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**Yellow-legged gulls as indicators of changes in
environmental conditions: a long-term study at Berlenga
Island, western Portugal**



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environmental conditions: a long-term study at Berlenga
Island, western Portugal**

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YELLOW-LEGGED GULLS AS INDICATORS OF CHANGES
IN ENVIRONMENTAL CONDITIONS: A LONG-TERM STUDY
AT BERLENGA ISLAND, WESTERN PORTUGAL

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Resumo

A gaivota-de-patas-amarelas *Larus michahellis* (YLG) é uma espécie de gaivota grande e generalista, com peso corporal adulto de 900-1.200g e comportamento agressivo e predatório. Como outras gaivotas grandes, a gaivota de patas amarelas frequentemente depende de alimentos de depósitos de lixo, aterros e rejeições da pesca para complementar sua dieta. Em Portugal, a maior colónia reprodutora de YLG situa-se na Ilha da Berlenga, cujos números populacionais são monitorizados desde 1974, sendo que a população atingiu cerca de 4.800 casais reprodutores em 1983. Foi então possível notar o início de um crescimento exponencial da população até 1994, quando o número de ca. 22.000 pares reprodutores foram registados. De 1994 a 1996, o Instituto de Conservação da Natureza e Florestas (ICNF) autorizou o abate de aves adultas, o que diminuiu consideravelmente o número de reprodutores pela metade em 1996. Desde então, um controle populacional é realizado, e o abate de posturas (ou seja, ovos) em toda a ilha é executado (exceto nas áreas de controle) durante a época de reprodução. A tendência dos números da população ficou mais ou menos estabilizada até 2009 e começou a diminuir lentamente desde então. Em 2018, foi registada uma população de 5.661 reprodutores (ICNF).

As gaivotas-de-patas-amarelas da colónia da Berlenga alimentam-se principalmente de rejeições da pesca do porto de Peniche (10 km de distância) e têm um alto nível de consistência de curto e longo prazo na sua ecologia alimentar. As espécies de peixes mais relevantes na dieta de adultos e filhotes são comercializadas no porto de Peniche, e incluem a sardinha *Sardina pilchardus*, a cavala *Scomber colias*, o carapau *Trachurus* spp., o verdinho *Micromesistius poutassou* e a pescada-branca *Merluccius merluccius*. No entanto, estudos realizados nesta colónia observaram que a dieta de adultos durante a época de reprodução também é composta em grande parte de fontes naturais, como o caranguejo nadador de Henslow *Polybius henslowii*. Na verdade, esse caranguejo pode ser a presa mais frequente, correspondendo a até 95% da dieta quando disponível.

A dieta das YLG tem sido cada vez mais estudada com a análise de isótopos estáveis (SIA) como valiosa abordagem complementar, fornecendo informações de curto e longo prazo sobre dietas de gaivotas e permite identificar grupos tróficos de presas, estimar o consumo de presas, calcular níveis tróficos e avaliar nichos tróficos.

As atividades humanas, como a pesca, estão associadas a mudanças na dieta das gaivotas, podendo levar à diminuição ou aumento de seus níveis tróficos dependendo dos recursos consumidos. Por serem espécies generalistas e oportunistas, as gaivotas podem mudar de presa e fonte de alimento, adaptando-se a novas condições. Para aproveitar os recursos disponíveis, as gaivotas são capazes de mudar seu comportamento rapidamente, e os efeitos disso, por exemplo mudanças tróficas, são vistas nos níveis individual e populacional.

O objetivo desta tese foi investigar se determinadas condições ambientais estão relacionadas com a ecologia trófica, a densidade populacional e o sucesso reprodutivo de uma grande colônia de uma espécie de ave marinha oportunista, a gaivota de patas amarelas (*Larus michahellis*), que cria na ilha da Berlenga (Portugal). Para isso, uma base de dados de longo prazo incluindo densidades populacionais (desde 1974), parâmetros de reprodução (desde 2002) e nichos tróficos (de isótopos estáveis, desde 2011) de gaivotas de patas amarelas foi relacionada com parâmetros ambientais (Índice de Oscilação do Atlântico Norte - Oeste (wNAO), clorofila a (Chla) e temperatura da superfície do mar (SST)) e dados de pesca (desembarque das espécies mais importantes na dieta de YLG) para avaliar a ecologia desta espécie de gaivota em relação às condições ambientais e atividade antropogénica.

Durante o período de 2002-2018, as variáveis utilizadas foram aquelas obtidas nos censos, sucesso reprodutivo, postura de ovos, desembarques pesqueiros e parâmetros ambientais. Para o período de 2011-2018, foram utilizadas as variáveis relacionadas com ecologia trófica, medidas biométricas de gaivotas (ou seja, IMC, índice de massa corporal) e volume de ovos. Ao contrário do que era esperado, não se encontrou relação significativa entre o número de indivíduos reprodutores e wNAO desde 2002. Essas duas variáveis tiveram variação semelhante até 2010, quando o wNAO teve uma queda muito acentuada. A partir de então, o número de reprodutores não regressou aos valores anteriores e iniciou uma descida contínua até 2018. No entanto, outras correlações com o wNAO foram observadas, como a data em que o primeiro ovo foi depositado, que mostra que quanto mais negativo for o wNAO, mais tarde esse ovo irá surgir, mostrando uma relação com a dependência de recursos. Em 2010 foi observado um valor extremamente baixo do wNAO, que atingiu -4,64 e que impactou claramente o ecossistema, causando uma queda abrupta da produtividade marinha naquele ano e consequentemente afetando consumidores de níveis tróficos

mais elevados, como as aves marinhas, e os dados observados sugerem que o ano de 2010 foi um ponto de viragem (*tipping point*) para as gaivotas na Ilha da Berlenga.

Neste estudo, várias correlações foram observadas entre as variáveis do SIA e as variáveis ambientais. A correlação de Chl- α com RBC $\delta^{13}C$ sugere que quanto mais altos os níveis de Chl- α , mais YLG se alimenta de recursos terrestres, ao contrário do que era esperado, pois quanto maior a produtividade marinha, maior a disponibilidade potencial de recursos (ou seja, peixes). Além disso, Chl- α também apresentou uma relação significativa, mas negativa, com RBC TA (nicho de gaivotas amostradas) e RBC SEAB (modelo de nicho estatístico), indicando que quanto maior a concentração de clorofila, menor o tamanho do nicho, que sugere uma dieta mais especializada. 2013 mostrou um valor de $\delta^{13}C$ muito mais negativo no plasma do que em outros anos, grandes nichos isotópicos em ambos os tecidos analisados e alta variação nos valores isotópicos estáveis. Os baixos valores de $\delta^{13}C$ sugerem que durante este ano as gaivotas consumiram mais fontes de origem terrestre. Outra variável utilizada neste estudo também apresentou um outlier em 2013, um valor extremamente alto de Chl- α (9,15 mg.m⁻³), que indica que a produtividade marinha pode estar relacionada com essas observações marcantes deste ano. Ainda, as análises mostraram que wNAO tem uma correlação negativa com três variáveis do plasma, TA (área total da elipse), SEAb (modelo Bayesiano da área da elipse), e SEAc (área da elipse corrigida), particularmente do plasma, métrica que representa a área do nicho populacional da YLG. Essa correlação é muito significativa e sugere que quanto maiores os valores de wNAO, menores os valores de SEAc e, conseqüentemente, maior a especialização de nicho. Em anos de wNAO positivo, o nicho era maior, então as gaivotas adotaram hábitos alimentares mais gerais. Este estudo deve ser continuado para que se possa aprofundar o conhecimento sobre a ecologia alimentar das YLG, assim como investigar a influencia dos aterros e do afloramento costeiro (*upwelling*) na disponibilidade de recursos para a colônia da Ilha da Berlenga.

Palavras-chave: Gaivotas-de-patas-amarelas; Nichos isotópicos; Ecologia Alimentar,

Abstract

The yellow-legged gull *Larus michahellis* (YLG) is a large and generalist gull species and with aggressive and predatory behavior, and most of its breeding populations have grown significantly over the last decades. Since 1994, a population control has been carried out on Berlenga Island (Portugal), where population numbers stabilized until 2009 and started to decrease slowly since then. In 2018, 5,661 breeding individuals were recorded there. Gulls have a high level of short- and long-term consistency in their feeding ecology, however, long-term studies correlating simultaneously their abundance and breeding success with oceanographic conditions, fisheries and feeding ecology are lacking. The aim of this thesis was to investigate how environmental conditions might influence the trophic ecology, density population and breeding success of YLG, breeding at Berlenga. For that aim, a long-term data base including population densities, breeding parameters, and trophic niches of YLG was related with environmental parameters (western North Atlantic Oscillation index (wNAO), Chlorophyll- α (Chl- α) and Sea Surface Temperature (SST)) and fishery data (landing of the most important species in the diet of YLG) to assess the ecology of this gull species in relation to environmental conditions and anthropogenic activity. Contrary to what was expected, there was no significant linear relationship between the number of breeding individuals and wNAO, however, the data suggest that the decrease in the number of gulls was caused by a year in which the value of wNAO was extremely low. SIA results suggest that the higher the Chl- α levels, the more YLG feeds on terrestrial resources and the smaller the niche size, suggesting a more specialized diet. Also, in years of positive wNAO, the niche is larger, so YLG have adopted a more generalist diet. Further research should focus on investigating the role of landfills and upwelling for this population.

Key words: Yellow-legged gull; Isotopic niches; Feeding ecology

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List of abbreviations

BI	Breeding Individuals
BMI	Body mass index
Chl- α	Chlorophyll α
NAO	North Atlantic Oscillation index
NB	Non-breeding individuals
SEAB	Bayesian model of the standard ellipse area
SEAc	Size of the standard ellipse obtained by Bayesian inference
SST	Sea surface temperature
TA	Total convex hull area
YLG	Yellow-legged Gull
wNAO	Extended winter NAO

1 Introduction

Seagulls are social seabirds that typically form large colonies during the breeding season. In Europe, some populations of seagulls showed a high increase in the last decades and it has been a matter of concern due to the impacts caused on ecosystems and urban areas (Faria et al., 2021; Martínez-Abraín et al., 2003; Vidal et al., 1998). The increase in the populations of large gulls (*Larus* spp.) has been documented since the beginning of the XX century (Chabrzyk & Coulson, 1976; Lockley., 1952), and were related to the proliferation of open refuse dumps and increase of discards from fisheries (Duhem et al., 2008; Hebert et al., 2002; Oro et al., 1995; Paracuellos & Nevado, 2010; Ramos et al., 2009). Some species are considered pests in several regions (Belant, 1997; Rock, 2005).

Because large gulls are opportunistic scavengers and top predators, often dependent on anthropogenic activities and natural prey (e.g. Ceia et al. 2014) , they can be used as bioindicators of human activity and marine environmental changes (Arizaga et al., 2014; Moreno et al., 2013). For this reason, they can be used to monitor abundance of fish and other marine resources (Gremillet & Charmantier, 2010; J. A. Ramos et al., 2013) as well as resources from anthropogenic activities (Lopes et al., 2020; Matos et al., 2018). Several studies focusing on the spatio-temporal variation in the use of resources have been performed to understand the feeding ecology of large gulls (Ceia et al., 2014; Ronconi et al., 2014; Stenhouse & Montevecchi, 1999; Watanuki, 1992), including identification of ecosystem changes through modification of food web structure (Hebert et al., 2008, 2009; Laurich et al., 2019). However, long-term studies correlating simultaneously their abundance and breeding success with oceanographic conditions, fisheries and feeding ecology are lacking.

The yellow-legged gull *Larus michahellis* (YLG) is a large and generalist gull species, with adult body weight of 900-1,200 g, and with aggressive and predatory behavior. YLG populations have grown significantly over the last decades (Martínez-Abraín et al., 2003; Paracuellos & Nevado, 2010), an effect which is closely related with the increase in the availability of food resources from human activities (Ramos et al., 2009). The breeding distribution of YLG ranges between northern Africa and southern Europe, including Morocco, the Iberian Peninsula, the Atlantic coast of France, Mediterranean and Macaronesian regions (Equipa Atlas, 2008; Louzao et al., 2011). Like

other large gulls, the yellow-legged gull often relies on foods from refuse dumps, landfills and discards from fisheries to complement its diet (Arizaga et al., 2010; J. G. Calado et al., 2018; CéLine Duhem et al., 2003; Louzao et al., 2011).

In Portugal, the largest breeding colony of YLG is located in the Berlenga Island (Ceia et al., 2014), whose population numbers has been monitored since 1974 (Figure 1). However, it is documented that there were about 1,000 breeding pairs in 1939 (Lockley., 1952). In more recent years, it was estimated ca. 1,300 breeding pairs in 1974 (Vicente, 1987), ca. 3,000 in 1981, and the population reached around 4,800 breeding pairs in 1983. It was then possible to notice the beginning of an exponential growth of the population up to 1994, when the number of ca. 22,000 breeding pairs was recorded (Figure 1), with a higher growth rate than those predicted by models (Morais et al., 1998). However, there is evidence that this growth was in part due to the immigration of seagulls from other colonies, with the Berlenga colony being part of a metapopulation that also includes Galicia's colonies (Morais et al., 1998). From 1994 to 1996 the Institute of Nature Conservation and Forestry (ICNF) authorized the culling of adult birds, which decreased considerably the number of breeding individuals by half in 1996 (Morais et al., 1998). Since then, a population control is carried out, and the culling of postures (i.e. eggs) in the whole island is executed (except in control areas) during the breeding season (Alonso et al., 2015). The trend in population numbers was more or less stabilized until 2009 and started to decrease slowly since then. In 2018 a population of 5,661 breeding individuals was recorded (ICNF).

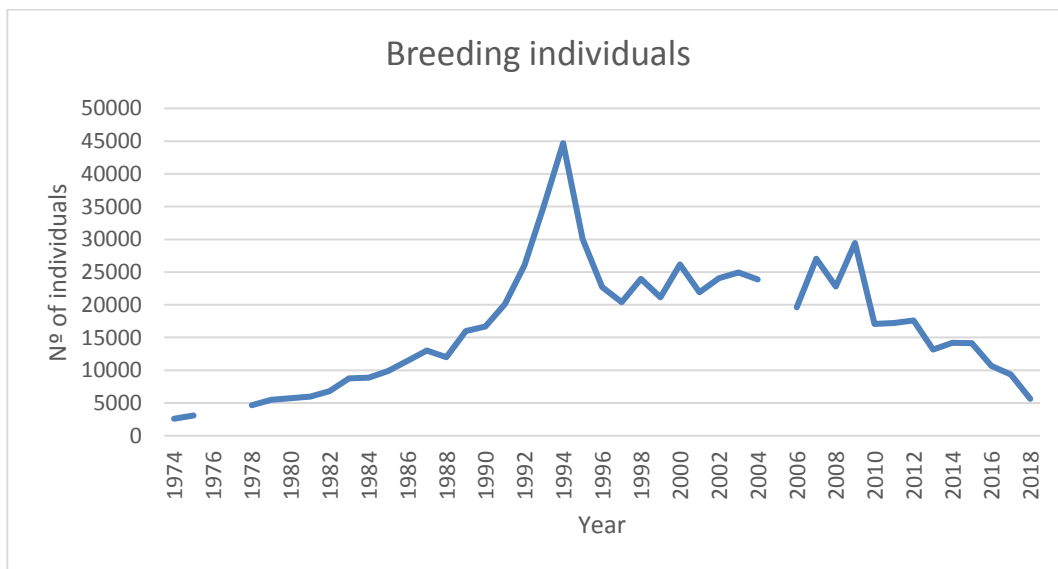


Figure 1.1: number of breeding individuals in the Berlenga Island colony, from 1974 to

2018. Data obtained from ICNF annual population control expeditions (ICNF)

Yellow-legged gulls from the Berlenga colony feed largely on fishery discards from Peniche harbor (10 km away) and have a high level of short- and long-term consistency in their feeding ecology (Ceia et al., 2014). The most relevant fish species in the diet of adults and chicks are commercialized in the Peniche harbor, and includes the European pilchard *Sardina pilchardus*, the chub mackerel *Scomber colias*, the horse/blue jack mackerel *Trachurus* spp., the demersal blue whiting *Micromesistius poutassou* and the European hake *Merluccius merluccius* (Alonso et al., 2015; Calado et al., 2018; Ceia et al., 2014). However, studies conducted in this colony observed that the diet of adults during the breeding season is also largely composed of natural sources, such as the Henslow's swimming crab *Polybius henslowii* (Alonso et al., 2015). In fact, this crab can be the most frequent prey, corresponding up to 95% of the diet when available (Alonso et al., 2015; Ceia et al., 2014). There is evidence that seagulls fed on a greater proportion of food from refuse dumps when *P. henslowii* was absent or in low densities (Ceia et al., 2014). Although the adult diet mostly relies on this swimming crab during the breeding season, substantial temporal variations in the oceanographic conditions determining the abundance of this resource might occur, such as the concentration of chlorophyll a (Chl- α), which was found to be positively correlated with the density of *P. henslowii* (Signa et al., 2008). As a consequence, the crab consumption can decline or even be absent of the diet, being replaced by fish and terrestrial food resources (Ceia et al., 2014; Mendes et al., 2018). These fluctuations influencing the availability of marine resources are common across years and even during different periods of the breeding season (Alonso et al., 2015; Ceia et al., 2014), and are related to fluctuations in oceanographic conditions, such as the North Atlantic Oscillation index (NAO) (Mendes et al., 2018).

While using seagulls as bioindicators, a parameter that is often analysed is the breeding success, since under conditions of limited resources the condition status of adult birds will be poorer, reducing for instance the number of breeding individuals, egg clutches, egg volume, hatching success, fledging success and overall reproductive success (Hebert et al., 2002, 2009). Reproductive success is closely related to the diet as the female stocks the resources consumed for weeks before egg laying for egg formation and food supplementation, so the quality of the food ingested during pre-laying

will be reflected on egg clutches and egg-volume (Annett & Pierotti, 1999; Bolton et al., 1992; Martin, 1987). It was shown that the quality of eggs in colonies of herring gulls (*Larus argentatus*) is affected by dietary and environmental factors (Hebert et al., 2020), and a decrease in egg volume of *L. argentatus* was correlated with a drop in productivity in the Great Lakes that have become more oligotrophic in the last decades (Bunnell et al., 2014), and consequent decrease in the quality and abundance of prey (Paterson et al., 2014; Hebert et al., 2008). Moreover, a long-term study showed that, egg clutches and egg volume of black-legged kittiwakes (*Rissa tridactyla*) have changed substantially in response to oceanographic conditions, specifically variations in influx of warm Atlantic Water into the Barents Sea (Barrett et al., 2017). The effect of food quality on the breeding success was also observed in YLG. In the Illas Cíes, NW Spain, an experiment was performed in the colony of YLG and supplementary feeding was provided to the parents during the egg formation period. This study demonstrated that the parental food condition influences the egg volume, which was greater on the nests where supplementary food was provided to adults. In addition, a higher hatching success was observed in the supplemented group (Pérez et al., 2006).

In several bird species, it is observed a declining size in last laid eggs. Together with hatching asynchrony, this generates a difference in size between the siblings and, consequently, when there is competition for food, it can lead to the death of smaller and weaker individuals (Hillström et al., 2000; Ricklefs, 1993). In order to verify the effect of food quality on the last laid egg of the YLG (i.e. the third egg), a research was conducted in the colony of Comacchio lagoon, NE Italy. The results showed that the mass of the third egg is enhanced due to an increase in the content of albumen, indicating that when ecological conditions are favorable, the third egg has greater chances of survival, and thus the entire clutch (Saino et al., 2010).

1.1 *Assess the Feeding Ecology of Yellow-legged gulls*

As a generalist species, YLG consumes a great diversity of prey, but during the breeding season they are central-place foragers, which makes them more limited in relation to the food available near the colony site. Gulls' chicks are mostly fed with fish (Arizaga et al., 2013; Moreno et al., 2010), however the contribution of this food source to the chick's diet is dependent on its availability, therefore a higher consumption of

refuse tips is observed when the availability of fish and other marine resources are limited (Arizaga et al., 2013). This species can travel up to 100 km in search of food (Ceia et al., 2014). However, longer trips might influence their breeding success, since the time seagulls spend foraging affects the time spent in the nest (Bukacinski et al., 1998). Besides, the dependence on anthropogenic food resources is directly related to its distance from the nesting colonies, so the closer a source is to the colony the more it will be exploited (Alonso et al., 2015; Ramos et al., 2009).

A comparison of the feeding ecology of yellow-legged gulls from two distinct colonies in Portugal, in Berlenga Island and in Deserta Island (Algarve), revealed that even when the colony is close to the mainland, this species tends to use a marine foraging strategy, especially when environmental conditions are favorable (i.e. higher winter NAO index values; Mendes et al., 2018). Nevertheless, yellow-legged gulls exhibited a high flexibility at both individual and population levels, and frequently vary between marine, mixed and terrestrial strategies, foraging on land more often when marine resources are scarce and the oceanographic conditions are poor (Mendes et al., 2018).

The diet of YLG can be efficiently assessed using two complementary techniques: pellet/regurgitated samples and stable isotope analysis (SIA). Pellets are undigested regurgitated masses, which may contain bones, otoliths and hard parts of crustaceans, and is the more traditional method, that allows a taxonomic study of prey items (González-Solís et al., 1997). However, it has the disadvantages of allowing only the analysis of prey ingested in a short-term, and during specific periods, and thus an incomplete method that not fully addresses the feeding ecology and foraging niches of seabirds throughout the year (Barrett et al., 2007; Votier et al., 2003). Within this context, SIA has been increasingly used as a valuable complementary approach providing short- and long-term information on assimilated diets of gulls and allows to identify trophic groups of prey, estimate prey consumption, calculate trophic levels and evaluate trophic niches (Arizaga et al., 2013; Calado et al., 2018; Hobson et al., 1994). Carbon stable isotope values ($\delta^{13}\text{C}$) can indicate the relative contribution of marine or terrestrial food sources to the diet of seagulls; e.g. marine prey typically show higher $\delta^{13}\text{C}$ values than terrestrial food sources (Hobson et al., 1994; Mendes et al., 2018). This allows to identify foraging habitats and strategies (Ceia et al., 2014; Mendes et al., 2018), including the importance of fishery discards in their diet (Calado et al., 2018; Matos et al., 2018).

Nitrogen stable isotope values ($\delta^{15}\text{N}$) are used as a proxy to establish the trophic level since the consumer tissues are more enriched in ^{15}N s isotope compared to prey tissues (Hobson et al., 1994; Hobson & Clark, 1992), with an enrichment of around +2.0 to +5.0 ‰ at each trophic level (Hodum & Hobson, 2000). On the other hand, $\delta^{13}\text{C}$ values change very slightly between trophic levels, around 0 to +1.0 ‰ between prey and consumers, and thus an excellent proxy for habitat use (Inger & Bearhop, 2008). In addition, SIA can be used to infer changes in the bird's diet during different periods of the year by analysing tissues with different turnover rates (Hobson & Clark, 1992).

Through the study of gulls diet it is possible to monitor shifts in marine resource availability, which in turn may reflect long-term variations in the environment, i.e. different structure of biological communities (Cairns, 1992). Using a retrospective measurement of biochemical indicators of organism trophic position, temporal changes can be described, as pathways of energy and critical nutrients flow through food webs will also be altered (Eby et al., 2006; Hebert et al., 2006). Human activities, such as fisheries, are associated with changes in the diet of seagulls, and it can lead to a decrease or increase of their trophic levels depending on the resources consumed (Farmer & Leonard, 2011). As generalist and opportunistic species, seagulls can change prey and the sources of food, adapting to new conditions (Ronconi et al., 2014). To take advantage of available resources, gulls are able to change their behavior quickly, and the effects of these changes, e.g. trophic changes, are seen at the individual and populational levels (Bosch et al., 1994; Oro et al., 1995). Thus, YLG might respond differently to food availability changes caused by human impacts (e.g. Matos et al, 2018). Currently, there are large populations of YLG that have a large part of their diet dependent on human activities, nevertheless, some studies show that with decreasing fisheries discards or other sources related to fishery, such as spawning baitfish, seagulls tend to adapt and change main prey (Stenhouse & Montevecchi, 1999).

Other examples show different effects of changing food availability. A study carried out with Yellow-legged gulls *L. michahellis atlantis* compared the past and present feeding ecology of this subspecies that occurs in the Azores Archipelago, through the use of stable isotopes analysis of feathers from modern birds (2009-10) and museum-preserved birds (1921-1928; Pedro et al., 2013). This population was found to be currently feeding at a lower trophic level and at more coastal/terrestrial prey, and one of the reasons was the increase in the consumption of food of anthropic origin, such as

refuse, and a decrease in the predation of seabirds, a resource widely used during periods of poor marine conditions. With the close of landfills, there is the possibility to understand how seagulls can adapt to this sudden change in the availability of foods. In Gipuzkoa, northern Iberian Peninsula, after the closure of a landfill, an increase in feeding on terrestrial prey during breeding period, but not in winter, was observed (Zorroza et al., 2020). Interestingly, and contrary to what was expected, there was no increase in fish consumption (Payo-Payo et al., 2015).

These studies exemplify how trophic ecology of gulls are affected by several factors, in addition to reflecting human impacts on the environment. Therefore, through the study of gull's diet, it is possible to assess distribution of prey at a local scale (Moreno et al., 2010; Ramos et al., 2009), changes in marine ecosystems over long time scales and the responses of these birds to oceanographic changes, making them good bioindicators of environmental conditions and anthropogenic activities.

1.2 Environmental conditions affecting trophic ecology and breeding success

North Atlantic Oscillation (NAO) index is a large-scale atmospheric pattern of variability that affects the mid and high latitudes of the Northern Hemisphere, which is defined as movements of air masses between the Arctic and the sub-tropical Atlantic, affecting the speed and direction of the winds, carrying heat and humidity (Hurrell et al., 2003). The NAO can be divided in two phases, the positive phase is characterized by an increase in precipitation and sea-surface temperature in northern Europe, with the strengthening of the winds from west that begin to head north, while Southern Europe undergoes opposite changes. The negative phase of the NAO, in turn, is characterized by Southern Europe being hit by the storm's return, causing increased heat and wind (Pinto & Raible, 2012).

These shifts in atmospheric variables lead to alterations in the entire North Atlantic environment and influences marine ecological processes such as productivity of phytoplankton and fish stocks, since it changes the salinity and temperature of the seawater, as well as circulation patterns and ice formation in the North (Best et al., 2011; Visbeck et al., 2003). Such shifts in environmental parameters may thus be reflected in

the availability of marine resources of seabirds, including seagulls, that are affected by a bottom-up effect due to changes in prey abundance and distribution (Crawford et al., 2015; Grémillet & Boulinier, 2009; Mendes et al., 2018).

A low value of the extended winter (December to March) NAO index is related to poor oceanographic conditions and lower marine prey abundance (Paiva et al., 2013). Analyses of stable isotopes and pellet samples from a colony of Audouin's gulls (*L. audouinii*) in the Deserta Island (Ria Formosa) in 2014, revealed that the chicks experienced a decrease in the trophic level, when compared to other years (Calado et al., 2018). This was related to unfavorable oceanographic conditions, which might have led adults to increase foraging effort into more offshore areas, coincident with a much lower value (-1.97) of the extended winter (December to March) NAO index than usual. Also, in a colony of Little Terns (*Sternula albifrons*) in Ria Formosa, climatic conditions affected diet and breeding parameters of individuals, as negative NAO conditions led to earlier breeding and lower SST during winter-spring stimulated the abundance of prey fish, consequently influencing their reproductive parameters (Ramos et al., 2013).

1.3 Objectives

The aim of this thesis is to investigate how environmental conditions can drive the trophic ecology, density population and breeding success of a large colony of an opportunistic seabird species, the yellow-legged gull (*Larus michaellis*), breeding at Berlenga island (Portugal). For that aim, a long-term data base including population densities (since 1974), breeding parameters (since 2002), and trophic niches (from stable isotopes, since 2011) of yellow-legged gulls will be related with environmental parameters (North Atlantic Oscillation index, Chlorophyll a and Sea Surface Temperature) and fishery data (landing of the most important species in the diet of YLG) to assess the ecology of this gull species in relation to environmental conditions and anthropogenic activity.

It is expected that YLG is suitable to be used as an indicator of environmental changes in the long-term, and the specific questions to be assessed are:

- 1) Is the long-term variation in annual breeding population size related to large scale variations in a) the NAO (and winter NAO) index, b) Sea Surface Temperature (monthly SST mean, annual SST mean), c) Chlorophyll a concentration (monthly Chl a mean, annual Chl a mean) d) fisheries landings (by species and month, total landings)?

- 2) Is the long-term variation in annual reproductive success and trophic niche of YLG related to large scale variations in the a) b) c) and d) above-stated variables?

2 Materials and Methods

2.1 Study Area

The Berlengas Archipelago consists of three islands: Berlenga Grande (i.e. Berlenga Island), Estelas and Farilhões (Figure 2.1), and data was collected on Berlenga Island, Portugal (39°24'N, 009°30'W). Berlenga is a small rocky neritic island of ca. 78.8 ha, located within the continental shelf about 11 km off western Portugal coast, surrounded by shallow waters. Berlengas Archipelago is characterized by having a high marine productivity due to upwelling regime, more intense from April to September with high concentration of chlorophyll *a* and low sea surface temperature (Paiva et al., 2013; Sousa et al., 2008).

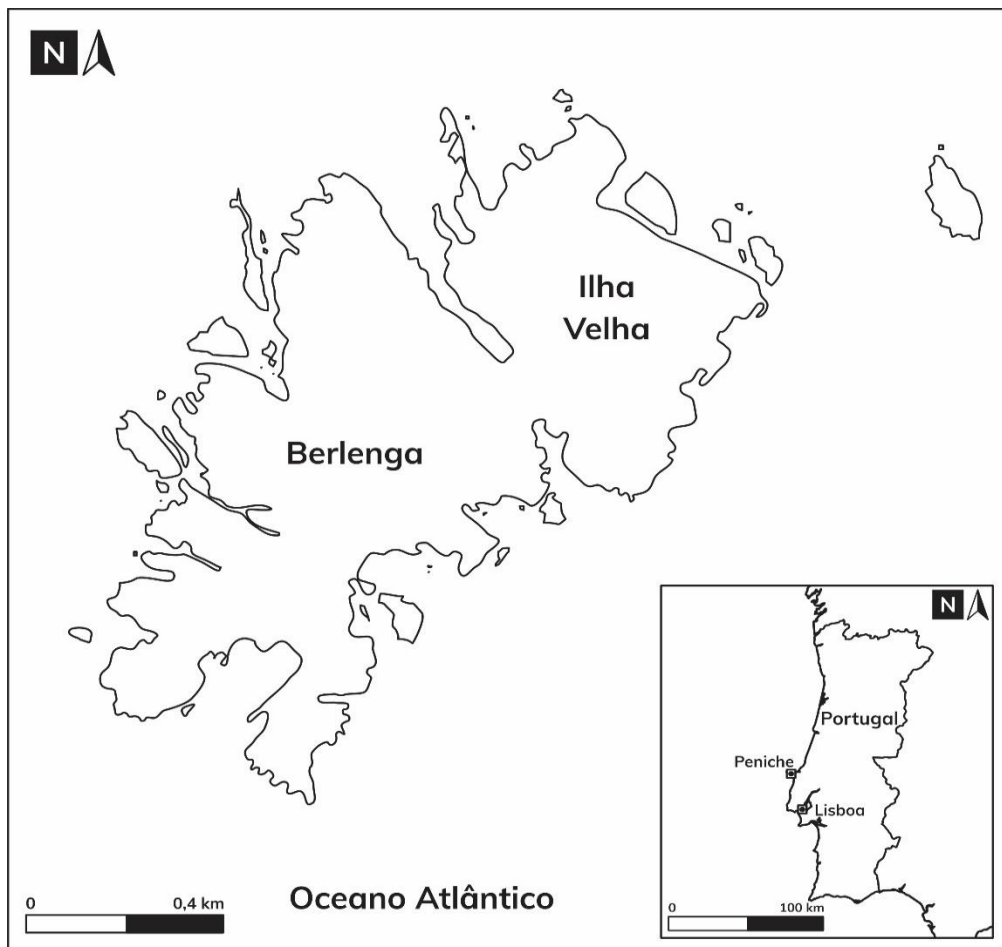


Figure 2.1: Berlenga Island, west central Portugal.

The estimated population of yellow-legged gulls was 5,661 breeding individuals in 2018 (ICNF) and data was collected annually, during the breeding period, from 1974 to 2018.

2.2 *Sampling Procedures*

Data concerning the counts of breeding individuals of YLG in the colony and their postures (i.e. number of breeding individuals and total number of eggs), were collected during the annual census and population control campaigns for yellow-legged gulls carried out by the Nature and Forest Conservation Institute (ICNF), and started in 1974 (census of breeding birds) and in 1999 (counts of the eggs). Non-breeding individuals were also counted since 1996. Egg culling is carried out on Berlenga Island since 1999 in the entire island, excluding two permanent control areas of 800 m² each to assess annual breeding success of gulls (Carreiro dos Cações and Fortaleza). Because YLG can replace their postures and are not completely synchronized in the first laying, three campaigns are performed by the wardens of ICNF, with 21-day intervals, to ensure the maximum effectiveness of the method. The information collected during these campaigns were: number of breeding and non-breeding individuals (BI and NB), total number of eggs laid (Total Eggs), number of eggs per breeding individual (estimated), number of eggs hatched in controls areas (breeding success) and total days from the beginning of the year to the date of the first egg was laid (First Egg).

During 2011-2018, a total of 140 breeding individuals were sampled for approximately two weeks in May/June, when adults were incubating. By using square traps over the nests, adults with 3 egg clutches were captured and ringed. Each bird was weighted, and the wing length was measured. The individuals were chosen randomly and blood samples of ca. 0.5 ml from the tarsal vein were collected, using 27G needles, for isotopic analyses. The time between capture and release took about 10 minutes for each bird. Up to 3 h after collection, the blood samples were then separated into plasma and blood cells, using a centrifuge for 15 min at 1250 x g and stored frozen (-20°C) until isotopic analyses. To estimate the volume of eggs each year, nests with 3 eggs from the first clutch were randomly measured (length and width, to the nearest 0.1 mm) in the control areas since 2012 (except in 2013).

The Portuguese Institute of the Sea and the Atmosphere has provided data collected from 1996 to 2018 on *P. henslowii* in its research campaigns covering the Portuguese continental coast from Caminha to Vila Real de Santo António, from 20 to 500 meters, with a variable frequency each year, with the aim of estimating abundance and biomass indices of the main species on the Portuguese continental coast, as well as studying their distribution pattern. The gear used is a bottom trawl.

2.3 *Body mass index*

The body weight and wing length of each individual was used to calculate the body mass index (BMI), considered as a value of Darwinian fitness and an indicator of energetic reserves (Brown, 1996). The BMI was obtained using a regression analysis of the sampled individuals (pooled data) and extracting the residuals from this regression ($N = 139$, $r = 0.492$, $p = 2.2 \cdot 10^{-16}$). The mean BMI value for each study year was then used for subsequent analyses.

2.4 *Stable isotope analysis (SIA)*

For this work, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in the blood plasma and cells of YLG were analysed, and a Bayesian dual-isotope mixing model was used to estimate the annual isotopic niches of this generalist species.

Lipids were removed from plasma by successive rinsing in a 2:1 chloroform-methanol solution, to avoid depletion of $\delta^{13}\text{C}$ values that high lipid content in plasma may cause. However, this procedure is not necessary for analysis of red blood cells (RBC) due its low lipid content (Cherel et al., 2005). C/N mass ratios are a proxy of lipid content since a lipid structure is made up of large amounts of carbon and little or no nitrogen (Barnes et al., 2007). The C/N mass ratio during this study was $3.5 \pm 0,3$ (mean \pm SD) in RBC and $3,7 \pm 0,2$ in plasma, below the 4.0 threshold which correspond to a low lipid concentration in the tissues (Post et al., 2007)

In order to determine carbon and nitrogen stable isotopic composition of the plasma and blood cells, a Flash EA1112 Series elemental analyzer was used, coupled online via Finnigan conflo II interface to a Thermo Delta V S mass spectrometer. For the determination of nitrogen and carbon isotope ratios, 0.3 mg of each sample was

combusted in a tin cup. The usual δ notation of isotope ratios are used, based on the Vienna-PeeDee Belemnite (V-PDB) for carbon, and for nitrogen, atmospheric N₂ (AIR), expressed as ‰. $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$, where $R = {}^{13}\text{C} / {}^{12}\text{C}$ or ${}^{15}\text{N} / {}^{14}\text{N}$, respectively. Precision of <0.2 ‰ were indicated by replicate measurements of internal laboratory standards (acetanilide), for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Stable isotopes values reflect what the consumers have been consuming, and the values obtained from blood samples inform on the diet and at-sea foraging habitat selection (Pearson et al., 2003). Because different blood components have specific turnover rates, the analyses of plasma provides values referring to the diet from 1-2 weeks before sampling, i.e. incubation period, while RBC provides information referring up to 4 to 5 weeks before sampling, i.e. pre-laying period (Hobson & Clark, 1992).

2.5 *Environmental parameters*

Three environmental parameters were used to evaluate oceanographic conditions around the breeding colony: Surface Chlorophyll *a* (Chl-*a*), Sea Surface Temperature (SST) and the North Atlantic Oscillation Index (NAO). To characterize marine productivity, data on Chl-*a* and SST during the period from 2003 to 2018 were downloaded from the Giovanni website <https://giovanni.gsfc.nasa.gov/giovanni/>, for the months of March, April, May and June. These months were selected to correlate with data obtained in this study because coincides with the sampling of birds and the respective breeding period (i.e. May and June). However, March and April were also analysed since the previous months to the breeding season might have a strong influence for the body reserves and fitness of birds, determining their breeding success. Data prior to 2003 were not available, as the Aqua-MODIS satellite, the one responsible for collecting these data, only became operational in July 2002. For both environmental predictors the plot type selected was “Time Series, Area-Averaged” and the coordinates (Bounding Box and Shape) were -9.57 W, 39.35 N, -9.45 W, 39.46 N, the area around the colony. For both parameters, the option chosen was Level 3 at 4 km spatial resolution, monthly composites of satellite-derived Chl-*a* and SST. For the SST the period of the day chosen was the night-time, because of the lower amplitude of variation than diurnal SST (Paiva et al., 2010).

The extended winter NAO index (wNAO; Hurrell & Staff, 2012) – December to March – was used because it has long-lasting effects on oceanic productivity patterns during that year (Paiva et al., 2013), and confirmed to affect YLG foraging strategies (Mendes et al. 2018). A year with negative wNAO presents low recruitment and prey availability for seabirds in this area of the North Atlantic, due to an increase in warm conditions, wind speed and vertical water mixing along the Iberian Peninsula, and a consequent decrease in the marine productivity. On the other hand, a positive phase of the wNAO is associated with a typical increase of productivity and consequent availability of marine resources, although very high values can induce strong upwelling events during the spawning season of small pelagic fish that move plankton and fish larvae away from the continental shelf, compromising the availability of resources (Santos et al., 2007).

2.6 Fisheries Data Collection

Data on fisheries landings were obtained from the main fishing harbor on the area, the Peniche harbor (Figure 2.1) since 2002 (no available data for earlier years), as a proxy of the fluctuations in fish availability in the study area each year, during the period of interest: March, April, May and June. The pooled period of March-June was also used to assess an overall proxy of the fluctuations in fish availability related with the sampling of YLG. The fish species taken into account were the nine most consumed by the YLG from Berlenga Island (Alonso et al., 2015): the pelagic *S. pilchardus*, *Scomber spp.*, *Trachurus spp.*, *M. poutassou* and the garfish *Belone belone*, and the demersal *M. merluccius*, the bogue *Boops boops*, the pouting *Trisopterus luscus* and the European conger *Conger conger*. Data regarding Peniche Harbor's fisheries landings were analysed by fish species separately, and by groups of Pelagic and Demersal fish species, and Other Species, which includes all the other species landed on Peniche Harbor, during March-June. Fish Landings were also analysed during March-June for each fishing gear, i.e. Trawl, Purse-seine and Polyvalent fishing.

In order to estimate abundance and biomass of the main prey species of YLG, i.e. the Henslow's swimming crab *P. henslowii* (Ceia et al. 2014), data was obtained from the Portuguese Institute of the Sea and the Atmosphere. Data was collected from 1996 to 2018 during fishery research surveys using a bottom trawl from 20 to 500 meters and

covering the Portuguese continental coast from Caminha to Vila Real de Santo António, with a variable frequency each year.

2.7 Data Analysis

Data were divided according to the available variables into two periods, i.e. 2002-2018 and 2011-2018. As most of the data did not follow a normal distribution, the analysis was performed using nonparametric statistics using Spearman's correlations.

During the period 2002-2018, the variables used were those obtained from the censuses, breeding success, eggs laid, fisheries landings and environmental parameters. For the period 2011-2018, the variables related with trophic ecology, biometric measurements of gulls (i.e. BMI) and egg volume were used.

Data were separated into explanatory and response variables. The explanatory variables were those related to fisheries and oceanographic conditions. Response variables included the data collected in the colony, i.e. number of breeding individuals, egg parameters, breeding success and BMI. The variables obtained through SIA were analysed both as explanatory variables and response variables. SIA variables were considered as explanatory when relationships were assessed for number of breeding individuals, egg parameters, breeding success and BMI. When the variables analysed were the fisheries and oceanographic conditions, SIA metrics were considered as response variables.

To analyze stable isotopic data in order to build isotopic niches and perform comparisons between years, it was used metrics based in a Bayesian framework SIBER: Stable Isotope Bayesian Ellipses in R (SIBER, Jackson et al., 2011). This methodology subjects the data to a robust Bayesian statistical analysis and three main metrics are obtained: TA, SEAc and SEA.B. TA refers to total convex hull area, constructed from the extreme values of the isotopic niche, that represents the total amount of niche space occupied, but it is very sensitive to sample size (Layman et al., 2007). SEAc is the size of the standard ellipse obtained by Bayesian inference, that is, statistically corrected for sample size and represents 40% of the data from the population of interest. The SEA.B is the Bayesian model of the standard ellipse area, that is the result of a repetition of a thousand times the model, and thus can be used to compare statistically the isotopic

niche width of the different years of study. The package *SIAR* was used to calculate the metrics from SIBER using R. All analyses were performed using the package Commander under R 4.0.3 (R Core Team, 2020) RStudio, and the significance level was set at $P < 0.05$.

3 Results

3.1 Analyses 2002-2018

Contrary to what was expected, the abundance and biomass of *P. henslowii* did not show significant correlations with any variable from the YLG from Berlenga Island.

3.1.1 Relationships with Environmental Parameters

A small decrease in Chl- α values, and an increase in SST, was recorded from March to June in the Portuguese coast, and particularly around the Berlenga Island (Figure 3.1).

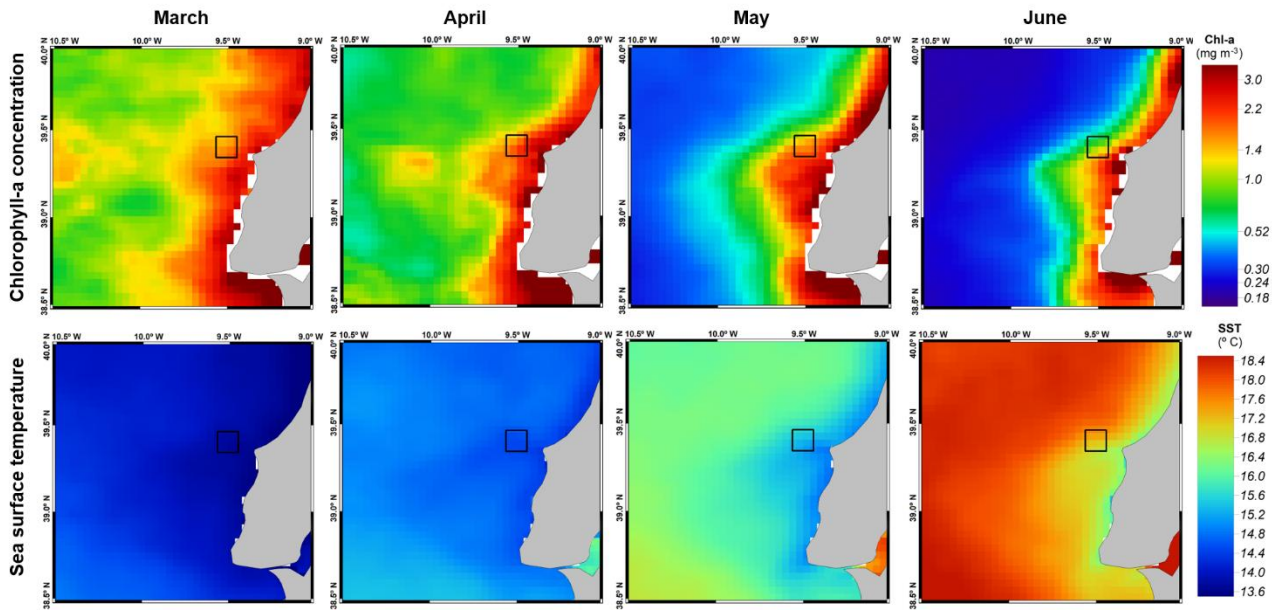


Figure 3.1: climatological averages of chlorophyll-a (above) and SST (below) in the months of March, April, May and June, during the period 2002-2018. The black square indicates the region of Berlenga Island and the gray region represents land, in this case, Portugal.

Significant negative correlations were found for the 2002-2018 period between Breeding Success and June SST ($r_s = -0.6$, $p = 0.029$, Figure 3.2.A) and First Egg and wNAO ($r_s = -0.5$, $p = 0.041$, Figure 3.2.B). Positive significant correlations were detected between June Chl- α and Eggs per individual ($r_s = 0.68$, $p = 0.020$; Figure 3.2.C) and Non-breeding individuals and June SST ($r_s = 0.6$, $p = 0.027$, Figure 3.2.D). No other potential relationships between population numbers/breeding success and environmental variables were detected.

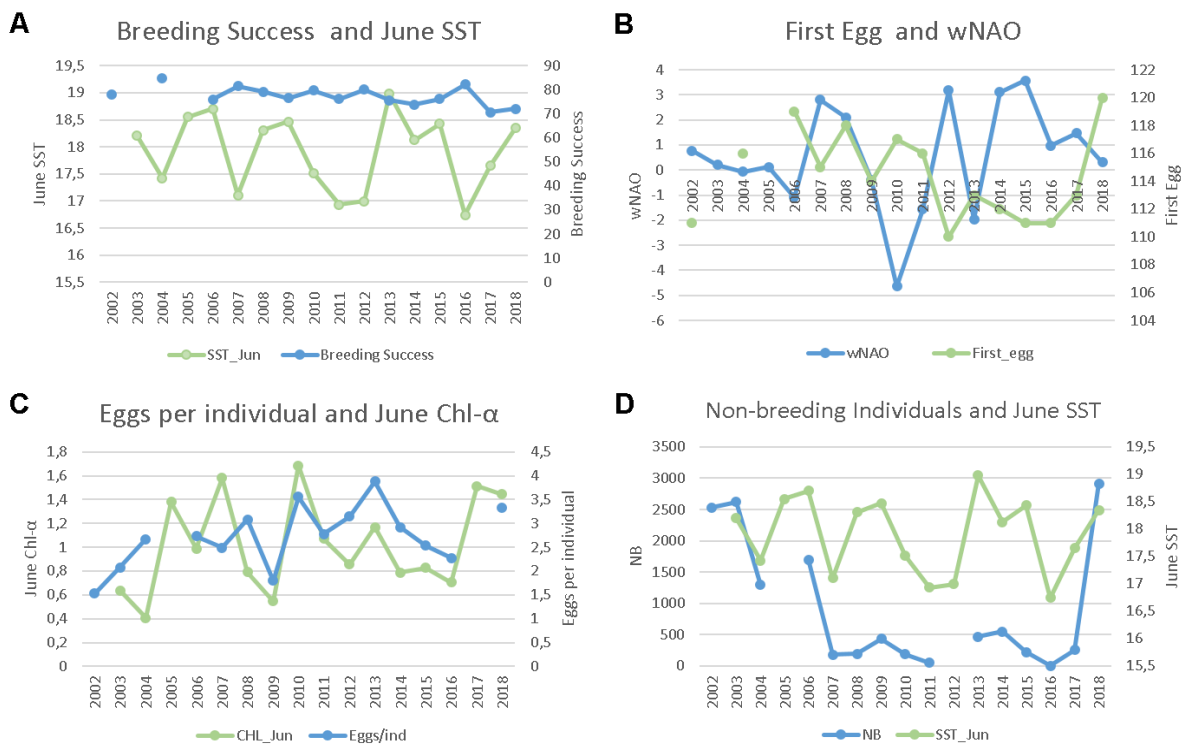


Figure 3.2: 2002-2018 period A) Breeding Success (%) and June SST (C°), B) First Egg and wNAO, C) Eggs per individual and June Chl- α (mg.m⁻³), and D) Non-breeding individuals and June SST (C°).

Contrary to what was expected, there was no significant relationship between the number of breeding individuals and wNAO since 2002 (Figure 3.3). These two variables had a similar variation until 2010, when the wNAO had a very pronounced drop. From then on, the number of breeding individuals has not been able to recover to previous numbers and started a continuous descending until 2018.

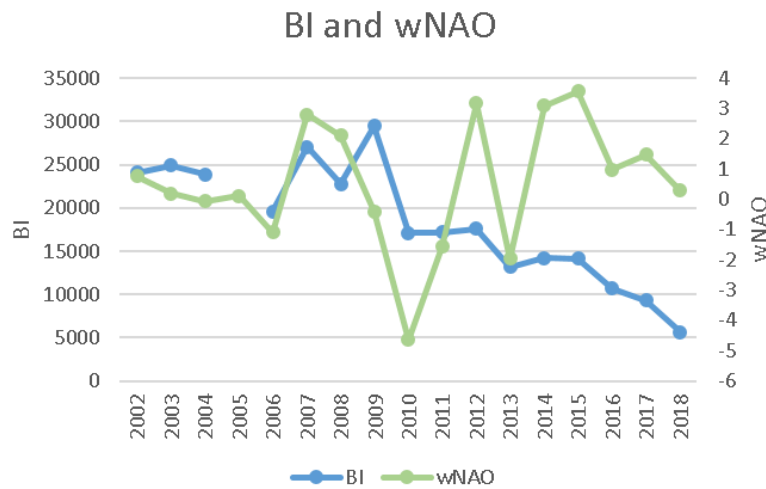


Figure 3.3: Number of breeding individuals and wNAO values during the period of 2002-2018

3.1.2 Relationships with Fisheries Landings

The variable Total Eggs showed several significant correlations with fisheries variables. Significant positive correlations were observed with Fisheries Total Landings ($r_s = 0.6$, $p = 0.03$; Figure 3.4.A) and with Pelagic Species ($r_s = 0.6$, $p = 0.03$; Figure 3.4.B).

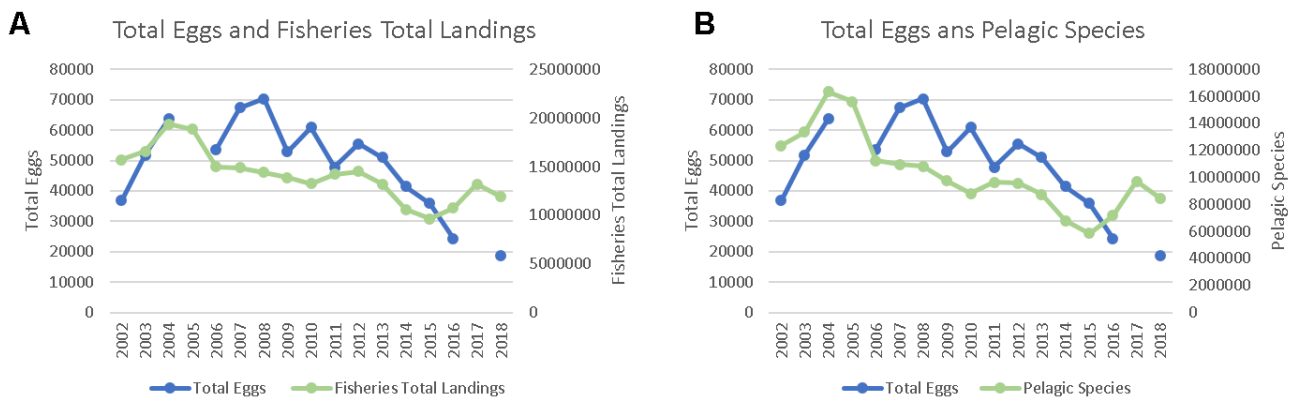


Figure 3.4: Graphs of A) Total Eggs and Fisheries Total Landings (kg) and B) Total Eggs and Pelagic Species

Regarding the fishing gear, it was possible to observe a positive correlation with Purse-seine ($r_s = 0.6$, $p = 0.03$; Figure 3.5.A) and a negative correlation with Trawl ($r_s = 0.6$, $p = 0.03$; Figure 3.5.B)

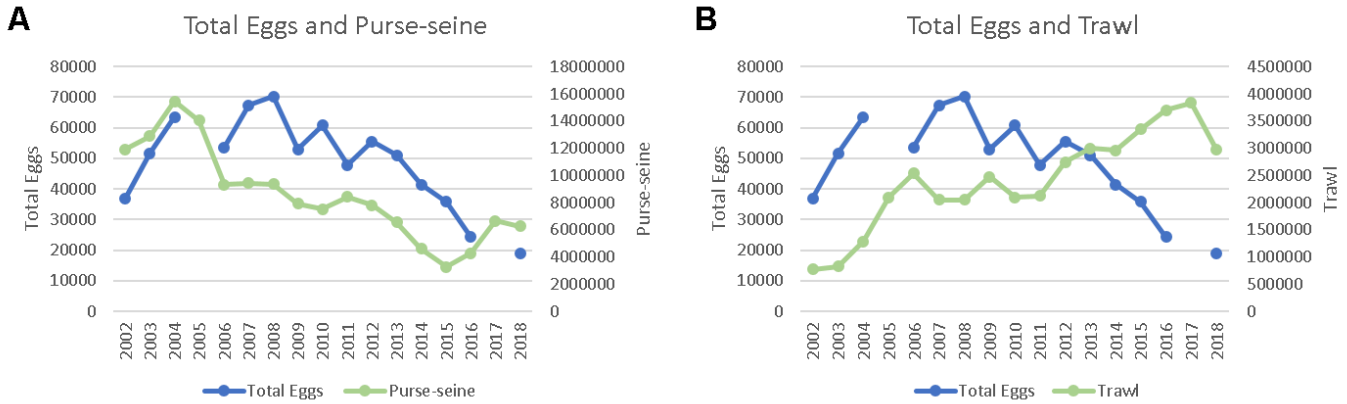


Figure 3.5: Values of Total Eggs and Purse-seine, kg (A) and Total Eggs and Trawl, kg (B)

Concerning fish species analysed separately, significant correlations were found between Total Eggs and two demersal species: negative with *B. boops* ($r_s = -0.7$, $p = 0.004$; Figure 3.6.A) and positive with *C. conger* ($r_s = 0.5$, $p = 0.038$; Figure 3.6.B).

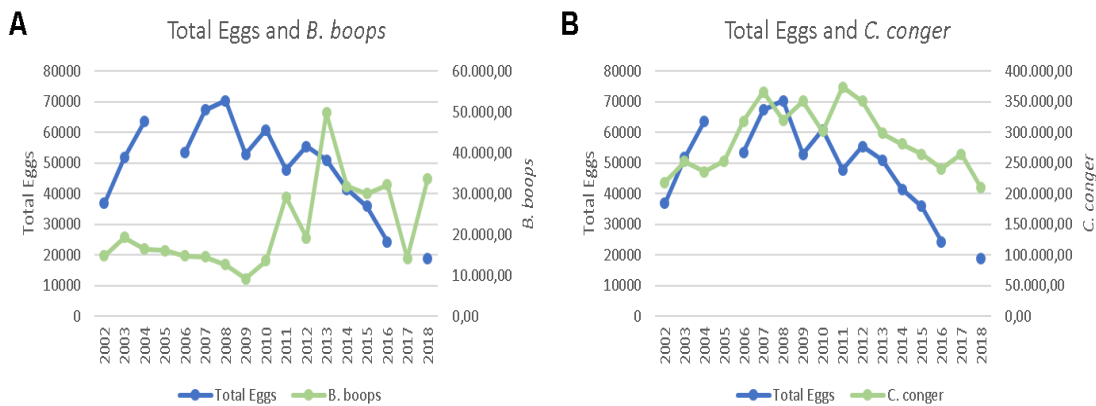


Figure 3.6: Graphs of Total Eggs and *B. boops*, kg (A) and Total Eggs and *C. conger*, kg (B).

Analyses correlating Total Eggs with pelagic species showed three significant correlations: with *S. pilchardus* ($r_s = 0.5$, $p = 0.036$; Figure 3.7.A), *Trachurus* ssp. ($r_s = -0.6$, $p = 0.026$; Figure 3.7.B) and *M. poutassou* ($r_s = 0.6$, $p = 0.030$; Figure 3.7.C).

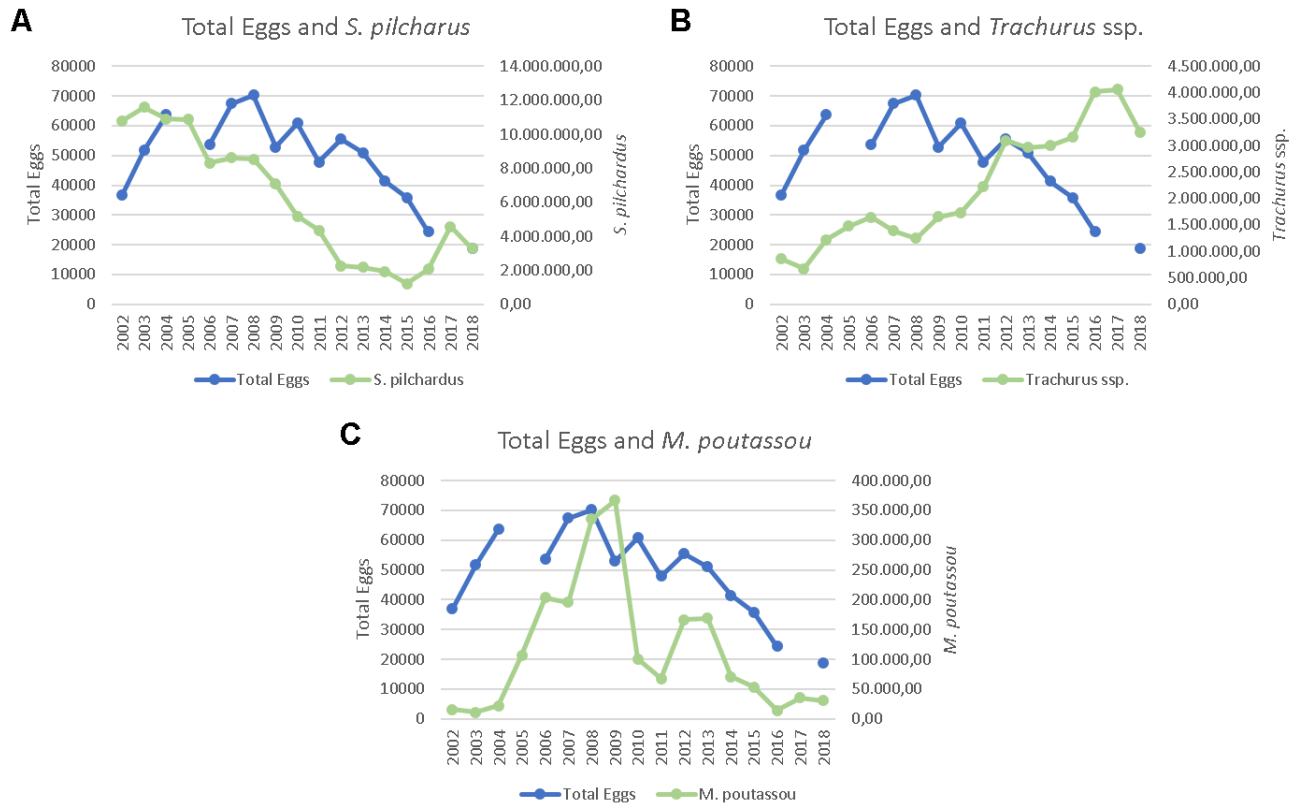


Figure 3.7: Values of A) Total Eggs and *S. pilchardus*, (kg) B) Total Eggs and *Trachurus ssp.* (kg) and C) Total Eggs and *M. poutassou* (kg), during the period of 2002-2018.

Analyses considering the Eggs per Individuals and fisheries landings resulted in three significant correlations with fish species (Figure 3.8): negative correlation with *T. luscus* ($r_s = -0.59$, $p = 0.022$), and positive correlations with *M. merluccius* ($r_s = 0.56$, $p = 0.031$) and *Scomber ssp.* ($r_s = 0.64$, $p = 0.012$).

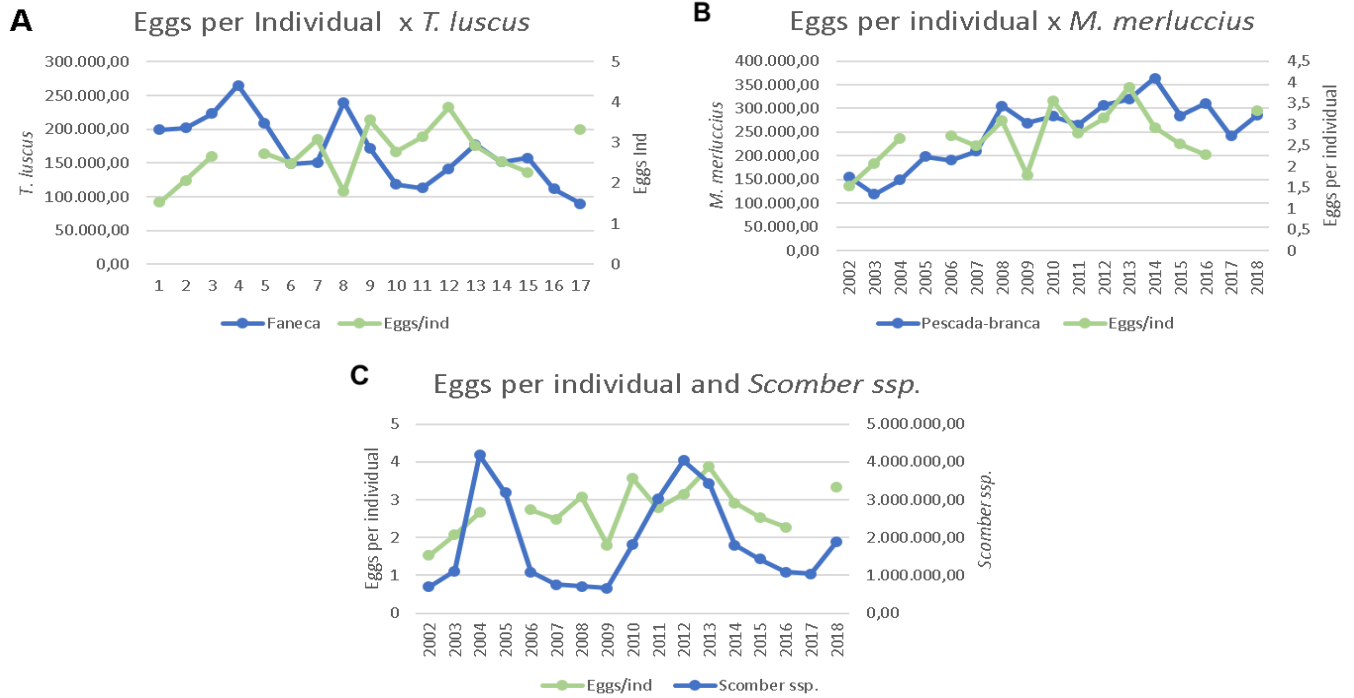


Figure 3.8: 2002-2018 Period A) Eggs per individuals and *T. luscus*l (kg) B) Eggs per individuals and *M. merluccius* (kg) and C) Eggs per individual and *Scomber ssp.*(kg)

Non-breeding Individuals (NB) showed two similar negative correlations with *C. conger* and Other species ($r_s = -0.56$, $p = 0.031$; Figure 3.9.A and B). Breeding success has a negative correlation with Trawl ($r_s = -0.53$, $p = 0.043$; Figure 3.9.C), and Days to First Egg has a positive correlation with June Fisheries ($r_s = 0.55$, $p = 0.033$; Figure 3.9.D).

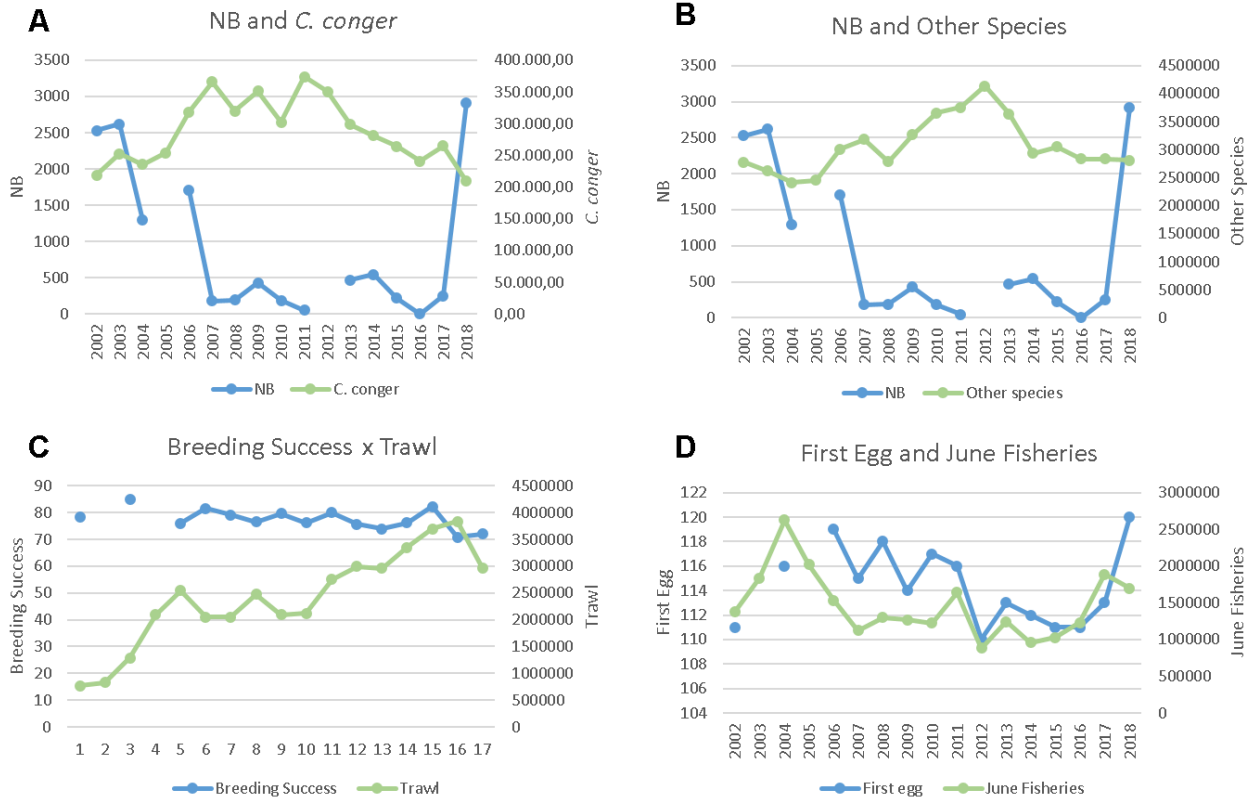


Figure 3.9: Values of A) Non-breeding individuals and *C. conger* (kg), B) Non-breeding individuals and Other Species (kg), C) Breeding Success and Trawl (kg) and D) First Egg and June Fisheries (kg).

3.2 Analysis 2011-2018

The analysis of this period focused on the results of SIA and the comparison between the results of the SIA and the other variables, that is, environmental parameters, censuses and breeding success, and fisheries landings.

3.2.1 Isotopic Values of YLG

Among the years of study, 2013 was the one when the population showed the lowest value for mean $\delta^{13}\text{C}$ and highest standard deviation (i.e. $-20.6\text{‰} \pm 2.0$) in plasma samples. This occurred mainly because there was an outlier in 2013, with a more negative $\delta^{13}\text{C}$ value (-24.7‰) and a higher $\delta^{15}\text{N}$ (14.6‰). Moreover, the effect of this

outlier is intensified because this was the year with the lowest number of individuals sampled (i.e. n=6). On the other years, the values of $\delta^{13}\text{C}$ of plasma varied from -19,3‰ to 18,4‰ (Table 1). The $\delta^{15}\text{N}$ values of plasma varied between 12.0 ‰ in 2015 and 13.4 ‰ in 2014, which also presented the lowest standard deviation (i.e. 0.5 ‰).

In the RBC samples, the $\delta^{13}\text{C}$ values ranged between -19,5 ‰ in 2016 and -18,4 ‰ in 2018, and the highest standard deviation was in 2013 (i.e. 1.3 ‰). The $\delta^{15}\text{N}$ values of RBC ranged from 11,4 ‰ in 2012 and 12.7 ‰ in 2011 (Table 1).

Table 1: Isotopic values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in Plasma and RBC, and body mass index (BMI) for the 2011-2018 period. The numbers in parentheses refer to the sample size

	2011 (n=26)	2012 (n=26)	2013 (n=6)	2014 (n=18)	2015 (n=9)	2016 (n=22)	2017 (n=16)	2018 (n=17)
$\delta^{13}\text{C}$ (‰)	-18,9 ± 0,8	-18,7 ± 0,5	-20,6 ± 2,0	-18,4 ± 0,4	-18,8 ± 0,3	-19,1 ± 0,7	-19,3 ± 0,5	-18,8 ± 0,7
Plasma								
$\delta^{15}\text{N}$ (‰)	12,9 ± 1,5	12,2 ± 1,0	12,4 ± 1,9	13,4 ± 0,5	12,0 ± 0,9	12,7 ± 1,1	12,4 ± 0,8 (15)	12,2 ± 1,2
Plasma								
$\delta^{13}\text{C}$ (‰) RBC	-19,3 ± 0,7	-19,2 ± 0,6	-19,1 ± 1,3	-18,6 ± 0,6	18,5 ± 0,2	-19,5 ± 0,7	-18,8 ± 0,7	-18,4 ± 0,3
RBC								
$\delta^{15}\text{N}$ (‰) RBC	12,7 ± 1,4	11,4 ± 1,1	11,6 ± 1,2	12,5 ± 0,9	11,7 ± 0,7	12,0 ± 1,2	12,6 ± 0,7	11,6 ± 0,9
RBC								
BMI	-10,1 ± 48,9	36,6 ± 50,6	88,2 ± 50,6	-10,2 ± 52,2	-29,7 ± 52,5 (8)	9,2 ± 51,9	-20,1 ± 51,9	-39,8 ± 52,0

3.2.1.1 Plasma

The metrics calculated by SIBER analysis (TA, SEAc and SEA.B) showed that the highest values of niches parameters correspond to 2013 followed by 2011, and that 2014 and 2015 were years with the lowest values (Table 2).

Table 2: Isotopic niche metrics calculated based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of Plasma during the 2011-2018 period. TA indicates the dashed limits, SEAc is the ellipse area and SEA.B is the ellipse statistical mode and were estimated using SIBER analyses (see methods).

Year	TA	SEAc	SEA.B
2011	8.1	3.0	3.1 ± 0.6
2012	4.6	1.5	1.5 ± 0.3
2013	10.4	13.1	13.0 ± 6.5
2014	1.9	0.7	0.7 ± 0.2
2015	1.6	1.0	1.0 ± 0.4
2016	5.7	2.5	2.5 ± 0.5
2017	4.1	1.5	1.5 ± 0.4
2018	5.4	2.7	2.7 ± 0.7

Isotopic niches represented by ellipses clearly represent these differences in plasma $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The larger isotopic niche of the year 2013, contrasts with the year 2014, which has the smallest isotopic niche width (Figure 3.10).

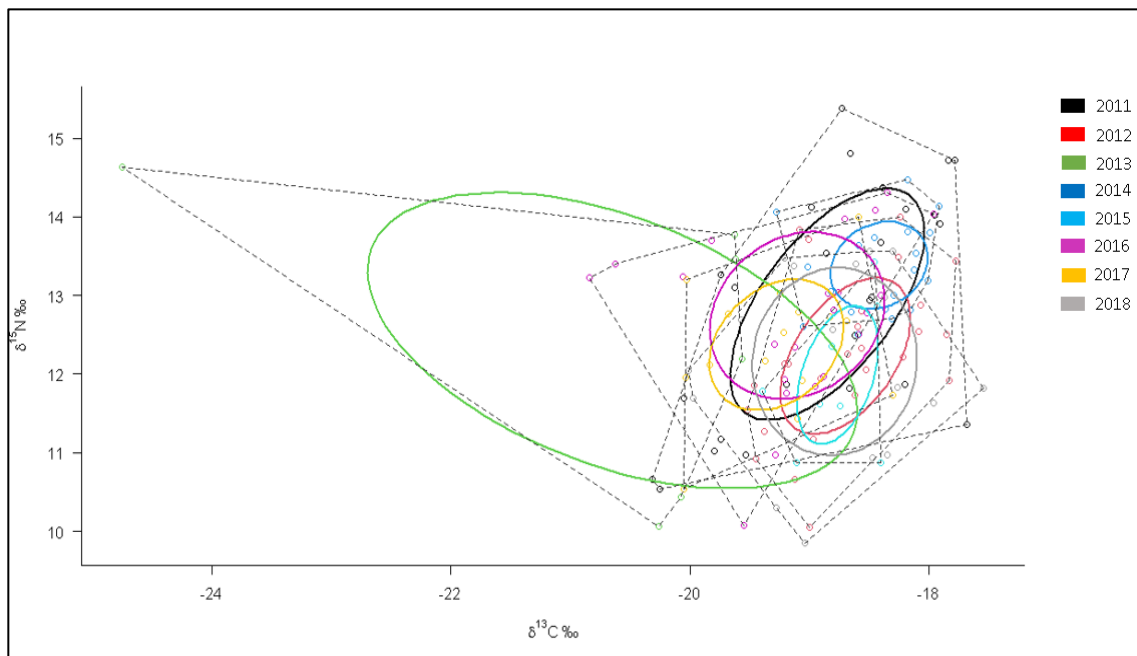


Figure 3.10: Stable isotope ratios in plasma of YLG breeding on Berlenga Island, from 2011 to 2018. The area of the standard ellipses (SEAc) is represented by solid lines,

each color represents a year and each point represents a sampled individual. The TA is represented by the dotted lines.

SIBER analyses revealed significant differences in niche width between years, and the 2013 ellipse revealed a significant difference compared to all other years (SEA.B; Plasma: all $p < 0.001$).

Table 3: Statistical comparison between ellipses size representing isotopic niche width using plasma. Values represent p-value. In bold, significant values.

	2012	2013	2014	2015	2016	2017	2018
2011	0.007	0.99975	0	0	0.003	0.21425	0.30075
2012		1	0.008	0.102	0.94575	0.4375	0.96125
2013			0	0	0	0	5e-04
2014				0.71675	0.99975	0.978	0.99975
2015					0.98975	0.86475	0.99175
2016						0.0705	0.589
2017							0.9515

3.2.1.2 RBC

SIBER analyses revealed that the isotopic niche metrics calculated with the values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in RBC were also higher in 2013. The highest TA was in 2016 (i.e 7.3), but the SEAc was the highest in 2013 (i.e. 6.3), as well as the SEA.B. The lowest value was recorded in 2015 followed by 2018 (Table 4).

Table 4: Metrics calculated with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of RBC, presented by year (2011-2018).

Year	TA	SEAc	SEA.B
2011	7.3	2.2	2.2 ± 0.5
2012	4.7	1.4	1.5 ± 0.3
2013	5.2	6.3	6.2 ± 3.2
2014	2.9	1.1	1.2 ± 0.3
2015	0.6	0.4	0.4 ± 0.2

2016	7.8	2.7	2.7 ± 0.6
2017	2.8	1.4	1.4 ± 0.4
2018	1.9	0.8	0.8 ± 0.2

Similar to plasma, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of RBC showed a marked difference in the width of the niche in 2013, but there was more overlap of the niches. The smallest width was in 2015, meaning that YLG were more specialized in the resources exploited in that year (Figure 3.11).

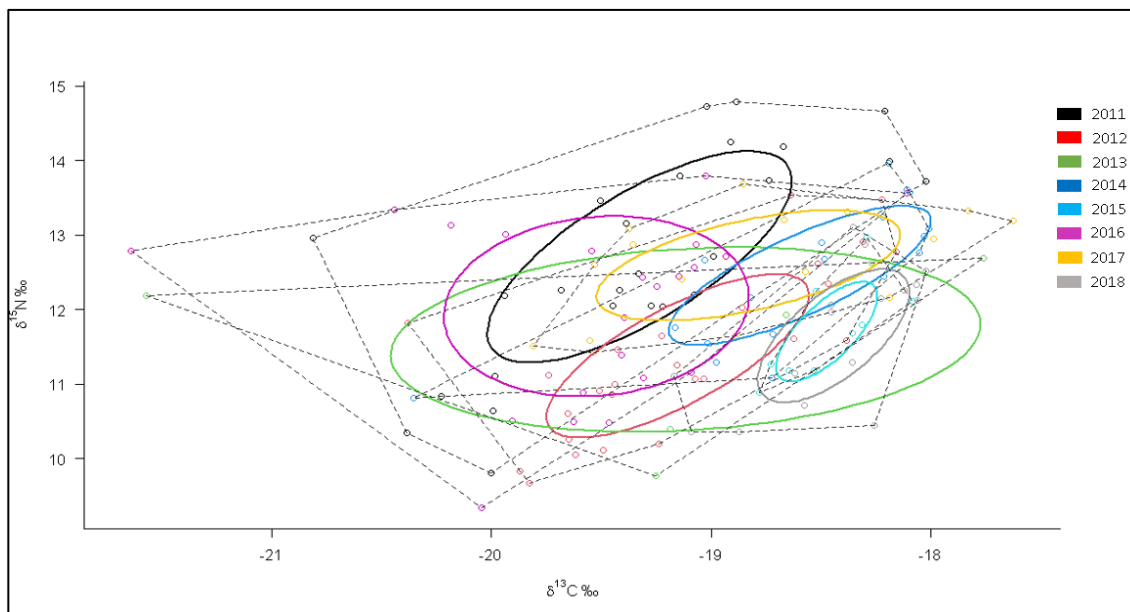


Figure 3.11: Stable isotope ratios in RBC of YLG breeding on Berlenga Island, from 2011 to 2018, and respective isotopic niche widths represented by standard ellipses (SEAc, solid lines) and convex hull areas (TA, dotted lines).

Accordingly, the isotopic niche width in 2013 is highly significantly larger (i.e. all $p < 0.001$) than those from all other years, and significantly higher than 2016, which is the second largest (SEAB; RBC: $p = 0.048$; Table 5).

Table 5: Statistical comparison between ellipses size representing isotopic niche width using RBC. Values represent p-value. In bold, significant values

	2011	2012	2013	2014	2015	2016	2017	2018
2011		0.055	0.9815	0.021	0	0.7135	0.07375	0.00075
2012			0.999	0.26275	0.001	0.984	0.46725	0.031
2013				5e-04	0	0.04825	0.00075	0
2014					0.00675	0.9935	0.6805	0.11175
2015						1	0.997	0.9435
2016							0.031	0
2017								0.049

3.2.2 Body mass index

The BMI (Table 1) showed a strong negative correlation with May Chl- α ($r_s = -0.9$, $p = 0.010$) and could be observed that the oscillations of these two variables were practically opposite during the years (Figure 3.12.A). Besides, and contrary to what was expected, the analyses showed another negative correlation between BMI and Egg volume ($r_s = -0.9$, $p = 0.017$, Figure 3.12.B). Still, when correlating BMI standard deviation with Eggs per Individual the results showed a strong positive correlation ($r_s = 0.8$, $p = 0.034$; Figure 3.12.C).

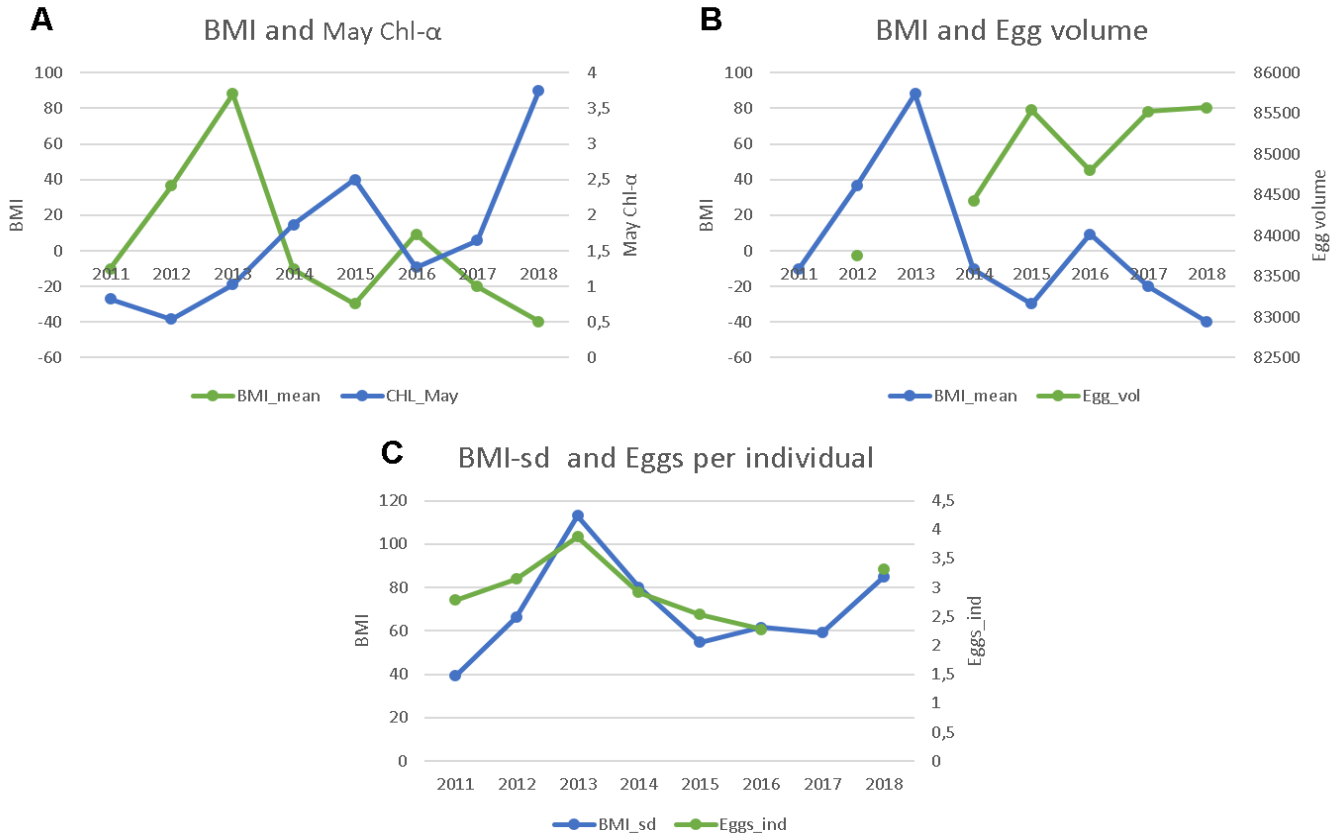


Figure 3.12: Values of BMI and A) May Chl- α ($\text{mg}\cdot\text{m}^{-3}$), B) Egg Volume and C) BMI standard deviation and Eggs per Individual.

Considering the potential relationships between BMI and stable isotopes measured in RBC, it was found a negative correlation with the RBC $\delta^{13}\text{C}$ ($r_s = -0.8$, $p = 0.037$; Figure 3.13.A) and positive correlation with the SEAc ($r_s = 0.8$, $p = 0.028$; Figure 3.13.B), SEA.B ($r_s = 0.9$, $p = 0.007$) and TA ($r_s = 0.8$, $p = 0.028$).

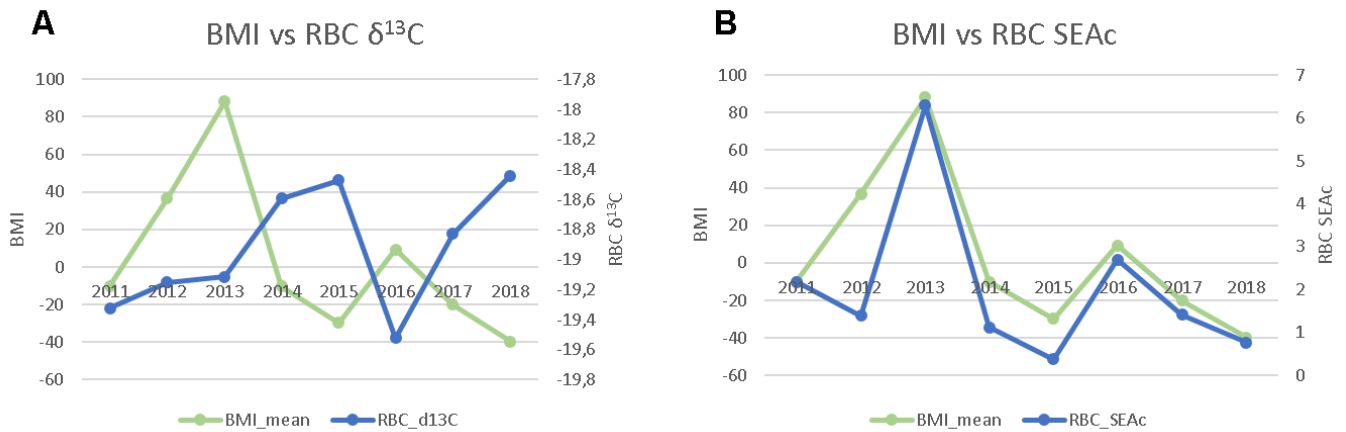


Figure 3.13: Values of BMI and A) RBC $\delta^{13}\text{C}$ and B) RBC SEAc, during the period of 2011-2018.

When correlating BMI standard deviation, two significant correlations were observed: a positive correlation with Eggs per Individual ($r_s = 0.8$, $p = 0.034$; Figure 3.14.A) and a negative correlation with March Fisheries ($r_s = -0.8$, $p = 0.022$; Figure 3.14.B).

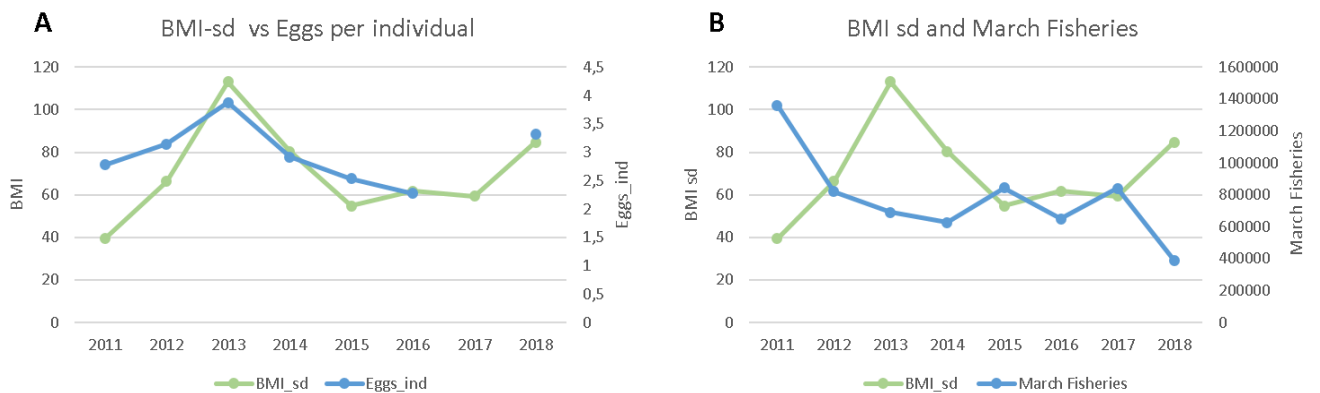


Figure 3.14: Correlation between BMI-sd and A) Eggs per Individual and B) March Fisheries (kg).

3.2.3 Relationships with Environmental Parameters

Positive significant correlations were found between RBC $\delta^{13}\text{C}$ and May Chl- α ($r_s = 0.8$, $p = 0.015$; Figure 3.15.A) and RBC $\delta^{15}\text{N}$ and March SST ($r_s = 0.8$, $p = 0.022$; Figure 3.15.B), and negative between RBC SEA.B and May Chl- α ($r_s = -0.8$, $p = 0.037$; Figure 3.15.C) and RBC TA and May Chl- α ($r_s = -0.7$, $p = 0.046$; Figure 3.15.D).

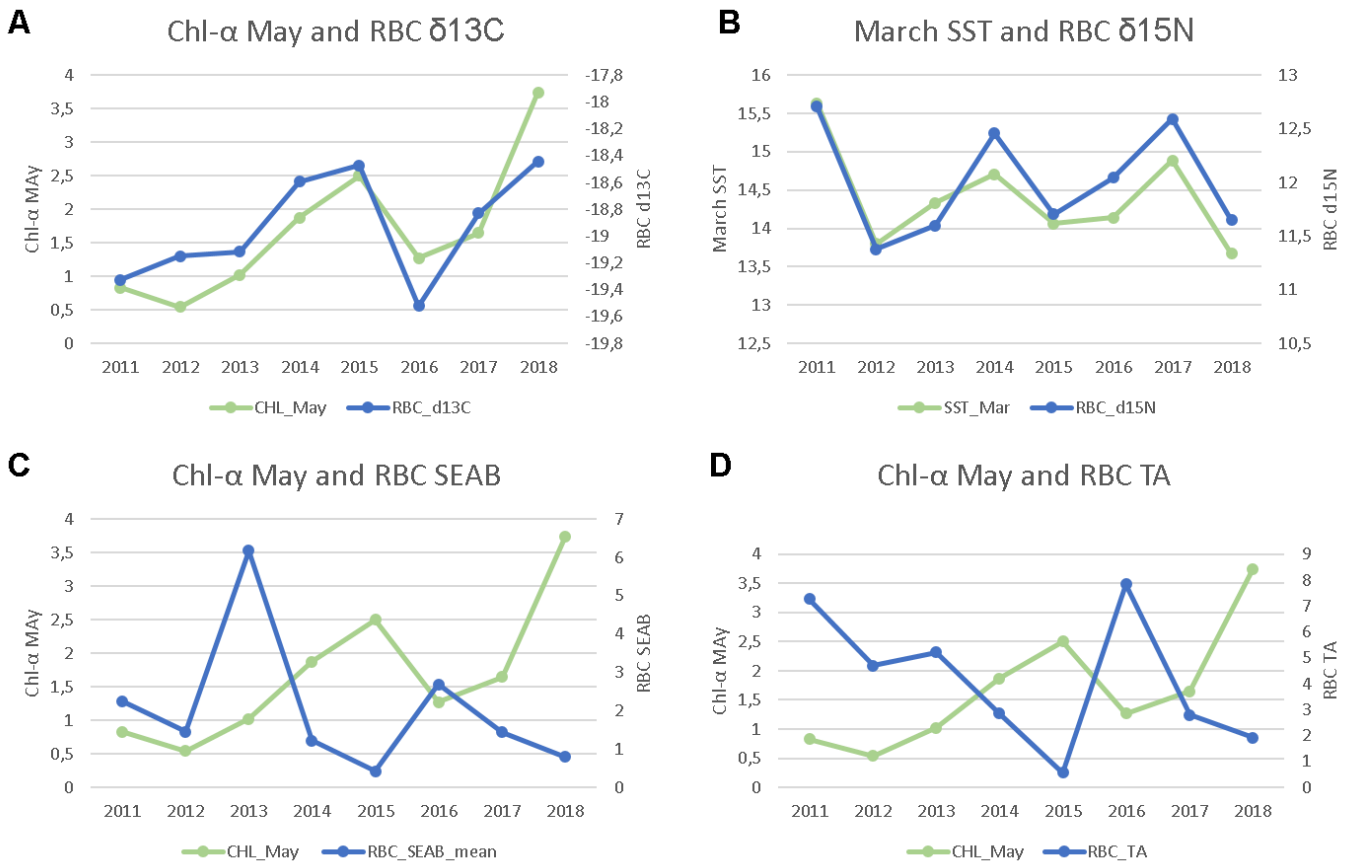


Figure 3.15: Values of A) Chl- α and RBC $\delta^{13}C$, B) RBC $\delta^{15}N$ and March SST (C°), C) RBC SEA.B and Chl- α ($mg.m^{-3}$) and D) RBC TA and Chl- α ($mg.m^{-3}$).

Moreover, the wNAO was significantly correlated (negative) with SEAc Plasma ($r_s = -0.9$, $p = 0.007$; Figure 3.16.A), and corroborated by the wNAO correlations with TA ($r_s = -0.9$, $p = 0.004$), SEA.B ($r_s = -0.9$, $p = 0.007$) and SEA.B standard deviation ($r_s = -0.9$, $p = 0.007$). In addition, negative correlations between w NAO and the standard deviations of $\delta^{13}C$ ($r_s = -0.9$, $p = 0.004$; Figure 3.16.B) and $\delta^{15}N$ ($r_s = -0.8$, $p = 0.022$; Figure 3.16.C) were found.

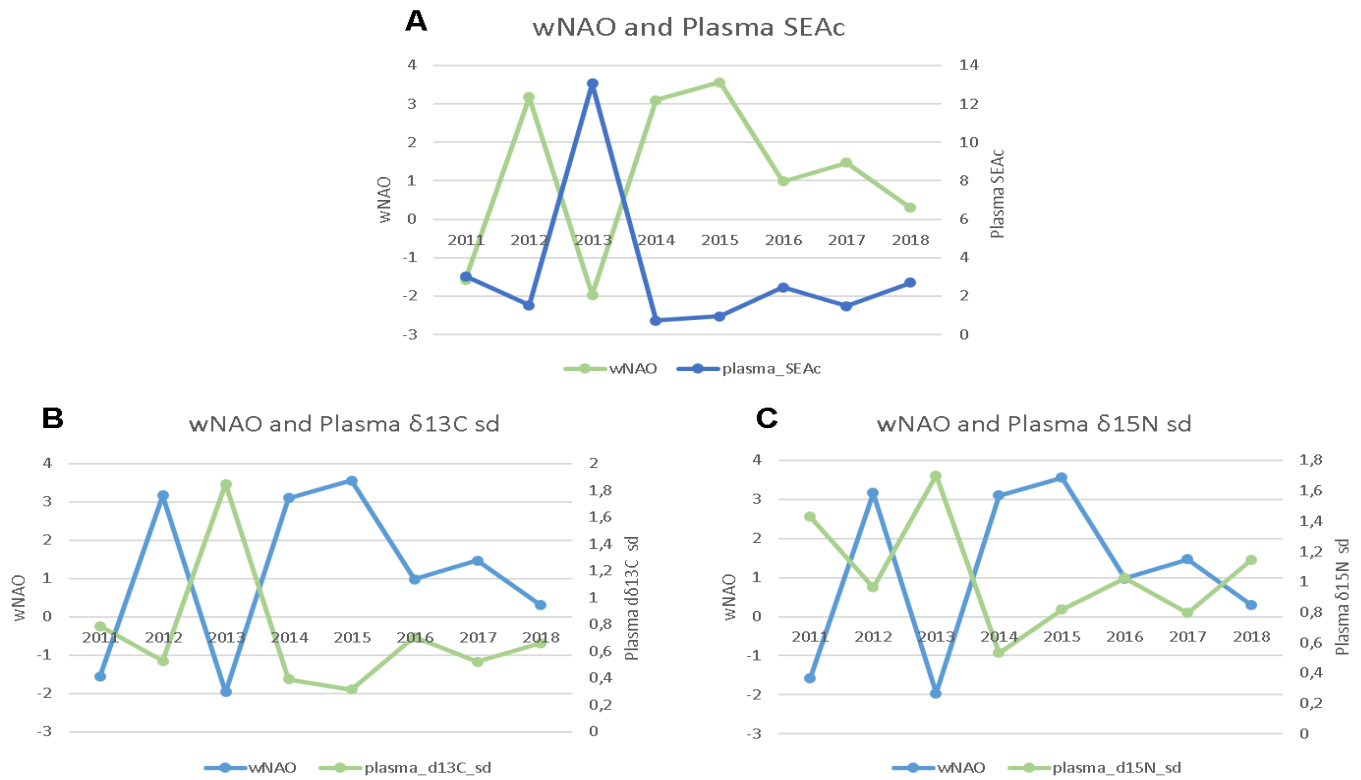


Figure 3.16: Values of wNAO and A) SEAc in plasma, B) standard deviation $\delta^{13}C$ in plasma and C) standard deviation $\delta^{15}N$ in Plasma, from 2011-2018.

3.2.4 Egg Volume

The correlation between Egg Volume and data from fisheries landings resulted in only one negative correlation with Demersal Species ($r_s = -0.9$, $p = 0.033$). No correlation was detected between isotopic signatures and egg volume.

4 Discussion

The colony of YLG at the Berlenga Island is the largest in Portugal, but in the last decade the number of breeding individuals have dropped consistently. Population data (density and breeding success) from the Berlengas Island were used to understand which oceanographic factors may influence the fluctuation of the breeding individuals since 2002, and stable isotopes were used since 2011 to understand their trophic ecology. The main objective of this study was to identify factors associated with the marine environment (i.e. oceanographic variables and fisheries data) that were related with the long-term oscillation of the reproductive population in the Berlenga colony and its trophic ecology. This study confirmed the influence of oceanographic variables on marine productivity around Berlenga Island and consequently on the trophic ecology of YLG.

4.1 Relationships with Environmental Parameters (2002-2018)

In this study, several significant correlations with environmental variables for the month of June were detected. Some of these correlations may not have a direct cause-effect but may indicate other relationships that are reflected also in June. That is the case with the negative correlation with June SST and Reproductive Success and the positive correlation with June SST and the number of Non-Breeding Individuals. Other variables are potentially correlated, but the relationship between them cannot be drawn, such as Eggs per individuals and June Chl- α , since in June the chicks have already hatched. However, the eggs are destroyed at Berlenga Island, and gulls can lay more eggs to try to replace postures if conditions are still favourable in June.

The North Atlantic Oscillation index (NAO) has a broad influence on marine ecosystems of the North Atlantic. One of the effects of a positive NAO value is an increase of the upwelling phenomenon in the Portuguese West coast, and particularly at the Berlenga Island. In general, the upwelling increases the marine productivity and fish availability due the lower SST and nutrient-rich waters (Mann & Lazier, 2013), although very strong events may push fish larvae to the open sea, causing high mortality (Santos et al., 2007). On the other hand, lower (i.e. negative) values of NAO index are associated

with poor oceanographic conditions, higher SST and low marine productivity (Sousa et al., 2008). Accordingly, this study shows that the First Egg is negatively related to wNAO, which means the lower the wNAO, the more days it took for the colony's first egg to be laid that year.

Overall, during the study period (2002-2018) there was no correlation between Breeding Individuals and wNAO. However, when analysing the graph that shows the values of both variables (Figure 3.2), it is possible to observe that until the year 2010 the two variables seemed to be oscillating in a similar way. From then on, the number of breeding individuals has not been able to recover to previous numbers and started a continuous descending up to 2018.

In 2010 an extremely low value of the wNAO was observed, which reached the value of -4,64 and which clearly impacted the ecosystem, causing an abrupt drop in marine productivity in that year and consequently affecting consumers of higher trophic levels, such as seabirds (Paiva et al., 2013). The observed data suggest that the year 2010 was a tipping point for the gulls at Berlenga Island (Scheffer et al., 2009), but current estimations in 2018 still suggest around 5,600 breeding individuals in this colony.

Several correlations were observed between the SIA variables and environmental variables. May Chl- α correlation with RBC $\delta^{13}\text{C}$ suggests that the higher the Chl- α levels, the more YLG feed on terrestrial resources, contrary to what was expected since the higher the marine productivity, the greater the potential availability of resources (i.e. fishes). In addition, Chl- α also showed a significant but negative relationship with RBC TA (niche of sampled gulls) and RBC SEAB (statistical niche model), indicating that the higher the concentration of Chl- α , the lower the niche, a more specialized diet. The RBC $\delta^{15}\text{N}$ values were positively correlated with March SST, suggesting that the higher sea surface temperatures in March, the higher the trophic level of YLG prey.

Moreover, the analyzes showed that wNAO has a negative correlation with three plasma variables (TA, SEAc and SEAb), and particularly with Plasma SEAc, which represents the bulk area of the YLG population niche. This correlation is very significant and suggests that the higher the wNAO values, the lower the SEAc values, and consequently higher niche specialization. In years of positive wNAO, the niche was higher, so the gulls adopted more generalist feeding.

For future studies, it would be of great relevance to include upwelling among the

studied variables, due to its relevance and influence on marine conditions and productivity. In addition, gulls can travel distances up to 100 km in search of resources (Ceia et al. 2014), and it is thus possible that oceanographic variables from adjacent regions also have an influence on YLG foraging ecology and breeding success.

4.2 Relationships with Fisheries Landings

It is already known that the gulls from the Berlenga Island colony exploit the Peniche Harbour to feed on fisheries leftovers (Ceia et al., 2014), and that during the breeding season they tend to feed more on fish, as well as to feed their chicks. Therefore, it was expected that the variables from the colony were correlated with fisheries variables. Total Eggs showed a significant correlation with Total Landings and Pelagic Species, showing that the more fish in general and the more pelagic species, the greater the number of total eggs. It can be considered that a greater availability of resources would be reflected in a greater egg productivity. It should be taken into account that YLG lay an average of 2-3 eggs per breeding posture (M. Bosch et al., 2000), but as on Berlenga Island the eggs are culling, the gulls continue to replace postures.

From Figure 3.5 it is possible to observe an alternation of the dominant fishing gear, as the Purse-seine decreases and the Trawl increases. The decrease in Total Eggs may be also related to this, but as regardless of the fishing gear, fish are still being collected, there is not enough evidence to consider that this shift is the reason for this decrease.

The stock of commercial fish species is declining in the oceans due the effects of large-scale fishing and global changes caused in large part by anthropogenic activities (Pauly et al., 1998). A species of extreme ecological and economic value is the sardine *S. pilchardus*, which is one of YLG's most important prey (Alonso et al., 2015; J. G. Calado et al., 2018). Thus, the decrease in the amount of sardine caught since 2005 (Figure 3.X) could have impact the population of YLG at Berlenga Island. Other pelagic species such as *Trachurus* ssp., which have been increasingly landing up to 2018 (Figure X), and *M. poutassou* are also consumed by YLG. The landings of *C. conger* have decreased since 2012 and the landings of *B. boops* have increased but not enough to meet the needs of the YLG that would feed on sardines, considering that they may not

be able to replace the niche that sardine occupies with other fish species (Cardona et al., 2015). The number of Total Eggs may reflect resource availability; however, results suggest that the decrease in Total Eggs is very likely to just accompany the decrease in Breeding Individuals.

4.3 *Trophic Ecology of YLG (2011-2018)*

Since the late 1980s, stable isotopes have been increasingly used to study the diet of birds and their trophic relationships (K. A. Hobson, 1987; Keith A. Hobson et al., 1994; Keith A. Hobson & Clark, 1992). Isotope values of plasma and RBC reflect the diet of an individual from two weeks to one month before collection, respectively, enabling the inference of an isotopic niche at the species or population level, through the tissue's sampling of individuals. Two stable isotopes (i.e. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were used to assess the foraging niche of a population of yellow-legged gulls. Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values showed a large variation over the years, in both RBC (representing the diet approximately 1-2 months before sampling) and plasma (representing diet ca. 7-15 days before sampling), reinforcing its already well-known generalist habit at Berlenga Island.

2013 was an atypical year in terms of sampling procedures because it had the lowest number of samples since 2011, but it also showed a much more negative $\delta^{13}\text{C}$ value in plasma than in other years, large isotopic niches in both tissues analyzed and high variation in stable isotopic values. The low values of $\delta^{13}\text{C}$ suggest that during 2013 the gulls consumed more sources from terrestrial origin. Another variable used in this study also presented an outlier in 2013, an extremely high value of Chl- α (9,15 $\text{mg}\cdot\text{m}^{-3}$), that by directly indicates marine productivity may be related to these remarkable observations of this year. In 2014, the highest value of plasma $\delta^{15}\text{N}$ was recorded, but the plasma $\delta^{13}\text{C}$ value was the lowest, indicating a higher consumption of prey from higher trophic levels, and lower proportion of sources from marine origin.

In general, during the study period, SIA showed a large variation in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, suggesting that food sources were varied in terms of terrestrial vs. marine origin and from different trophic levels. The results of this study are in accordance with Mendes et al (2018), that studied the feeding ecology of the YLG colony in Berlenga during the period 2011-2016 and concluded that the foraging strategy was not consistent over the years, presenting a large annual variation in their use of marine, mixed and terrestrial foraging strategies. This lack of consistency is attributed to interannual

fluctuations in the availability of marine resources (Ceia et al., 2014; Mendes et al., 2018).

SIBER analyses revealed significant differences in niche width among years, but in general niches overlapped at some degree. In 2013, seagulls had the largest niche based on the values of plasma, and it was also the year that the mean chlorophyll concentration had the highest value (3.13 $\mu\text{g/l}$ on March-June period) and a negative wNAO (-1.97). Although the high value recorded in 2013 was largely influenced by the outlier, a large isotopic niche for 2013 was also detected in analyses of RBC (Table 2 and 4, and Figures 3.10 and 3.11), corroborating the large isotopic niche in such year.

Some studies have used the $\delta^{34}\text{S}$ isotope to supplement dietary information, as this isotope helps to distinguish the marine species that have been consumed such as fish, crabs and mussels (Moreno et al., 2010). Therefore, in future studies it would be advantageous to include this variable in the analyzes so that more in-depth studies can be carried out, in order to infer in more detail its feeding ecology and the source of resources.

4.4 *Body mass index*

The BMI is a proxy of overall fitness of individuals, and thus of great relevance to the conditions of survival and breeding success. During this research it was possible to measure BMI and evaluate how it is related to the exploited environment and respective food sources of YLG from Berlenga Island. The results suggest that the consumption of food from terrestrial origin could have an influence on the BMI of seagulls, showing why they have such a high preference for this type of food source, and consequent increase in the exploitation of landfills. This was shown by the negative correlation between RBC $\delta^{13}\text{C}$ (representing the timescale before incubation period) and BMI.

A recent survey revealed that females have a different feeding pattern than males, being more generalist and with $\delta^{13}\text{C}$ values lower than males (Calado et al., 2020a). Therefore, the correlation of BMI with $\delta^{13}\text{C}$ values may suggest that females have this different diet due to their different nutritional needs, i.e. due to egg production, although this cannot be corroborated since the sex was not evaluated during this study. However, these results are in accordance with a study carried out with YLG in order to analyze carotenoid-based bill coloration, which found that the greater the red spot on the gulls'

beak, the lower the $\delta^{13}\text{C}$ values, suggesting that a higher consumption of terrestrial prey might be more advantageous (Zorrozua et al., 2020). Furthermore, another study showed that after the closure of several landfills that were used by seagulls, there was an increase in the consumption of terrestrial prey instead of marine prey during the breeding season (Zorrozua et al., 2020a).

However, some limitations regarding the use of BMI have already been described, such as that the use of residuals from regressions might generate misleading results or that the mass of females captured during incubation period would drop, being suggested that the cholesterol would be the best predictor of mass optimum (Alonso-alvarez & Velando, 2003).

5 Conclusion

Despite the decreasing trend in population size at the Berlenga Island in recent years, population control will continue, as number of breeding individuals are still high. During this study it was possible to uncover some clues on the influence of environmental factors in population size and breeding success of yellow-legged gulls, and to infer the effects that climate environment changes may have on the species trophic ecology. Using Stable Isotope Analyses, it was possible to unveil some aspects of the trophic Ecology and to confirm that the Yellow-legged gulls possesses great flexibility in its foraging tactics, varying from marine, mixed and terrestrial foraging strategies in accordance with the availability of marine food sources. More studies are needed so that we can continue to investigate the extremely plasticity of the foraging ecology of this generalist and opportunistic species, and it would be very relevant to carry out studies relating the topics covered here with other environmental variables, such as upwelling, as well as data on discharges in landfills close to the colony.

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