body condition revealed at this particular colony in 2019. It is well known that coastal breeding gulls may be subjected to periodic infections by botulism (Newman et al. 2007). The most common clinical symptoms of botulism infection are loss of equilibrium (inability to stand and keep head up), moderate to severe paralysis, motor incoordination, vomiting and diarrhea (Fire and van Dolah 2012), and individuals with these symptoms were observed in Deserta and also in Berlenga Islands.

Chicks from Deserta also exhibited higher H/L index values than in other colonies denoting physiological stress amongst chicks too. WBC data confirms this evidence with urban colonies showing lower values than natural colonies. The higher stress in chicks from natural colonies can be explained also by the higher breeding density that can pose threats to chicks from other adults possibly including aggressions (Siegel 1980). For young chicks this might also be the first immune system response in the form of inflammatory reaction representing higher responses to stress. Then again as adults already presented signs of stress it has been shown that stressed individuals living in social groups can act as stimuli for other individuals with whom they interact (Noguera et al. 2017). Through this process, parents can induce their brood to develop physiological stress signs. Chicks from Deserta Island also showed lower haemoglobin concentration compared to urban chicks, reinforcing that these were in poorer health condition. On the contrary, chicks from Porto reported a higher concentration of reactive oxygen metabolites, denoting higher oxidative stress. Oxidative damage can be induced by several human-derived stressors such as chemical pollution, noise pollution, artificial light pollution, traffic and diet quality (Silva et al. 2001; Forero et al. 2002; Isaksson 2015; Grace and Anderson 2018; Injaian et al. 2018). These factors can induce oxidative stress and are especially prominent in an urban environment. On the other hand adults did not show significant differences regarding oxidative stress, which could be attributed to the fact that oxidative damage increases with age (Constantini et al. 2014a) and also because breeding is a costly activity and oxidative stress may be an inherent cost to it (Constantini et al. 2014a; Colominas-Ciuró et al. 2019). Therefore, breeding adults have increased values of oxidative stress which can mask any possible differences between colonies. Results may also be influenced by the development of habituation to stressful conditions. It has been shown that urban individuals normally subjected to the different stressors present in urban environments tend to develop resistance meaning they are better able to cope with environmental stressors (Constantini et al. 2014c). Hence, individuals will show reduced values of stress parameters because of the constant exposure to stressors (Cyr and Romero 2009; Injaian et al. 2018).

Additionally, there are other factors that may influence the results such as the amount of time taken to collect and handle blood samples. Physiological parameters, especially blood related, are prone to suffer alteration depending on the time it takes to handle the sampled individual. As different field workers took samples from different colonies the time it took to collect samples could be different, which could affect the results (Norte el al. 2008; Cīrule et al. 2011). However, we do not think that this was an important factor, because the different fieldworkers were previously trained to take blood samples in the same way as quickly as possible. In addition, the small sample sizes in some colonies and parameters make it imperative that results should be analyzed and interpreted with caution.

#### 4.4. Final Remarks

With this study we were able to discuss some of the advantages and disadvantages of urbanization for breeding gulls. Our results do not show a clear picture as to whether urban habitats are consistently poor-quality areas for breeding individuals. The effects of urbanization on breeding gulls and possibly on other wildlife, should be regarded as a strategy implying costs and benefits.

Natural colonies should present advantages towards a higher reproductive success, particularly the higher clutch size and egg volumes (this study) as well as a rich diet comprised by high quality food items such as pelagic prey fish (Blight 2011; Steigerwald et al. 2015; Bennett et al. 2017).

The urban breeding birds showed a great adaptability to the stressful environment enjoying more space for each nest and consequently less intraspecific aggression and exhibiting lower values of physiological stress. The easy adaptation to urban life by gulls brings rapidly increasing population numbers in cities around the world. Conflicts with humans are now frequent as gulls are not portrayed "as a great neighbour" due to noise, attacks on people, faeces. etc. Actions have been taken in order to mitigate this interaction. In some cases, control measures resorted to destruction of nests and eggs and even gull killing. However, there are several non-lethal measures that can be taken. For instance, better waste and debris management should be a priority to diminish the availability of food to gulls and the amount of anthropogenic litter they bring to their nests. Simple measures can be implemented by city halls and even at a personal level that should mitigate conflicts between gulls and people, and even deter gulls from nesting in urban locations.

Further work is recommended to better understand the effects of urban conditions on the ecology of breeding gulls. Future studies should also include measures of fledging success to better evaluate reproductive success. In this study we also experimented with heart rate measurement, although raw data collection was successful, data extraction and analysis was not possible due to time limitations. However, in the future, heart rate can be implemented has a proxy of stress, with further improvements in software for a more automated data extraction. Emphasize sampling uniformization to collect equal samples in every colony with minimal methodological variation between fieldworkers and it would be recommended to increase the number of samples in urban colonies although it is difficult to get access to nests in tall buildings.

It would be very positive and productive to continue this work for more years as a multi-year experiment would give an overall status of each gull population and the adaptions observed through the years.

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