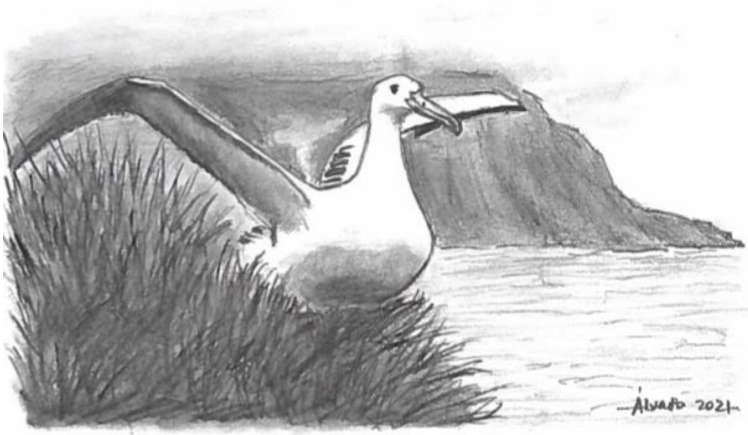
Universidade de Aveiro
2020

INÊS TERRAQUENTE RIBEIRO

PERFIL BIOQUÍMICO DE MACHOS E FÊMEAS DE ALBATROZES-VIAGEIROS

BIOCHEMICAL PROFILE OF MALE AND FEMALE WANDERING ALBATROSSES



Universidade de Aveiro
2020

INÊS TERRAQUENTE RIBEIRO

PERFIL BIOQUÍMICO DE MACHOS E FÊMEAS DE ALBATROZES-VIAGEIROS

BIOCHEMICAL PROFILE OF MALE AND FEMALE WANDERING ALBATROSSES

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Marinha Aplicada, realizada sob a orientação científica da Doutora Ana Marta dos Santos Mendes Gonçalves, Investigadora do Centro de Ciências do Mar e do Ambiente, Departamento de Ciências da Vida da Universidade de Coimbra e do Departamento de Biologia & CESAM da Universidade de Aveiro, e do Doutor Fernando José Mendes Gonçalves, Professor Associado com Agregação do Departamento de Biologia & CESAM da Universidade de Aveiro

o júri

presidente

Prof. Doutora Maria Marina Pais Ribeiro da Cunha

Professora Associada da Universidade de Aveiro

arguente

Doutora Cláudia Sofia Cordeiro Nunes

Investigadora Doutorada da Universidade de Aveiro

orientadora

Doutora Ana Marta dos Santos Mendes Gonçalves

Investigadora Auxiliar da Universidade de Coimbra

agradecimentos

Primeiramente gostaria de agradecer aos meus orientadores, Ana Marta Gonçalves e Fernando Gonçalves, por me darem a oportunidade de trabalhar na área da bioquímica, e ganhar ainda mais conhecimentos na parte laboratorial, o que será sempre importante para o futuro.
Ao José Carlos Xavier, que apesar de não ser o meu orientador oficial na dissertação também me ajudou ao longo do trabalho e ainda tornou possível que eu conseguisse trabalhar com albatrozes. O seu entusiasmo e dedicação sempre foram contagiantes.
À British Antarctic Survey e, em particular, a Richard Phillips, Gabriele Stowasser e Phil Trathan pelo apoio científico e logístico no contexto dos projetos CEPH-BAS, PROPOLAR, ICED, SCAR anT-ERA e SCAR EGBAMM.
Ao meu pai, por ter feito especialmente o desenho da capa de um Albatroz-viageiro, que vou guardar para sempre como recordação.
Aos meus pais e irmã que me aturaram desde o primeiro dia em que comecei a dissertação, que lidaram com as mudanças de humor, desde a felicidade aos stresses constantes.
Ao Tiago que sempre que eu precisava, tinha as palavras certas para me motivar a continuar e a dar o meu melhor neste trabalho.
Aos meus amigos, Cláudia, Jorge e Figueiredo que compreenderam a minha ausência constante e mesmo longe foram importantes para me darem motivação, e combinarem encontros quando precisava de espairecer e conviver.

palavras-chave

albatroz-viageiro, predador, ácidos gordos, hidratos de carbono, Geórgia do Sul, plasma, sangue, tecidos, sexos, meses, SFA, MUFA, PUFA, glucose, cria, reservas.

Resumo

O albatroz-viageiro *Diomedea exulans*, devido ao seu comportamento e biologia, é muitas vezes utilizado como modelo de predador de topo. Esta espécie passa grande parte do tempo no oceano à procura de alimento e apenas vem a terra na época de reprodução. Neste estudo pretendeu-se determinar, e comparar, o perfil em ácidos gordos e açúcares de machos e fêmeas de albatrozes viageiros, entre Maio e Outubro de 2009, de modo a verificar potenciais diferenças na dieta e inferir sobre a condição corporal dos indivíduos de ambos os sexos e os meses de amostragem durante o período de reprodução e cuidados com as crias. Analisámos 34 adultos reprodutores (18 fêmeas e 16 machos). As amostras foram recolhidas na ilha de Bird, na Geórgia do Sul, para posterior análise do perfil bioquímico em amostras de plasma e sangue de cada individuo. Na análise dos ácidos gordos observou-se que existiam diferenças entre tecidos (plasma e sangue) ($F=68.31$; $p<0.01$) mas não entre sexos e os meses de amostragem. Observou-se uma maior abundância de ácidos gordos no plasma (98%) em relação às amostras de sangue. Os ácidos gordos saturados (SFA) e monoinsaturados (MUFA) foram os mais abundantes. Ácidos gordos poli-insaturados (PUFA) foram apenas observados em amostras de sangue de indivíduos de ambos os sexos. Apesar de se terem observado diferenças ao longo dos meses tanto no sangue como no plasma, estas não foram significativas. Os machos apresentaram uma maior quantidade de ácidos gordos (63%) em julho e as fêmeas em Maio (68%), nas amostras de plasma, enquanto que nas amostras de sangue este padrão foi observado em Outubro para os machos (85%) e em Setembro para as fêmeas (67%). Na análise de açúcares também se observaram diferenças significativas entre tecidos ($F=56.02$; $p<0.01$) mas não entre sexos e os meses de amostragem. Registou-se uma maior abundância de açúcares em amostras de plasma do que em amostras de sangue, com a Glucose a apresentar uma maior abundância. No plasma, os machos apresentaram um pico em Outubro (71%) e as fêmeas em Julho (55%), assim como no sangue. Estes resultados evidenciam que tanto os machos como as fêmeas contribuem nos cuidados da cria, não se observando diferenças entre sexos, e que ambos têm de gastar reservas energéticas nesta época de reprodução. No entanto, os machos parecem conseguir recuperar mais rapidamente.

keywords

Wandering albatross, predator, fatty acid, carbohydrates, South Georgia, plasma, blood, tissues, sexes, months, SFA, MUFA, PUFA, glucose, young, reserves.

abstract

The Wandering albatross *Diomedea exulans*, due to its behavior and biology, is often used as a top predator model. This species spends a great time in the ocean searching for food and only comes ashore during the breeding season. This study aimed to determine and compare the fatty acid and carbohydrates profile of male and female traveling albatrosses, between May and October 2009, in order to verify potential differences in the diet and infer about the body condition of individuals of both sexes and the months of sampling, during chick rearing. We analyzed 34 breeding adults (18 females and 16 males). The samples were collected on Bird Island, South Georgia, for further analysis of the biochemical profile in plasma and blood samples from each individual. In the analysis of fatty acids, it was observed that there were differences between tissues (plasma and blood) ($F = 68.31$; $p < 0.01$) but not between sexes and months. A greater abundance of FA in the plasma (98%) was observed in relation to blood samples. Saturated (SFA) and monounsaturated (MUFA) fatty acids were the most abundant FA. Polyunsaturated fatty acids (PUFA) were only observed in blood samples from individuals of both sexes. Although differences were observed over the months in both blood and plasma, these were not significant. Males showed a greater amount of FA (63%) in July and females in May (68%), in plasma samples, whereas in blood samples this pattern was observed in October for males (85%) and in September for females (67%). In the analysis of sugars, significant differences were also observed between tissues ($F = 56.02$; $p < 0.01$) but not between sexes and months. There was a greater abundance of carbohydrates in plasma samples than in blood samples, with Glucose showing greater abundance. In plasma, males showed a peak in October (71%) and females in July (55%), as well as in blood. These results show that both males and females contribute to the care of the young, not showing differences between sexes, and that both have to spend their energy reserves during the breeding season. However, males seem to be able to recover more quickly.

CONTENTS

1. GENERAL INTRODUCTION

1.1. The relevance of the Southern Ocean

The Southern Ocean is an extension of the Atlantic, Indian and Pacific oceans but also distinguishes itself as a marine area, in both physical (e.g colder waters, around 0 ºC) and ecological terms (e.g. endemic species such as emperor penguins, *Aptenodytes forsteri*) (Rintoul, 2018; Xavier & Peck, 2015). Its boundary is defined by the transition of waters with different characteristics, in which the cold, low salinity water in the surface of the polar Antarctic front zone faces Subantarctic warmer waters, with high salinity (Scully, 1978). Also exists a strong interaction with the deep circulation of the waters coming from the other Oceans, thus playing an important role in the global ocean ("*thermohaline circulation")* (Sarmiento *et al.*, 2004; Griffiths, 2010), where the deeper waters that arrive in the Southern Ocean are rich in nutrients (Rintoul *et al.*, 2018).

Dominant West cold winds push the water to the cooler and less salinity waters of East (Scully, 1978), which combined with Coriolis effect, creates a system of circumpolar currents that allows the transport of nutrients as the deeper waters will ascend (Griffiths, 2010). Not only the currents, but also the fronts, upwelling, water column mixing and stratification, will influence the transport and concentration of nutrients thus delimiting the spatial pattern of primary production (Lecomte *et al.*, 2010) and a continuous source of nutrients in the water (Scully, 1978). Beyond these physical factors are also ice formation (ice shelves or icebergs), depth, temperature (lower temperatures allow oxygen levels to be significantly higher than in other regions of the world) and geomorphology (Griffiths, 2010). These characteristics, together with climatic and solar regimes (e.g. amount of light that changes seasonally), create an environment providing different ecological systems, that combined with nutrient supply, create favorable conditions for primary production (Scully, 1978). In Austral summer, 24 hours of light make available greater amount of nutrients in water, leading to phytoplankton blooms, an advantage to predators, influencing all trophic chains and compensating the winter season.

Phytoplankton is the main source of food for the primary organisms in the trophic chain; however, other primary producers also benefit from solar radiation, namely algae and microalgae that produce energy for primary consumers (Xavier & Peck, 2015). Another important feature of this region is the provision of nutrients that are associated with ice shelves. In Austral summer, when ice shelves melt, organic matter that was trapped is released, thus allowing more nutrients to be available to primary producers, such as phytoplankton (Xavier & Peck, 2015), which serves as food for a wide variety of species from krill and copepods, being most relevant the zooplankton, namely, Antarctic krill (*Euphausia superba*), usually concentrated near the continent, forming hotspots, which allows a better location by predators such seabirds and fishes (Scully, 1978). In Austral autumn, this species, as well as heterotrophic microalgae and protists, associate with the platforms that start to freeze, to forage for food (Arrigo & Thomas, 2004). So, the seasonality in the Antarctic region, promotes the development of large areas of ice formation (Griffiths, 2010), being a habitat for several species of organisms as bacteria, algae and grazers (Xavier & Peck, 2015).

The large primary production that occurs in the Southern Ocean allows several species to concentrate in this region, namely species of penguins, seals, whales, albatrosses, fishes and cephalopods (Xavier & Peck, 2015). Although there is a great diversity of large organisms, this occurs mainly in organisms of small dimensions or even microscopic, such as amphipods and bryozoans (Griffiths, 2010). In relation to the largest organisms of the Southern Ocean, there are only four species of seals in Antarctica, which depend on the ice shelves during their life (Scully, 1978). In seabirds, there are about 18 species, which are classified as threatened or endangered in the Red List of the International Organization for the Conservation of Nature (Griffiths, 2010). For cephalopods, about 70 species of cephalopods are documented, mainly pelagic species of squid (Xavier *et al.*, 2018). They play an important role in ecology because they feed on fish and crustaceans, but themselves are predated by organisms from higher trophic levels (Xavier & Peck, 2015). Regarding fish species, around 200 were identified, although only those that live above the continental shelf show greater endemism (Xavier & Peck, 2015), while other species migrate seasonally from more northern waters to feed on zooplankton and Antarctic krill. Although nowadays we know more about this region, only a few taxa, such as the chordates, are more studied, mainly species of penguins, seals and albatrosses, while certain organisms (e.g. fish, cephalopods and ascidians) are less studied, namely because of the difficulty of obtaining samples (Griffiths, 2010). As well as the ocean, the terrestrial zone is quite different from other regions due to the hostile conditions that feel mainly during the harsh winters. The islands around Antarctica have very distinct characteristics and a great abundance of species, which may be a consequence of large-scale environmental variations, such as the period of glaciation or the colonization of other nearby islands (Selmi & Boulinier, 2001; Bergstrom & Chown, 1999). Among the various organisms that can be found, the islands are the main habitat for seabirds such as Procellariforms (e.g. shearwaters and albatrosses) and penguins that are concentrated here, mainly to reproduce (Bergstrom & Chown, 1999), being an integral part of the ecology of this region, as well as the Antarctic continent (Scully, 1978). Of the different islands around Antarctic continent, South Georgia (Figure 1) is one of those that displays greater density and richness of species, specially of albatrosses containing the second largest population of Wandering Albatross *Diomedea exulans* (Lewis *et al.*, 2012).

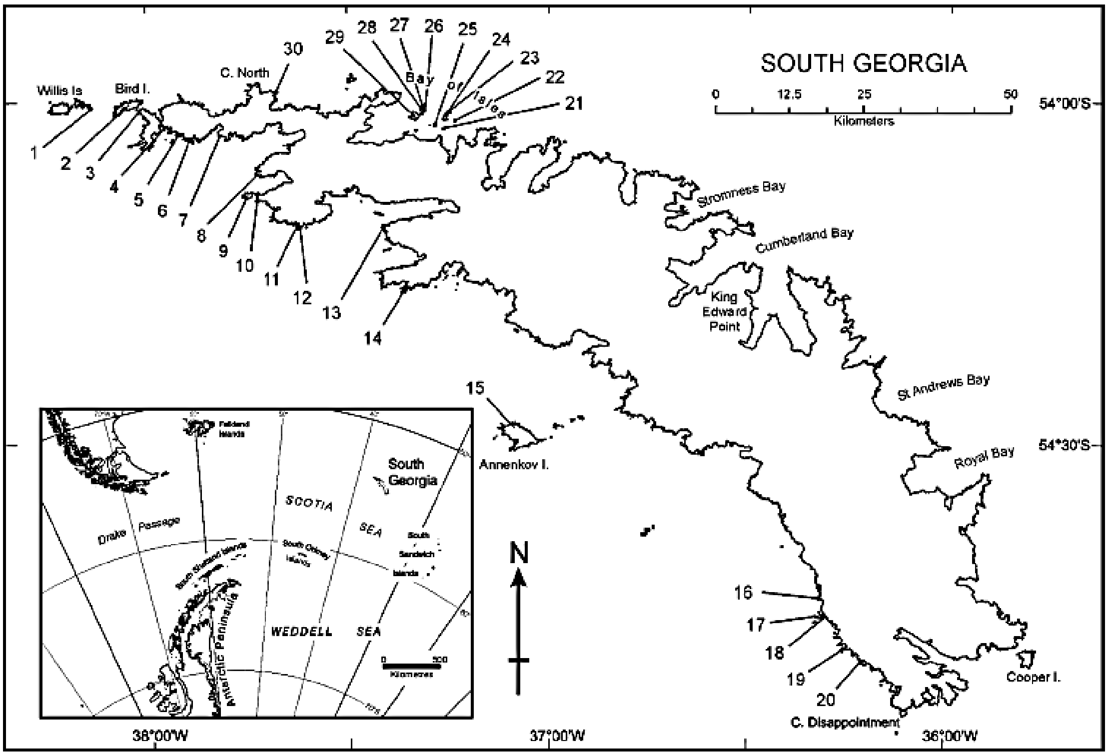


FIGURE 1 - MAP OF SOUTH GEORGIA AND LOCATION IN SCOTIA SEA (FROM PONCET *ET AL.*, 2006).

Southern Ocean special characteristics (e.g. connectivity with other parts of the world) allows more visible effects of climate change, being useful to forecast its impact and expected changes (Kennicutt *et al.*, 2014). Due to the different water masses passing through it and the effect they have on the linked oceans and in the global temperature (Sarmiento *et al.*, 2004; Xavier & Peck, 2015), with increasing values of about 1°C (Rintoul *et al.*, 2018). Anthropogenic activities even at large distance, may put their mark on Antarctica and in the Southern Ocean (Cossa *et al.*, 2011). Such changes can be observed in the Bellingshausen-Amundsen sector (Arrigo & Thomas, 2004). The emission of carbon dioxide into the atmosphere is one of the main factors that has potentiated climate changes and the fact that impact can take decades or centuries to arise makes it difficult to develop actions in time (Rintoul *et al.*, 2018). Carbon dioxide has led to rising temperature and acidification of the oceans. The Southern Ocean continues to extract large amounts of heat and carbon dioxide, helping to control the increase in global temperature. Although initially delaying warming around Antarctica, surface water and atmospheric temperatures eventually increased as well as in other parts of the globe, with a greater increase in winter than in summer (Rintoul *et al.*, 2018).

With the increase of temperature, glaciers begin to melt and no longer prevent the passage, allowing new areas of production (Xavier & Peck, 2015) as well as more accessibility to fishing and tourism activity (Rintoul *et al.*, 2018), which will require greater attention from the Antarctic Treaty governing the Antarctic region (Hughes *et al.,* 2018). In the case of fisheries, marine stocks have begun to be increasingly exploited, leading to declining populations, especially those species that take longer to mature and reproduce (Rintoul *et* *al.*, 2018). In addition to the fish that began to be more exploited also the Antarctic krill was highly sought after, which will consequently affect other top predators such as penguins and albatrosses, imposing a change to their diet, feeding on mesopelagic fish and squid (Rintoul *et al.*, 2018). Not to exhaust all the stocks, conservation measures and marine protected areas were created mainly for the most popular fish species such as *Dissostichus eleginoides* and *Dissostichus mawsoni*, belonging to the family Nototheniidae (Rintoul *et al.*, 2018). The Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) is the main regulator of fisheries in the Antarctic Ocean and was established to prevent Antarctic krill from disappearing from this region (Griffiths, 2010). By losing their food, source top predators can also undergo changes, especially the more sensitive species. Seals, penguins and other seabirds already exhibit differences in foraging moving further south due to the loss of ice shelves, as well as body mass and reproductive success, but currently appear to be more stable (Gutt *et al.*, 2015; Rintoul *et al.*, 2018).

On land, the fact that the soil is free of ice for longer time can facilitate colonization by native plants but also the introduction of new plants, which will lead to changes in the nesting areas of seabirds such as albatrosses and penguins (Rintoul *et al.*, 2018). But the fact that this thaw occurs leads to more serious consequences. The Southern Ocean is the largest contributor to the average rise in sea level because it is where there is a greater number of icebergs and ice shelves. Since 2000, sea level has risen more than 27 cm due to the loss of ice mass, and had led to a greater stratification, making it difficult to exchange deep waters rich in nutrients (Rintoul *et al.*, 2018). The collapse of ice blocks has consequences for the communities of marine organisms that found there a refuge and a source of food, thus creating a disturbance in that ecosystem (Griffiths, 2010). In addition to the impact observed by acidification and temperature increase, the hole in the ozone layer caused changes in the winds that weakened during the summer, which for some species such as Wandering Albatrosses that depends on the force of the winds to fly and forage, can have consequences on their breeding success and survival (Rintoul *et al.*, 2018), but is also linked to the acceleration of the West to South winds that will lead to changes in oceanography such as currents, stratification, nutrient transport and ice formation, which may lead to its reduction (Gutt *et al.*, 2015).

1.2. The role of Wandering Albatrosses in Antarctic marine food webs

In the Southern Ocean, the Wandering Albatross (Figure 2) is used as a top predator model because they are large seabirds, with a large distribution, able to travel long distances throughout their life by the oceans, breed in various island around the Southern Ocean and feed on a variety of poorly known prey, such as cephalopods and fish (Xavier *et al.*, 2004; Ceia *et al.*, 2012; Pereira *et al.*, 2018). This albatross species is the largest member of the Diomedeidae family, presenting sexual dimorphism with males being larger than females (Weimerskirch & Wilson, 2000) and the most pelagic seabird due to the time spent on open sea (Croxall *et al.*, 1990). Since its demography and abundance are influenced by the availability of food and association with fisheries, several studies have focused on its conservation (Xavier *et al.*, 2004).

FIGURE 2 - WANDERING ALBATROSS DURING THE EXPEDITION TO SOUTH GEORGIA (JOSÉ CARLOS CAETANO XAVIER).

This species of albatross reproduces mainly in Subantarctic islands in the Southern Ocean (Croxall *et al.*, 1990; Weimerskirch & Wilson, 2000). Among all the islands, South Georgia is the one that has the largest population of Wandering Albatross, and this species that reproduces here is known to forage between 28ºS (from Brazil) to 63ºS (Platform of the Antarctic Peninsula) and between 19ºW (from Tristan da Cunha) to 68ºW (Patagonian Platform and ocean waters south of Cape Horn), during the brood guard (Ceia *et al.*, 2012).

Using electronic devices, such as Platform Terminal Transmitters (PTT) or GPS devices, on Wandering Albatrosses, these are one of the best organisms to study the ecology of a diversity of marine species along the Southern Ocean such as the distribution of fish, crustaceans and cephalopods, which are included in their diet (Xavier *et al.*, 2006; Pereira *et al.*, 2017). In South Georgia, while adults take care of their offspring, they can travel up to 5 000 km on a trip, and as the herd becomes more independent, adults spend more time looking for food and can travel up to 15 000 km (Xavier *et al.*, 2004). Adults of both sexes take shorter trips, near the coast, in colder waters, but also travel longer in warmer Subantarctic and subtropical waters (Xavier *et al.*, 2004).

In Wandering Albatrosses, as in many other seabirds in which there is great sexual dimorphism, it is observed that there are differences in foraging behavior, being observed segregation usually by the male for the females that are smaller. Near the colony the competition is higher and this way the males will exclude the females, preventing them from forage in the same waters (Fauchald, 2009). To avoid this competition, there will be a different distribution of individuals, mainly in latitude, where females will explore different niches, which results in different diets since adults are subject to the availability of prey found in a certain region (Pereira *et al*., 2017; Croxall *et al*., 1988). This occurs mainly in the breeding season, shortly after the birth of the offspring, which is when adults cannot get too far from the colony to forage, thus having a great concentration of individuals in the area surrounding the colony (Croxall *et al.*, 1988). Although there are studies related to the biology of this species (Weimerskirch *et al.* 1997; Weimerskirch & Wilson, 2000; Xavier *et al.*, 2006), it is currently unknown if the effort of males and females in the breeding season is reflected in their body condition.

In studies conducted in the Southern Ocean, South Georgia, Kerguelen and Crozet, under normal oceanographic conditions, it has been observed that females tend to forage further north of the colony in subtropical waters, while males forage more to south (Weimerskirch *et al.*, 1997; Xavier & Croxall, 2005). But for shorter trips, there are no significant differences between males and females in foraging environments (Xavier *et al.*, 2004), although it is documented that females travel to more productive waters around the colony (Croxall *et al.*, 1988).

When the environmental conditions are unfavorable, greater differences between sexes have been observed. Between 1999 and 2000, in a study conducted in South Georgia, it was observed that females made longer trips and further north to Subantarctic waters while caring for the offspring, and males were concentrated near the colony (Croxall *et al.*, 1988).

About their feeding, this albatross species feeds especially in cephalopods (e.g. squid) and fishes, because since they do not have the capacity to dive, they only catch species of fish that are available at sea surface (Xavier *et al.*, 2004; Xavier *et al.*, 2003). The fact that there is no great specialization can minimize intraspecific competition (Pereira *et al.*, 2017), as well as the fact that males and females have different diet (Croxall *et al.*, 1988). Males have a less varied diet, feeding mostly on some species of fish found in less productive waters, while females present a more varied diet, looking not only for fish but also cephalopods (e.g squid), which can be an advantage to avoid competition (Xavier *et al.*, 2003; Pereira *et al.*, 2018). As Wandering Albatrosses have the largest wingspan among the various seabirds, they take advantage to minimize the energy costs using the wind to gain more speed during the flight. As the strength of the wind increases with latitude, the individuals take advantage to cover a larger area, making the search for food more efficient and also in their migration, being able to maintain their body condition (Mackley *et al.*, 2010; Weimerskirch *et al.*, 2006). Differences between males and females may influence habitat specialization, since females have smaller wings compared to males, but are better adapted to weaker winds in Subantarctic and Subtropical regions (Phillips *et al.*, 2009).

1.3. Wandering Albatross conservation

The Wandering Albatross is considered Vulnerable on the International Union for Conservation of Nature's Red List (IUCN Red List). In 2004, it was noted that there were fewer breeding pairs in South Georgia compared to previous years, with the population declining by about 30% (Lewis *et al.*, 2012; Grémillet *et al.*, 2012). This species has a high survival rate and adults are more sensitive to changes in the environment than juveniles (Prince *et al.*, 1992).

Despite the impacts of climate change on the Southern Ocean, the Wandering Albatross population in this region has fluctuated (Weimerskirch *et al.*, 2006), with its main threat being the interaction with fisheries, which has led to the decline of several Antarctic populations (Phillips *et al.*, 2011). Albatrosses will look for food at the same waters that the fishing boats and they are caught by the hooks (Phillips *et al.*, 2011). Nowadays, throughout the year several fishing boats are around South Georgia, being these seabirds more susceptible to be trapped in hooks (Xavier *et al.*, 2004). As females forage in farther north waters, they face a higher mortality rate than males due to an increase of vulnerability (Xavier *et al.*, 2004).

1.4. Fatty acids as a tool to address ecological issues in marine ecosystems associated to albatrosses

Knowing that females and males of Wandering Albatrosses may present different diets, different feeding areas and exhibit different efforts during the breeding season (e.g females must produce the egg and may forage further) (Xavier *et al.*, 2004; Pereira *et al.*, 2018), it is essential to understand how these different conditions may interfere with the body condition of the individuals (male and female) and with the quality of the diet for each gender in this context, and consequently for the offspring.

Through the diet of albatrosses, as in other organisms, lipids, including fatty acids, are essential molecules that play physiological functions fundamental to the individual's well-being, and allow the acquisition of energy necessary for the development of different activities (Filimonova *et al.*, 2016a). Fatty acids are important trophic markers, allowing to study the interaction between a predator and its prey(s) and thus, define about the quality of the diet (Filimonova *et al.*, 2016a). The trophic markers are calculated based on well-defined fatty acid ratios for a particular trophic group, allowing to obtain information about the diet and the use as bioindicators of the quality of the ecosystem (Filimonova *et al.*, 2018a). A healthy food chain means that there is an adequate source of food, rich in essential nutrients, fundamental to the good development of communities. If the primary producers are rich in nutrients and pass a significant amount of energy to the next trophic levels, the secondary consumers will present high nutritional values (Filimonova *et al.*, 2018b). The quality and quantity of lipids is related to the availability of nutrients (Arrigo & Thomas *et al.*, 2004).

Fatty acids are the main components of lipids and are found in all metabolic systems and play an important role in the physiology and structure of the organism (Moura *et al.*, 2016). These molecules pass along the trophic chain and are found at all trophic levels, from photosynthetic organisms to secondary consumers (Filimonova *et al.*, 2016a). Also, fatty acids have a high structural diversity and specificity (Filimonova *et al.*, 2016a). The structure and function of lipids defines the type of fatty acids that will be incorporated (Connan *et al.*, 2007). These can be classified as saturated fatty acids (SFA) without double bounds, monounsaturated fatty acids (MUFA) with one double binding, polyunsaturated fatty acids (PUFA) with two double bindings, and highly unsaturated fatty acids (HUFA) with three or more double bindings (Gonçalves *et al.*, 2012a).

The Essential Fatty Acids (EFAs) of which some PUFAs are part and the HUFAs, are synthesized by photosynthetic organisms, but also by some animals although they are only able to produce very small quantities with very high energy expenditure. This group of EFAs play an important role in the health and functioning of organisms (Filimonova *et al.*, 2018b). The formation of ice shelves is quite rich in PUFA for grazers, who will have a diet rich in these fatty acids. The essential fatty acids are produced by bacteria, micro/macroalgae and plants (Arrigo & Thomas, 2004).

The fatty acid profiles have already been analyzed in different organisms from the simplest to the most complex (Bergé & Barnathan, 2005). Due to the sensitiveness of Plankton to environmental changes, it is considered an interesting group in ecology studies to determine the consequences of extreme events and the response of fatty acids to climate changes, and the impacts along the trophic chain (Filimonova *et al.*, 2018a). Antarctic krill, as well as salps and copepods, serve as food for a wide variety of species found in Southern Ocean and contain a large reserve of lipids, providing a rich diet (Bergé & Barnathan, 2005). About Antarctic krill, lipid analyzes have already been carried out, not only general studies (Reinhardt & Van Vleet 1986), but also a comparison between individuals of different genera and ages (Virtue *et al.*, 1996) and differences at a spatial scale (Mayzaud, 1997). Although the quantity of lipids vary according to several factors, such as food availability in the surrounding environment that may increase the lipids or individual's growth, they mainly present PUFA, being more observed in adult males, however juveniles have a higher lipid content in relation to adults (Cripps *et al.*, 1999; Cripps & Atkinson, 2000). In addition to PUFA, it is also documented that Antarctic krill has MUFA, SFA, EPA, docosahexaenoic acid (DHA), and omega-3 have nutritional benefits for their predators (Ericson *et al.*, 2018). For copepods, according to analyzes, both in Antarctica and Arctic, it was observed that they presented essentially unsaturated fatty acids like PUFA, mainly EPA and DHA, which was also observed when analyzing the Antarctic krill (Bergé & Barnathan, 2005).

Algae are one of the main producers constituting an important source of food and energy for consumers in the different food chains. Fatty acid profiles can be good bioindicators of diet and energy reserves and adaptations to the surrounding environment (Filimonova *et al.*, 2016a). It has already been demonstrated that the composition of fatty acids can undergo changes with alterations in water parameters such as salinity, as happened with a species of microalga of the genus *Dunaliella* that can grow even when the salinity is much higher than expected (Xu *et al.*, 1998). Phytoplankton present a crucial role in the trophic chain and in the exponential growth phase, presenting about 50% of the total of lipids mainly as (n-3) PUFA (Bergé & Barnathan, 2005).

For fish species of the family Myctophidae (e.g *Electrona antarctica, Electrona carlsbergi, Gymnoscopelus braueri, Krefftichthys anderssoni, Protomyctophum bolini* and *Protomyctophum choriodon*) (Connan *et al.*, 2007; Stowasser *et al.*, 2009; Connan *et al.*, 2010; Richoux *et al.*, 2010), the species belonging to the suborder Notothenioidei (*Gobionotothen gibberifrons*, *Notothenia coriiceps*, *Notothenia rossii*, *Lepidonotothen squamifrons*, *Patagonotothen guntheri* and *Dissostichus eleginoides*) (Stowasser *et al.*, 2012) are very important in the diet of seabirds in Antarctica (Connan *et al.*, 2007; Stowasser *et al.*, 2009). For the species *Pseudosciaena crocea* and *Pampus argenteus* (Geng *et al.*, 2015) it was observed the presence of

MUFA and PUFA (Stowasser *et al.*, 2009). Ichthyofauna also plays a key role in the transfer of energy to higher trophic levels because, after Antarctic krill, they are the main source of food for a great diversity of species (such as pinnipeds and seabirds) and feed mainly on mesozooplankton (Stowasser *et al*, 2012; Connan *et al.*, 2010). In addition, they have nutritional benefits due to omega-3 and are important for the somatic growth of marine fish, as well as for the development of physiological functions (Filimonova *et al.*, 2016a).

In cephalopods, we can find a large proportion of fatty acids mainly in their digestive glands, such as polyenoic FA, EPA and DHA (Bergé & Barnathan, 2005). Squids are those with a higher lipid content, mostly in the digestive gland (up to 54%) and fatty acid profiles reflect their long-term diet because no major changes occur (Richoux *et al.*, 2010). In the species of squid *Illex argentinus* and in the deep-water species *Berryteuthis magíster* it was found that the tissues contain large amounts of PUFA, SFA, MUFA, DHA and EPA (Bergé & Barnathan, 2005).

For the seabirds, the fatty acid profiles for shearwaters and petrels have already been analyzed (Connan *et al.*, 2007) but, for albatrosses, the information is still scarce. To study the foraging behavior and diet of individuals, can not only analyze the stomach contents given that represents a recent diet, which may still be conditioned to a set of environmental variables and not match the regular diet of the individual. Thus, trophic markers, such as lipids, should be determined to describe and characterize individuals' diets (Connan *et al.*, 2007). In albatross' species like the Grey-headed albatross *Thalassarche chrysostoma*, were observed differences in diet and metabolism between adults and offspring (Richoux *et al.*, 2010), that can be observed through the quantities identified or the variety of lipids. A diet rich in lipids is important for the juveniles, after having the new plumage to survive as soon as they can fly and leave the colony (Richoux *et al.*, 2010).

Procellariforms in the Southern Ocean can travel long distances to forage and during breeding season require more energy, but the amount of food they can eat depends on the availability in the regions where these birds will seek. Analysis of fatty acids is therefore of paramount importance in assessing whether individuals can find enough food and whether it is nutritious, being possible to analyze through blood samples, feathers or tissues that demonstrate their diet (Richoux *et al.*, 2010). In blood samples, plasma can be separated from blood cells, allowing to be studied the diet at different time scales. In plasma it is possible to analyze the diet for the last days before the sample collection until one week, due to the lifetime (Ceia *et al.*, 2012), while blood cells provide information over a longer period, up to 4 weeks (Phillips *et al.*, 2009). This difference is due to the fact that different tissues renew themselves in different periods of time and corresponds to the time that a given molecule remains unchanged (Michael Allaby, 2021). The fact that several seabird populations in the Southern Ocean are declining, mainly due to accidental mortality from fisheries, makes it particularly important to assess the factors that lead to this consequences, the behavior and diet of individuals in order to be able to take conservation measurements and the preservation of their habitat so that these organisms do not have to make longer trips to search for food, which for the most vulnerable can be fatal (Richoux *et al.*, 2010).

There are some studies about fatty acid trophic markers (FATMs) where it was identified the fatty acids more abundant, also in albatrosses but the majority is in other seabirds as Procellariforms. In Procellariforms the fatty acids most common to be identified are cis-9-eicosenoic gadoleic acid (C20:1ω11), cis-11-docosenoic cetoleic acid (C22:1ω11), cis-9-octadecenoic oleic acid (C18:1ω9), cis-11-eicosenoic gondoic acid (C20:1ω9), hexadecenoic acid palmitic acid (C16:0), cis-9-hexadecenoic palmitoleic acid (C16:1ω7), tetradecanoic acid myristic acid (C14:0), eicosapentaenoic acid EPA (C20:5ω3), docosahexaenoic acid DHA (C22:6ω3), cis-11-octadecenoic vaccenic acid (C18:1ω7) (Wang *et al.*, 2009; Connan *et al.*, 2005; Connan *et al.*, 2007). In Albatrosses, the fatty acids are very similar to the ones identified in Procellariforms. In the papers, the fatty acids most abundant are C18:1ω9, C16:0, C20:5ω3, C22:6ω3, C16:1ω7, C20:1ω9 and octadecanoic acid stearic acid C18:0 (Connan *et al.*, 2014; Richoux *et al.*, 2010).

Squids are one of the preferences for albatrosses when they are searching for food, so analyzing the profile of fatty acids it is possible to link to the profile for albatrosses and try to understand what they are feeding. Phillips *et al.* (2002) analyzed four different squid species from Southern Ocean. The most abundant fatty acids in these species were PUFA either in mantle and digestive gland, in the mantle the fatty acids with more percentage were EPA (C20:5ω3), DHA (C22:6ω3), DPA and C16:0, while in digestive gland were C16:0, C18:0, EPA, C18:1ω9 and DHA.

The myctophid fishes are also important for seabirds from Southern Ocean. Lea *et al.* (2002) studied this group and the fatty acids identified for the individuals were very similar to the squids. However, the quantities were different since in this group of fishes the authors concluded that the individuals showed a higher percentage of monounsaturated fatty acids. The main fatty acids identified were C16:0, C18:1ω9, C22:6ω3, C20:5ω3, C14:0.

1.5. Sugars as a tool to address ecological issues in marine ecosystems associated to albatrosses

In addition to fatty acids, sugars are also important in the nutrition of individuals as energy reserves (Cotonnec *et al.*, 2001), being the most abundant and the most complex organic molecules in the ocean (Helbert, 2017). Sugars can be classified as simple (e.g glucose, trehalose) or complex (e.g starch, glycogen, chitin, cellulose) (Cuzon *et al.*, 2000). In animals' tissues we can find mainly the polysaccharides such as starch and glycogen (Cuzon *et al.*, 2000; Gutiérrez *et al.*, 2019). However, unlike fatty acids there are fewer studies conducted on marine ecosystems, especially on top predators. Most of the studies focus on primary producers, such as phytoplankton and algae, has its importance documented for marine species (Mühlenbruch *et al.*, 2018; El-Sheekh *et al.*, 2012). In studies where it was analyzed the composition of dissolved organic matter (DOM) from different sites and depths, it was observed that it is rich in polysaccharides representing a large fraction in samples near surface and about 25% in the deeper samples (Mühlenbruch *et al.*, 2018; Biddanda & Benner, 1997).

Phytoplankton uptake carbon in the form of carbohydrates for cellular synthesis and loss of DOM that will accumulate in the water or will sink (Biddanda & Benner, 1997), that will contribute for the vertical flux of matter and biochemical cycle in the oceans (Engel *et al.*, 2004). In spring when the nutrients are available and occurs the blooms of phytoplankton, it was detected a high level of glucose (Mühlenbruch *et al.*, 2018). Parsons *et al.* (1961) studied 11 species of phytoplankton during the exponential growth and found that carbohydrate content ranged between 6 and 37%. Biddanda and Benner (1997) also observed that during the growth of phytoplankton is when they have a higher concentration of carbohydrates.

As in other groups of organisms, the macroalgae are rich in polysaccharides mainly a great diversity of sulfated polysaccharides, that represent an important food source for heterotrophic species (Helbert, 2017; Jiao *et al.*, 2011). The sulfated polysaccharides are classified according their structural characteristics, position and number of attached sulfate ester groups and occurrence of α-anhydrogalactose (Helbert, 2017). In green seaweed of the order Ulvales, the constituents described are sulfate, rhamnose, xylose, iduronic and glucuronic acids (Percival & McDowell, 1967; Lahaye & Ray, 1996).

The use of pesticides that contaminate water can lead to biochemical changes in both lipid content, in primary producers (e.g. *Thalassiosira weissflogii*) and primary consumers (e.g. *Daphnia longispina* or *Artemia franciscana)* (Filimonova *et al.* 2016b; Neves *et al.*, 2015), but also leads to changes in carbohydrates as a response to chemical stress in estuarine bivalve species (*Scrobicularia plana*) (Gutiérrez *et al.*, 2019). Gutiérrez *et al.* (2019) observed the bivalve *Scrobicularia plana* when exposed to S-metholachlor and Terbutylazine releases mainly glucose because it accelerates the metabolism, thus increasing their concentration.

In scallop tissues, it was also observed that the glycogen values in the tissues changed after bacterial treatment, with changes in glucose metabolism to adapt to the new conditions. As a response, glucose degradation will be generated thereby generating extra energy (ATP) for the immune response, which leads to a decrease in glycogen in tissues. The same has already been tested in fish and crustaceans under stress conditions (Wang *et al.*, 2015). Some green algae can also develop a defense system by the production of polysaccharides to cope with oxidative stress, that indicated the involvement of polysaccharides in protection against toxic species (El-Sheekh *et al.*, 2012).

In crustaceans, under normal conditions, carbohydrates are important in reproduction and during the molt (Chang & O'Connor *et al.*, 1983), as well as in growth (Cuzon *et al.*, 2000), degrading glucose. In the shrimp *Macrobrachium rosenbergii* it was identified alfa-amylase and alfa – glucosidase (that hydrolyze starch and disaccharides to release glucose), alfa maltase, alfa saccharase, galactosidase, chitinase (biopolymer important in crustaceans that need to synthesize chitin before molting and happens in hypodermis where a chitinase activity exists), chitobiase and cellulase (Cuzon *et al.*, 2000). Most of these compounds are present in the epidermis, during the molt, not only in shrimp but in other crustaceans as crabs. It is also present in microorganisms that are important in the carbon and nitrogen cycle in the ocean and so for the organic matter used by other organisms (Zou & Fingerman, 1999; Beardsley *et al.*, 2011).

The ability of fish to digest carbohydrates differs according to the species and the type of diet. In carnivorous fish, the digestibility is very low and very poor for the complex carbohydrates (Cuzon *et al.*, 2000). In contrast, in herbivorous and omnivorous fish, the digestion is more developed hydrolyzing a variety of carbohydrates (Krogdahl *et al.*, 2005). Carbohydrates are stored in fish as glycogen and this can be used in all the activities that demands energy (Craig *et al.*, 2017). Being an important part of the diet, mostly for the fish that feed on crustaceans, because of the chitin, that is the second most abundant polysaccharide in marine and aquatic ecosystems (Krogdahl *et al.*, 2005).

In Procellariforms there are almost no studies on the sugar profiles. Niizuma *et al.* (2001) analyzed the fatty acid profiles for Leach's storm petrels *Oceanodroma leucorhoa*, however did not analyze polysaccharides because they represent less than 1% of body mass (Schmidt-Nielsen, 1997). Although it corresponds to a very low percentage, it is known that during the early phase, fasting birds use carbohydrates and lipids as the main metabolic fuels and in the later phase they use proteins, when the reserves of carbohydrate and lipid diminish (Cherel *et al.*, 1988).

In addition, the sugars are especially important in the polar regions, for some species of fish that adapt to extreme conditions, in which they can produce macromolecular antifreeze as proteins and polysaccharides, that control the ice crystals formation and their growth in circulation (Biggs *et al.*, 2017).

1.6. Aims of the study

The motivation for this study is strongly related with the fact that there are several studies that analyze the fatty acid and sugars profiles in other species of Procellariforms, but a lack of information about the Wandering Albatross breeding at South Georgia and its diet in short and long term. Moreover, this study intends to provide information in order to support research about biochemical profile of this species. To achieve this purpose, a set of main objectives was defined for this study, namely:

i) to determine and compare the fatty acid and carbohydrates profiles of plasma and blood samples from both male and female adults;

ii) to evaluate potential diet differences between male and female adults from different ages;

iii) to verify potential differences along the period in which the samples were collected (May to October 2009), corresponding to the period of chick breeding, under a context that females spend more energy during breeding to produce the egg;

iv) to infer about the body condition of individuals, adult males and females, when breeding, and discuss potential implications into the conservation of Wandering Albatrosses.

2. MATERIALS AND METHODS

2.1. Study Area and Sampling Collection

Samples were collected at Bird Island, South Georgia (54 °S, 38 °W) according to Ceia *et al.* (2012, 2015) and Pereira *et al.* (2017, 2018), between May and October 2009 (austral winter), during the period in which the Wandering Albatrosses are caring their chicks.

Blood samples were collected from a total of 34 randomly selected breeding adults. Of the 34 individuals, 18 were females and 16 were males identified by ringing at birth or through plumage and its morphology (Tickell, 1968), and it was identified the age of 29 adults who were already ringed. For the blood samples, 1 ml of blood from the tarsus vein was collected at the end of each trip when the individual returned to the colony after foraging (Pereira *et al.*, 2018).

2.2. Biochemical Analysis

The biochemical analysis was performed in the laboratory to determine the fatty acid and sugar profiles of adults Wandering Albatrosses. The samples were separated into plasma and blood cells in the centrifuge during 15 min at 3000 rpm. Subsequently, the plasma was separated from the blood cells and placed in separate eppendorfs', being stored at - 80 ºC in order to preserve the samples (Figure 3).

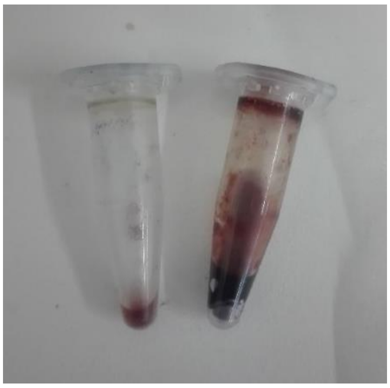


FIGURE 3 – EPPENDORF'S WITH PLASMA AND BLOOD CELLS SAMPLES RESPECTIVELY, AFTER BEING SEPARATED IN THE CENTRIFUGE AND STORED FROZEN.

2.2.1. Fatty Acids

The total lipid extraction and methylation to fatty acid methyl esters (FAMEs) was achieved using the method described by Gonçalves *et al.* (2012b), with a modified step, replacing the boron trifluoride-methanol reagent by a 2.5% H2SO4-methanol solution since BF3-methanol can cause artefacts or loss of polyunsaturated fatty acids (PUFAs) (Eder, 1995).

FAMEs present in the samples were separated and quantified using a Agilent 6890N Network Gas Chromatograph (Agilent Technologies, Santa Clara, CA, USA), equipped with a DB-FFAP capillary column (30m long × 0.32mm i.d. × 0.25 µm film thickness; Agilent Technologies, Santa Clara, CA, USA), associated to a 5973N Mass Selective Detector (Agilent Technologies, Santa Clara, CA, USA) at 70 eV electron impact mode, scanning the range m/z 40-500 in 1s cycle in full scan mode acquisition. The carrier gas He had a 4.4mL min-1 flow rate and 2.66 psi of column head pressure. 1 µL of sample was injected per run at the injector port, at a temperature of 250ºC, lined with a splitless glass liner of 4.0mm i.d. Each run had a 42.53 min duration.

The injection temperature was 220ºC and the oven temperature was programmed to start at 80ºC, increase to 160ºC at a 25ºC min^{-1} rate, increase to 210ºC at a 2ºC min^{-1} rate, increase to 250ºC min-1 at a 30ºC min^{-1} rate and finally maintaining this temperature for 10 min.

The detector starts operating 4 min after injection, corresponding to solvent delay. The injector ion source and transfer line were maintained at 220ºC and 250ºC, respectively. FAMEs were identified by comparison with the retention times and mass spectra of authentic standards and database available (WILEY Mass Spectral Libraries) (Figure 4).

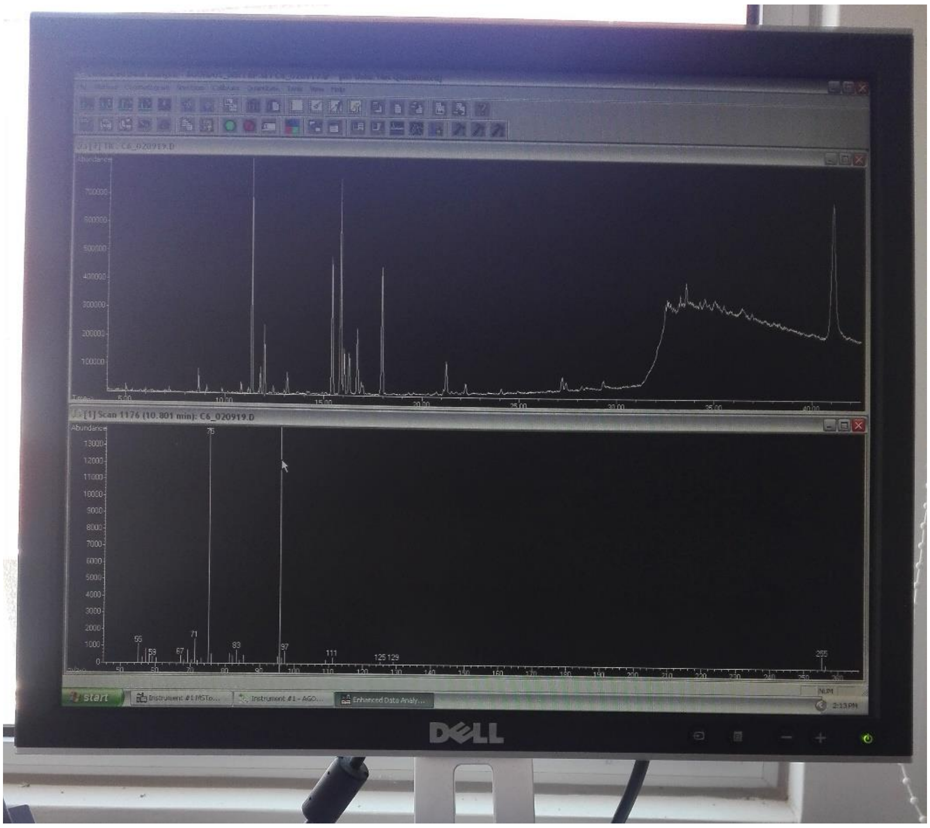


FIGURE 4 – AN EXAMPLE OF THE PEAKS OBTAINED DURING THE ANALYSIS IN GAS CHROMATOGRAPH.

Quantification of individual FAMEs was accomplished using an external standard (Supelco™ 37 Component FAME Mix, Supelco#47885, Sigma-Aldrich Inc., USA). The fatty acid Methylnonadecanoate C19:0 was the standard added in samples, to compare with the peaks obtained in the samples.

2.2.2. Sugar Analysis

For sugar analysis, samples were subjected to hydrolysis followed by reduction and acetylation, as described in Coimbra *et al.* (1996). The alditol acetate derivates obtained in the polysaccharides and neutral sugar analyses were separated in a Clarus 400 Gas Chromatography equipment (PerkinElmer®, Krakow, Poland) associated to a Flame Ionization Detector (GC-FID). A DB-225 capillary column (30m length × 0.25mm i.d. × 0.15µm film thickness; J&W Scientific, Folsom, CA, USA) was used. 2 µL of samples, dissolved in anhydrous acetone, were injected per run. Each run had a 11-min duration. The injection temperature was 220ºC and the oven temperature was set to increase from 200ºC to 220ºC at a 40ºC min-1 rate, stabilize at 220ºC for 7 min, and increase to 230ºC at 20ºC min^{-1} rate, finally maintaining this temperature for 1 min. The carrier gas was H_2, at a flow rate of 1.7mL min^{-1}.

Quantification of sugars was obtained by comparison of the sugar chromatographic peaks to the peaks obtained for the standard used (2-desoxiglucose).

2.3. Statistical analysis

To determine the differences between individuals and variables was performed a statistical analysis of variance, one-way analysis (ANOVA) in SPSS, allowing different groups to be compared. This test was performed for both fatty acid and carbohydrate samples, to compare and identify potential differences between variables. It was considered that two variables were significant different when *p* value was lower than 0.05.

The fatty acids and carbohydrate samples were subject to Primer 7 tests to check if there are differences between the different groups, trying to see which are the nearest and most distant. An analysis of similarities (ANOSIM) was performed, with Euclidean distance measure. The multidimensional scaling (MDS) for a graphical representation to the closer samples and the ones that are further apart, and the last analyzes, SIMPER, to see which individuals contribute to the dissimilarity between the groups.

These analyzes allowed us to relate the different groups that correspond to the months of the year, sex and tissue, of each individual.

3. RESULTS

3.1. Fatty Acids

The fatty acid samples examined through SPSS shows us that only exist significant differences between tissues (blood and plasma) samples ($F=68.31$; $p<0.01$) (Figure 5). However, beyond that differences between tissues ($R=0.70$; $p=0.001$) differences between sexes are also observed ($R=0.447$; $p=0.001$).

Comparing the total of FA, it was observed that plasma shows higher abundance of FA (98%) than blood cells (2%), being residual quantities in blood as shown by the differences in figure 5. In plasma and blood, the percentage of fatty acids are similar for males and females. There are differences between the sexes, since for some FA males show more quantities, but these differences are very small (Figure 5). In blood samples, it was identified more quantity of fatty acids in males (Table 1) and the difference in the percentage of fatty acids are quite larger, especially during October (85% of fatty acids identified in males) (Figure 7). Plasma shows a greater quantity of SFA (Males = 75%; Females = 81%) and MUFA (Males = 25%; Females = 19%) but none of PUFA (Table 1; Figure 8). Females have a greater diversity of fatty acids (for blood and plasma samples) since we can see that were identified 23 and 12 different fatty acids, respectively (Table 1). In plasma, the variable that more contributes for these differences inside the group were the months (34.78%) and in blood were the sexes (50.94%).

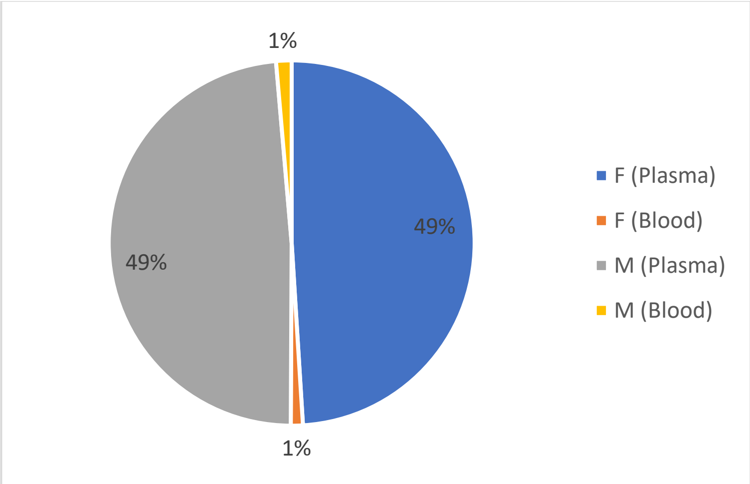


FIGURE 5 - TOTAL PERCENTAGE OF FATTY ACIDS IN BOTH SEXES (MALE AND FEMALE) AND BOTH TYPE OF TISSUES (PLASMA AND BLOOD). M - MALE AND F - FEMALE.

TABLE 1 - FATTY ACIDS PROFILE OF MALES AND FEMALES IN PLASMA AND BLOOD (%)

		Blood		Plasma	
	Fatty Acids	Males	Females	Males	Females
SFA	C16:0	28.77	28.83	32.00	27.46
	C18:0	21.97	18.85	21.71	18.58
	C14:0	1.13	0.82	0.00	0.27
	C15:0	0.19	0.23	0.00	0.08
	C8:0	0.19	0.17	0.00	0.00
	C20:0	0.13	0.07	0.00	0.00
	C10:0	0.07	0.02	0.00	0.00
	C12:0	0.03	0.01	0.00	0.02
	C24:0	0.03	0.00	0.00	0.00
	C17:0	0.00	1.86	0.00	0.80
	C13:0	0.00	0.00	0.51	0.52
	C6:0	0.00	0.00	0.01	0.00
	C21:0	0.00	0.01	0.00	0.00
MUFA	C18:1ω9c	51.90	49.31	50.36	24.25
	C18:1ω10	17.20	21.34	0.00	0.00
	C18:1ω9t	14.64	4.40	0.00	0.00
	C20:1ω9	10.28	9.33	0.00	0.00
	C16:1	4.81	4.52	42.80	70.90
	C16:1ω9	0.59	0.51	0.00	0.00
	C22:1ω9	0.41	1.15	0.00	0.00
	C24:1ω9	0.09	0.12	0.00	0.00
	C11:1ω10	0.05	0.01	0.00	0.00
	C18:2ω5	0.03	0.00	1.62	0.00
	C18:1ω6c	0.00	0.00	3.70	2.79
	C18:1ω8	0.00	8.77	0.00	0.00
	C16:1ω5	0.00	0.00	1.51	2.06
PUFA	C18:2ω6c	100.00	90.45	0.00	0.00
	C18:3ω3	0.00	9.55	0.00	0.00
Σ SFA		0.01	0.01	0.55	0.60
N SFA		10.00	11.00	5.00	8.00
Σ MUFA		0.01	0.04×10^{-1}	0.18	0.14
N MUFA		10.00	10.00	5.00	4.00
Σ PUFA		0.01×10^{-2}	0.01×10^{-2}	0.00	0.00
N PUFA		1.00	2.00	0.00	0.00

For the months of the study, there is not a segregation between groups (R=0.309; p=0.001), and that is visible in n-MDS (Non-metric Multidimensional scaling) the individuals are all mix together. There is a difference between plasma and blood samples, where the percentage of total fatty acids of female and male are higher in plasma than in blood. Table 2 shows that in plasma, males have a higher percentage of fatty acids during July comparatively with females (63%), with an increase from May to July and again from September to October. During May it was when male individuals present the lowest percentage of fatty acids (32%) (Table 2). Females show a higher percentage of fatty acids during May (68%) with an increase of fatty acids later than males, from July to September, before that period, the percentage decrease from May to July, where they have the lowest percentage of fatty acids (37%) (Table 2). For each month it was calculated the total of FA for females and for males.

In plasma, it is possible to observe that in September, females and males have the same percentage of fatty acids (50% of the total of fatty acids for each sex), and in majority of the months the males have a greater quantity of fatty acids with the exception of May (Figure 6). In males, the variable that most contributed to the difference within the group was the months (35.58%), while in females was the months (31.13%) but also the tissue (31.83%).

In blood samples, despite the quantities of fatty acids are very low, during September and October it is possible to see more differences between the sexes. In this tissue, males show a higher percentage in October (85%) and females in September (67%) (Table 3). Between September and October, the quantities of fatty acids are lower than the other months for both sexes, in September for males and in October for females (Table 3). As well as in plasma samples, males in the majority of months show a higher percentage of total fatty acids (Figure 7).

FIGURE 6 – TOTAL OF FATTY ACIDS IDENTIFIED IN PLASMA SAMPLES, IN PERCENTAGE.

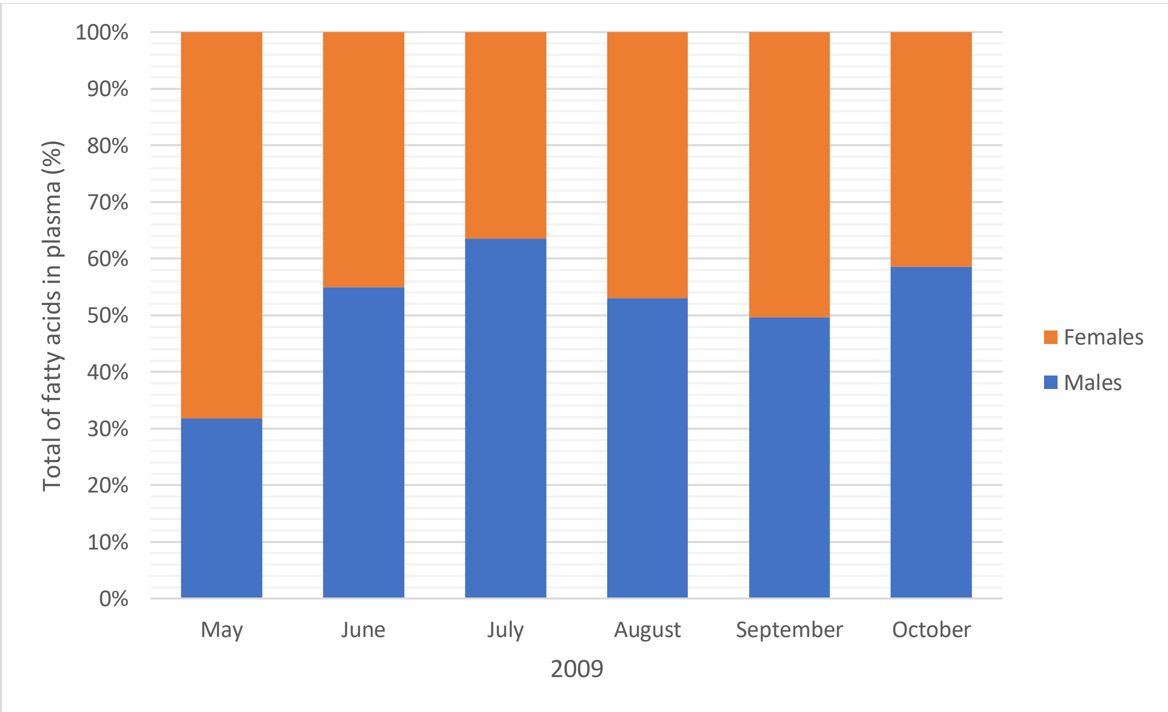


FIGURE 7 – TOTAL OF FATTY ACIDS IDENTIFIED IN BLOOD SAMPLES, IN PERCENTAGE.

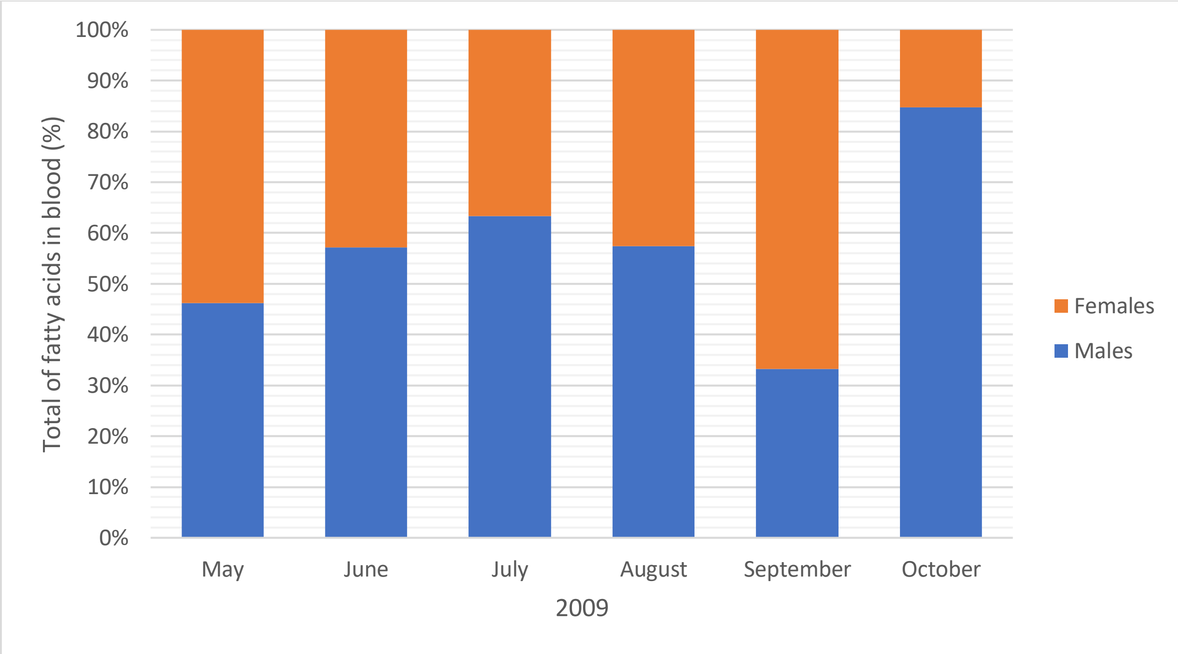


TABLE 2 – TOTAL OF FATTY ACIDS IN PLASMA SAMPLES (%)

	May	June	July	August	September	October
Males	32	55	63	53	50	58
Females	68	45	37	47	50	42

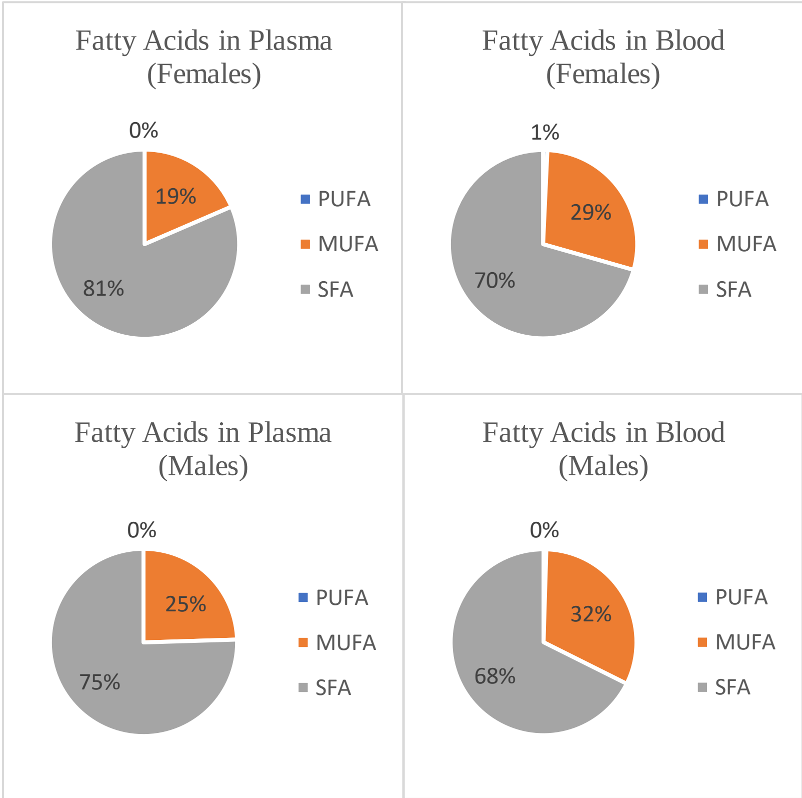
TABLE 3 – TOTAL OF FATTY ACIDS IN BLOOD SAMPLES (%)

	May	June	July	August	September	October
Males	46	57	63	57	33	85
Females	54	43	37	43	67	15

In both samples, plasma and blood, and both sexes, females and males, the more abundant fatty acids identified were saturated fatty acids (SFA) (Figure 8), being identified in a greater percentage in female plasma (81%) and male plasma (75%) and then in blood cells. This is followed by monounsaturated fatty acids (MUFA) that show a higher percentage in males' blood (32%) and lower percentage in females' plasma (19%) (Figure 8).

The quantity of polyunsaturated fatty acids (PUFA) are residual, in plasma these fatty acids do not appear in the samples and in blood only are identified two different PUFAs, being that females only counting 1% of these fatty acids (Table 1; Figure 8).

FIGURE 8 – PERCENTAGE OF THE DIFFERENT FATTY ACIDS (PUFA, MUFA AND SFA) FOR BOTH SEXES AND TISSUES



As in other articles with seabirds, Procellariforms and albatrosses, it was also identified cis-9-octadecenoic acid (C18:1ω9c), hexadecenoic acid (C16:0), tetradecanoic acid (C14:0) and octadecanoic acid (C18:0). However, the most abundant in the samples were hexadecenoic acid (C16:0), octadecenoic acid (C18:0), 9-octadecenoic acid cis (C18:1ω9c), 9-octadecenoic acid trans (C18:1ω9t) and 11-eicosenoic acid (C20:1ω9). The essential fatty acids as eicosapentaenoic acid (EPA, C20:5ω3) and docosahexaenoic acid (DHA, C22:6ω3) were not identified in these samples, only Alfa-linolenic acid (ALA, C18:3ω3) in blood samples and only in females (Table 1).

3.2. POLYSACCHARIDE ANALYSIS

For carbohydrates there are only significant differences between tissues in females ($F=56.02$; $p=0.000$) (Figure 9). However, in the program PRIMER beyond the differences between tissues ($R=0.786$; $p=0.001$) there are also differences between the sexes ($R=0.448$; $p=0.001$).

In plasma samples, 92% of the total of carbohydrates were identified in males and 8% in females (Figure 9), while in blood the quantities were very low. For example, the quantity of glucose in males were 0.30 µg/µl in blood samples, and 24.60 µg/µl in plasma (table 4). In females, the variable that contributes more to the differences within the group is the months (35.23%).

Males and females present more quantity of carbohydrates in plasma than in blood, with males showing, in general, higher quantities than females, with exception of July for both tissues. However, blood samples show a greater diversity of carbohydrates (N=9 for blood; N=7 for plasma) since in these samples it is possible to find D-Rhamnose (RHA) and 2-deoxyribose (DRIB) that are not identified in plasma samples (Table 4).

Glucose and xylose are the most abundant monosaccharides in plasma, in males the total of glucose is 24.60 µg/µl and xylose is 8.30 µg/µl, while in females the total of glucose is 20.90 µg/µl and xylose is 6.50

µg/µl (Table 4). The carbohydrates with lower concentration in plasma is fucose, with values different from the other carbohydrates (Males: 0.05 µg/µl; Females: 0.06 µg/µl) (Table 4).

In blood samples the most abundant monosaccharides are arabinose and glucose, for males, and glucose and galactose for females (Table 4). The carbohydrates DRIB and RHA appear only in the blood, for both sexes, but in low quantities (Table 4; Figure 10; Figure 11).

Males and females present a similar graphic in relation to the percentage of total of each carbohydrate, to plasma and blood samples. In plasma it is possible to observe a higher percentage of carbohydrates with the exception of fucose in which there is a percentage of almost 50% for each sex (plasma = 52% of total carbohydrates; blood = 48%) (Figure 10; Figure 11).

FIGURE 9 - TOTAL PERCENTAGE OF CARBOHYDRATES IN BOTH SEXES (MALE AND FEMALE) AND BOTH TYPE OF TISSUES (PLASMA AND BLOOD)

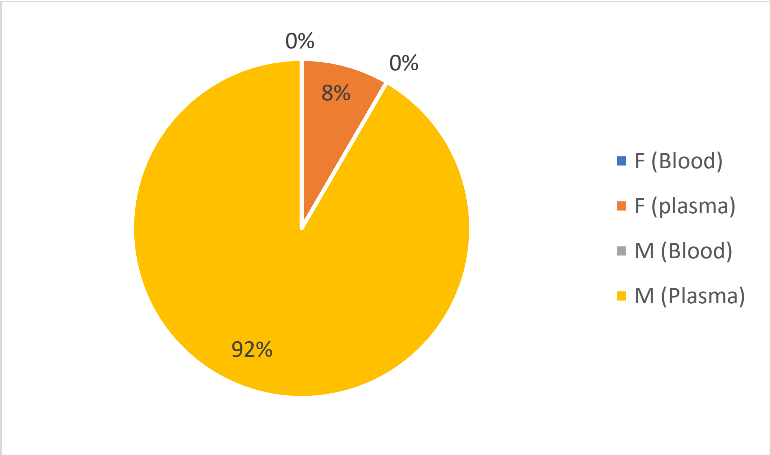


TABLE 4 - CARBOHYDRATE PROFILES OF MALES AND FEMALES IN PLASMA AND BLOOD CELLS (µG/µL)

	Blood		Plasma	
carbohydrates	Males	Females	Males	Females
Arabinose	0.34	0.27	2.97	2.74
Fucose	0.06	0.66	0.05	0.06
Galactose	0.08	0.29	2.59	2.09
Glucose	0.30	1.92	24.60	20.90
Mannose	0.05	0.05	3.28	2.69
Ribose	0.02	0.08	0.79	1.13
Xylose	0.07	0.20	8.29	6.50
2-Deoxyribose	0.08	0.01	0.00	0.00
Rhamnose	0.02	0.02	0.00	0.00
N	9.00	9.00	7.00	7.00

FIGURE 10 – TOTAL OF CARBOHYDRATES IN BOTH TISSUE SAMPLES FOR FEMALES, IN PERCENTAGE.

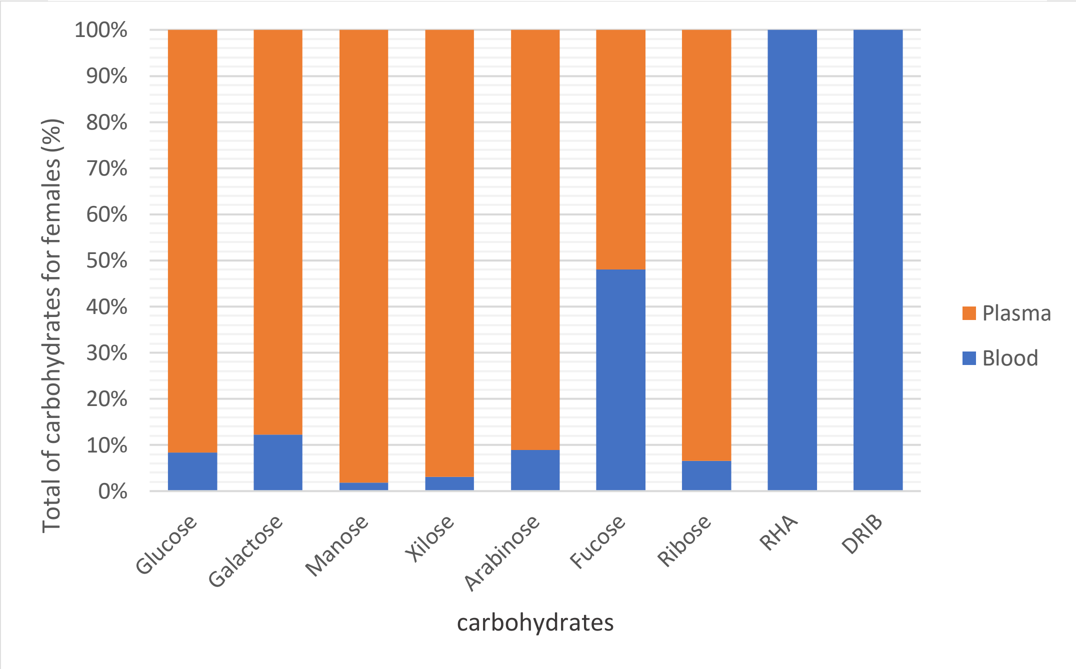


FIGURE 11 - TOTAL OF CARBOHYDRATES FOR BOTH TISSUE SAMPLES IN MALES, IN PERCENTAGE.

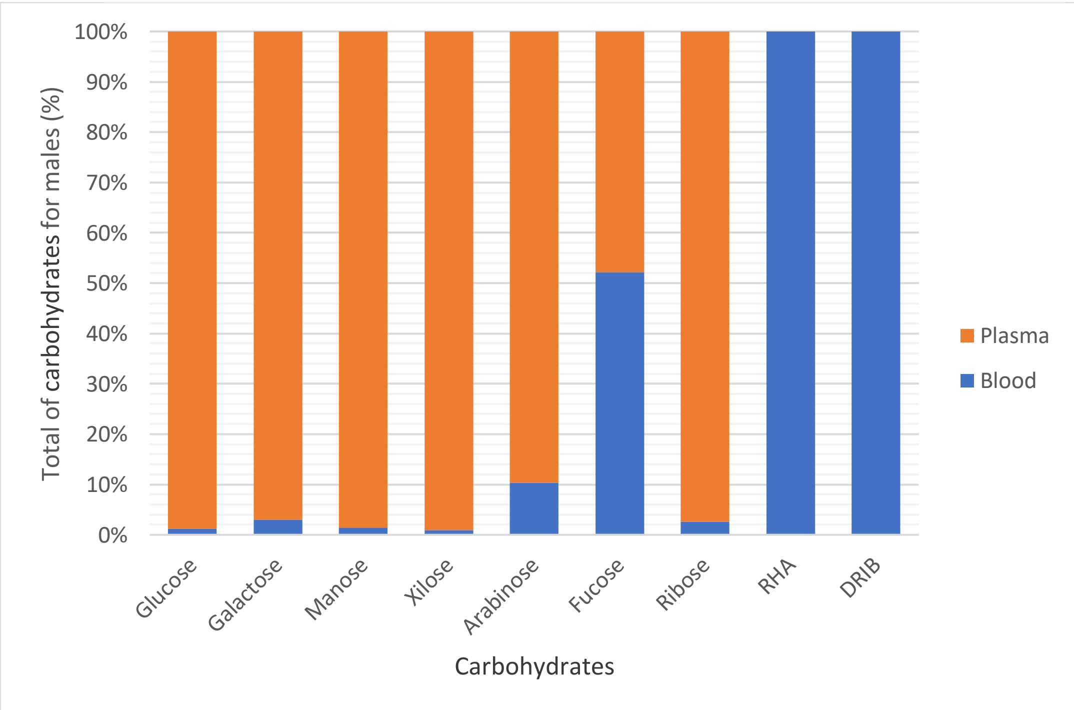


Table 5 shows that in plasma the peak of carbohydrates for males was in October (71%), while for females was in July (55%). The difference between sexes are not visible because the quantity is very close to each other, with exception of October that is when males show a higher percentage of carbohydrates (71%). In females, the percentage of carbohydrates has decreased from July to October and in males happened the inverse. For females, the lowest percentage occurred during October while in males was in July (Table 5).

In blood samples, males showed a higher percentage of carbohydrates than females during October (84%), and females, during July (95%) (Table 6). Males showed a greater decrease from May to July while females showed a decrease from July to October (Table 6).

In blood samples it is possible to observe more differences during the year since the percentages of carbohydrates in males and females differ more, especially during July (Figure 13; Table 6). While in plasma, the percentages of carbohydrates are close to 50% with an exception of October (Figure 12; Table 5).

Over the months, the differences are not considered significant (R=0.265; p=0.001), however, the variables sex and tissue have influence so that the values are different. For example, in August the sex contributed the most (42.87 %) and during September and October is the tissue that contributes more, 37.84 % and 43.21 %.

FIGURE 12 - TOTAL OF CARBOHYDRATES IDENTIFIED IN PLASMA SAMPLES, IN PERCENTAGE.

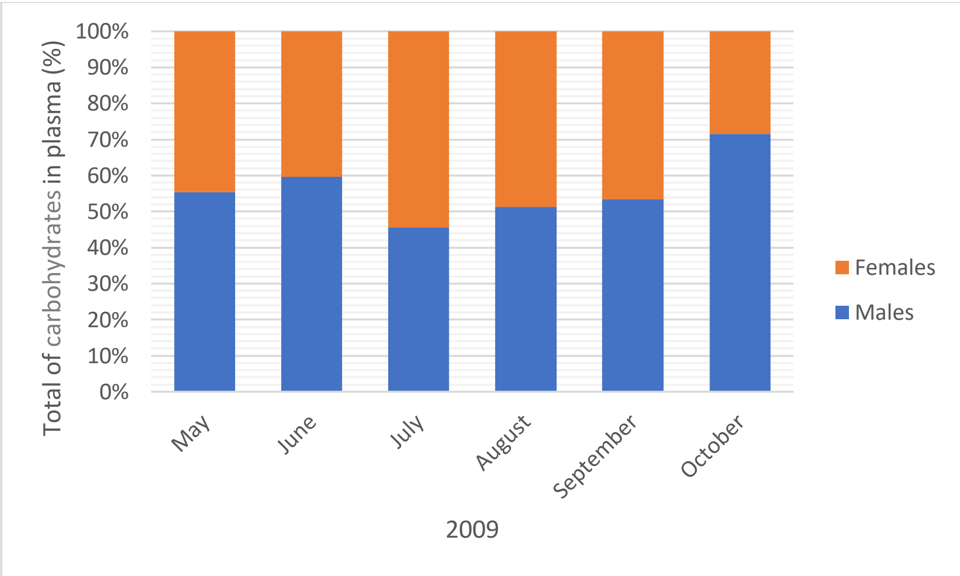


FIGURE 13 - TOTAL OF CARBOHYDRATES IDENTIFIED IN BLOOD SAMPLES, IN PERCENTAGE.

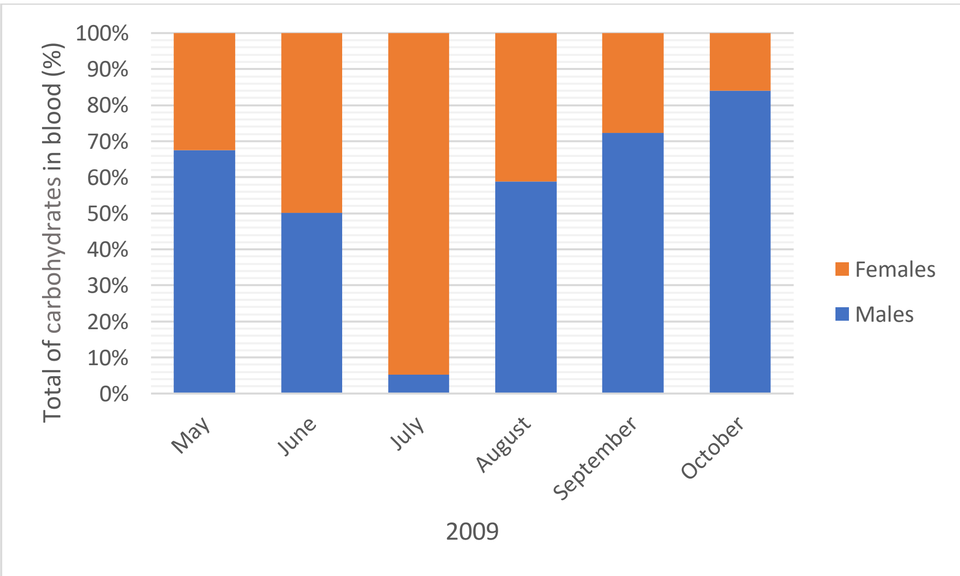


TABLE 5 - TOTAL OF CARBOHYDRATES IN PLASMA SAMPLES (%)

	May	June	July	August	September	October
Males	55	60	45	51	53	71
Females	45	40	55	49	47	29

TABLE 6 - TOTAL OF CARBOHYDRATES IN BLOOD SAMPLES (%)

	May	June	July	August	September	October
Males	67	50	5	58	72	84
Females	33	50	95	42	28	16

4. GENERAL DISCUSSION

The present work highlights the differences between tissues, plasma and blood samples, of male and female of Wandering albatrosses.

4.1. FATTY ACIDS

This study demonstrated that there are differences between tissues, with plasma having a greater amount of fatty acids compared to blood samples.

In plasma, the amounts of fatty acids are quite similar for males and females, however females have a greater diversity of fatty acids. This may indicate that both males and females, on short-trips, managed to capture nutritious prey and there was no advantage from one sex to the other, as both sexes forage close to the colony. However, the fact that in females samples it was identified some fatty acids that have not been identified in males, may indicate that they have captured different prey from males, with a different composition of fatty acids.

In blood samples, the amounts of fatty acids are residual compared to the values obtained for plasma samples. Males showed a greater amount of fatty acids than females, however, also in these samples, females have a greater diversity of fatty acids. This difference between tissues was expected since they correspond to different time periods. Plasma samples represent the food captured in a short period of time (up to 1 week), for that reason there are higher amounts of fatty acids in the circulation. While the blood samples correspond to a longer time, up to 4 weeks. In the case of individuals taking several short-trips and then a long-trip, the fatty acid values can correspond to the average of the trips that were made. Related to the different fatty acids, a greater amount of SFA and later of MUFA was identified. More SFA was identified in females and more MUFA in males, however the differences in values are not very large. When comparing blood samples for both sexes, it is possible to notice that there is a greater difference in the amount of identified saturated and unsaturated fatty acids, since in these samples PUFA is identified in addition to MUFA. Although PUFA was not identified in the plasma samples, it is also possible to observe this difference between saturated and unsaturated fatty acids. Males seem to find more beneficial food to them and their offspring having more unsaturated fatty acids that are more important for their body condition. However, the omega-6 (C18:2ω6c) and ALA (C18:3ω3) that are essential fatty acids were identified in females, both of them, but in low quantities. As Hulbert & Abbott (2012) mentioned in their work, females have worst body condition if they search for food that have lower essential unsaturated FA, and this will lead that they need to do a higher effort to improve their physical condition and energy providing good nutrition for their offspring. However, neither SFA nor

MUFA are essential in the diet, which are the fatty acids that were mostly identified in our samples, which may indicate that the diet specially of males does not have the most important fatty acids for which they are able to stay in good physical condition (Hulbert & Abbott, 2012).

Some of the species found in the Wandering albatross feed are *Moroteuthopsis longimana* (squid), *Euphausia superba* (krill), *Dissostichus eleginoides* (Patagonian toothfish) and *Antimora rostrata*. In the squid species referred, the main fatty acids identified in the literature are C14:0, C16:0, C18:0, C16:1ω7c, C16:1ω9c, C18:1ω9c, C18:1ω7c, C18:1ω5c, C20:1ω9c, C18:4ω3c, C18:2ω6, EPA and DHA. MUFA is the dominant fatty acid group identified, followed by SFA and PUFA (Wilson, 2004). In the species *Euphausia superba*, abundant in South Georgia, the fatty acids most identified in the literature are C14:0, C15:0, C16:0, C16:1ω7, C16:2ω6, C18:0, C18:1ω9, C18:1ω7, C18:2ω6, C18:3ω3, C18:4ω3, C20:1ω9, C20:4ω6, C20:5ω3 and C22:6ω3 (Cripps *et al.*, 1999). In the species *Dissostichus eleginoides*, the fatty acids are C14:0, C16:0, C16:1ω7, C18:0, C18:1ω9/C18:1ω11, C18:1ω7, C18:2ω6, C18:4ω3, C20:1ω9, C20:5ω3 and C22:6ω3. Since MUFA are the most identified with more than 50%, followed by SFA and lastly PUFA (Brown *et al.*, 1999; Moon *et al.*, 2011). In the latter species, *Antimora rostrata*, the fatty acids referred to are C14:0, C16:0, C18:0, C16:1ω7, C18:1ω9, C18:1ω7, C20:1ω9, C16:2ω4, C18:2ω6, C20:5ω3, C22:5ω3 and C22:6ω3. In this species, contrary to what was previously mentioned, it presents a higher percentage of PUFA, after SFA and MUFA (Stowasser *et al.*, 2012).

As in our study, Raclot *et al.* (1998) identified in king penguins more MUFA (47 to 55%) than PUFA, however in this study only 21-24% corresponded to SFA, that is the least identified, unlike what happened in our study in which more than 50% corresponded to these fatty acids. In the study with king penguins the fatty acids identified were C18:1ω9, C16:0, C22:6ω3, C22:1ω11, C20:1ω9, C16:1ω7 and C20:5ω3. As previously mentioned, a large percentage of their food were rich in MUFA, that is typical of myctophid fishes rich in oleic acid (C18:1ω9), palmitic acid (C16:0) and docosahexaenoic acid (C22:6ω3) (Raclot *et al.*, 1998).

Danckwerts *et al.* (2016) conducted a study with the species *Pterodroma baraui*, Barau's Petrel, where identified fatty acids during chick rearing. In this species, the most identified fatty acids were C16:0, C18:0, C18:1ω9, C16:1ω7, C18:1ω7, C18:2ω6, C18:3ω3, C18:4ω3, C20:1ω9, C20:4ω6, C20:5ω3, C20:5ω6, C24:0, C22:6ω3 and C24:1ω9. In this study, the percentages of MUFA, PUFA and SFA identified were quite close, with no major difference between them.

The fatty acids identified in this study and which are similar to the information obtained from other studies carried out with procellariforms such as albatrosses or other sea birds were cis-9-octadecenoic acid (C18:1ω9c), hexadecanoic acid (C16:0), tetradecanoic acid (C14:0), octadecanoic acid (C18:0), 9-hexadecenoic acid (C16:1ω7) and 11-eicosanoic acid (C20:1ω9) (Connan *et al.*, 2005; Connan *et al.*, 2007; Wang *et al.*, 2009; Connan *et al.*, 2014; Richoux *et al.*, 2010). The most identified fatty acids are possible to be found in greater quantities in the species referred above as *Moroteuththopsis longimana*, *Euphausia superba*, *Dissostichus eleginoides* and *Antimora rostrata*. The results obtained seem to be present in the diet of individuals myctophid fishes since it was possible to identify oleic acid (C18:1ω9) and palmitic acid (C16:0) (Raclot *et al.*, 1998).

Essential fatty acids (EFA) such as EPA and DHA were not identified in any of the samples. They are important for thermoregulation, basal metabolic rate and exercise performance (Hulbert & Abbott, 2012). It was only identified the essential fatty acid ALA. This may indicate that the individuals may have a worst body condition because of the greater effort that may do to find food for them and for their chicks. Chicks that are better fed and have all the nutrients that they need, will be able to grow and survive when they have to become independent and look for food on their own. The important thing in food is not to find large amounts of food, as they may not have what is essential for good body condition, but rather to find richer food even if it is in less quantity (del Rio & McWilliams, 2016).

In plasma samples, over the months, the males showed a greater amount of fatty acids with the exception of May, however the differences were not very large. In the case of males, they showed a peak of fatty acids during October and the lowest value in September. From May to July and from September to October, males showed an increase in the amount of fatty acids. In females, the peak was identified in September and the lowest value in July. The increase in fatty acids was identified from July to September, and from May to July there was a decrease. Although females showed their peak fatty acids during September, the contribution of both sexes was 50%-50%. The fact that males in some months have a higher percentage of fatty acids compared to females seems to indicate that they, as they do not have a greater expenditure of energy to form the egg as females, were in better physical shape, despite both sexes alternating in search of food while caring the egg in the nest. The greater amount of fatty acids was present in September and October for females and males, respectively, due to the fact that they start long-trips managing to seek more food mainly for themselves and not so much for the young.

In blood samples, the differences between the months was similar to the plasma samples with the exception of October, where a greater difference was observed between the sexes. As shown in the results, there are no significant differences in this variable, as the percentages of fatty acids over the months for males and females are close, only in a few months can one of the sexes have more fatty acids. In blood samples, males seem to benefit more from long-trips since the higher difference in relation to females occurs between September and October.

Ceia *et al.* (2015), analyzed the data of these same individuals under study and reported the duration of the trips, and in May it was when the individuals made the shortest trips (3.2 days), in June and July the duration of the trips increased (5.4 and 5.7 days respectively) and in August decreased again (4.8 days). In September was taken the longest trip (10.7 days), decreasing in October, but continuing to be a long-trip (6.5 days). This information is in line with our results, since from May to August both males and females could not get too far from the colony because they had the offspring to care and feed by doing short-trips, looking for food in the same waters, which usually presents low prey availability. These trips have a higher energy cost with landings and take-offs for males who are unable to take advantage of weaker winds. The females in this aspect have an advantage because they are smaller and take advantage of these winds. In September and October, as the offspring is becoming more independent, parents can take longer trips to improve their body condition again. This species, after making several short-trips in order to find food for the young, then performs a long-trip for self-maintenance. In our results we can observe that both sexes benefit from longer trips, since 50% of the total of fatty acids have been identified in males and the remaining 50% in females. In these trips, males have an advantage in flights because they travel longer distances and have a lower energy expenditure due to their large wings, while females have an advantage because they can find a greater diversity of food.

4.2. POLYSACCHARIDE ANALYSIS

In our study it was observed that there are differences between tissues. In plasma, a greater amount of carbohydrates was identified, mainly in males. This difference was expected, as previously mentioned, the tissues represent a different period of time. When individuals performed a short-trip, the plasma samples will indicate the carbohydrates that were part of the food that was captured during this trip because of the turnover time of plasma. While blood samples, as the turnover is up to 4 weeks, indicate all the carbohydrates in the diet that individuals had in more than one short-trip, for example.

In plasma, the monosaccharides present in greater quantity were glucose and xylose for both sexes. In blood samples, there is a greater diversity of sugars, since Rha and Drib were identified in these samples and were not identified in plasma samples. In blood, the highest percentage of monosaccharides were glucose and arabinose in males and glucose and galactose in females. Glucose is the most abundant monosaccharide in the oceans, found in the metabolism of several different organisms, from phytoplankton and suspended particles, but galactose is also quite common to be identified (Kirchman *et al.*, 2001). In algae, they present a cell wall made with cellulose and they are constituted of xylose, galactose and glucose (Raposo & Morais, 2014). These are derived from glucose and have structural functions. In animals is stored as glycogen, that stores energy in adipose tissue. In cephalopods, they are low in lipid and carbohydrates specially glycogen (Villanueva *et al.*, 2014). In the case of some seabirds such as Wandering albatross, on shorter trips they use this energy to survive while caring for their young. During these trips, individuals search for food for themselves but also for the offspring, not being able to move far from the colony in the first months as soon as the chick is born, and in this way the parents are unable to increase their reserves because the food they capture is not only for them and still have energy costs. Ribose also identified in this study is an important monosaccharide in coenzymes like ATP (Jabbari, 2020).

Kirchman *et al.* (2001) identified the different carbohydrates found in Ross sea, that are glucose, mannose and xylose, galactose, arabinose, rhamnose and fucose, in decreasing order of importance. But in the polar front, the order is different, the most abundant was glucose, galactose, mannose and xylose, arabinose, fucose and for last rhamnose. In spring the phytoplankton bloom can influence the sugars that are identified and their concentrations that will go through the food chain (Kirchman *et al.*, 2001). However, this only lasts until the beginning of summer and then it is degraded until in winter when there is less sunlight and there is no stratification of the water column, there is a limitation in the formation of phytoplankton.

According to the results, in plasma males and females present values close to the different carbohydrates. However, in blood samples it is possible to observe more differences between the sexes. In this tissue, males have a higher amount of carbohydrates, which seems to indicate that they have higher energy reserves. As the females have to form the egg and at the same time take care of the chick together with the males, they have a great expenditure of energy and spend some of the reserves they have.

When comparing the plasma and blood samples, it is possible to observe that in blood most carbohydrates have lower amounts than those identified in the plasma. These carbohydrates may not remain in the blood for a long time and therefore are found in smaller quantities.

In the plasma samples, the females showed a peak in July (during summer), and in October (during autumn) it was when it presented the lowest value. In the case of males, they show a peak in October (in autumn), and the minimum in July (in summer). Only in October is it possible to observe a greater difference between the sexes. In blood samples, males showed a peak in October and females in July. In blood samples, were observed more differences between genders. In both types of tissues, males showed a greater amount of carbohydrates in the most months, however this difference was not very significant, especially in plasma samples. Females in the first few months (spring/summer) after the offspring is born seem to have managed to increase their energy reserves over the months while making short trips near the coast. During these trips the females did not have as much energy expenditure and were able to feed the young more. The low quantities of carbohydrates in May and June may be due to the high level of parental investment required to feed the chicks within the first months post-hatching. However, as soon as they started making long-trips for self-maintenance, the amount of carbohydrates decreased, which may be due to the duration of trips that for females may have a higher energy cost and the food they managed to capture that may not be so rich in sugars. In the case of males, it seems to compensate for longer trips, as the amount of carbohydrates has increased.

Although both sexes were able to replace some reserves that were lost as soon as the chick was born, in these trips the males have a greater advantage due to their larger size. During September and October (autumn) it is possible to observe a greater difference between males and females, with males having a greater amount of carbohydrates, a greater difference than that observed in previous months. This may suggest that they maintain greater energy reserves during the breeding season than females. In this species, both parents invest in the care of the young, while one goes to search for food the other remains in the nest to take care of the young which cannot yet be alone. However, females end up with a lower amount of carbohydrates because they have a higher energy expenditure since they are the ones that have to form the egg. In the last months, the offspring is more developed and can stay longer times alone in the nest. In this way, parents are able to eat more and increase their energy reserves again.

The fact that the austral winter begins and decreases primary production also leads to a decrease in food availability and in the first months after the offspring is born, individuals have to stay close to the coast. As soon as they start making longer trips, they can look for food in more productive waters.

There are few studies on sugars at least to my knowledge, especially in top predators such as sea birds, in this case the albatross. Thus, it is difficult to make a comparison with other studies already carried out and with results obtained according to the feeding of these animals.

4.3. FINAL CONSIDERATIONS AND FUTURE PERSPECTIVES

The present study was conducted by several fundamental objectives that allowed us to reach the conclusion of our results. The first, and perhaps the most central, was to compare the fatty acid and sugar profiles of the plasma and blood samples of both sexes of the analyzed species. Next, it was also important to verify and analyze the different diets and parental care between males and females and whether they underwent changes over the months of study (May to October). Another condition that seemed to us fundamental to evaluate was to understand if there were variations in the biochemical profiles and in the body condition of the adults as the chick grows and what impacts could occur. Our results were in line with what has been described in other works with Wandering albatrosses. Although some differences were observed between males and females, these were not significant, contributing both parents to the growth and welfare of the young. The only differences that were observed in the present study were between tissue types, in which the amounts of both fatty acids and carbohydrates in the plasma were quite different from those identified in the blood samples. I am not aware of any study with Wandering albatrosses in which this comparison is made between these two types of tissues. However, it is possible to make a comparison of our results with other studies in other organisms. The expected difference from the beginning was mainly between the types of tissues because as they have different turnover life time, we would obtain different results according to the type of food throughout the trips and also depending on the duration of the trips.

In the case of Wandering albatross, both parents take care of the egg in the nest alternately and as soon as the hatchling is born, the two continue to search for food and take care of their chick. In this way and as confirmed by our results, there does not seem to be an individual who contributes more than the other does. What seems to happen, according to the results obtained, is that as males have larger dimensions than those of females and since females were the ones who had the energy expenditure when forming the egg, the males seem to be in better physical conditions and have more energy reserves. Ferrer *et al.* (2017) mentioned in his study with black-browed albatross, that the differences between sexes are usually not seen mainly in plasma samples.

During long-trips both parents can store body reserves to use during the short-trips, until the mass decreases again (Weimerskirch, 1999). This body fat consists of lipids such as fatty acids and polysaccharides such as glucose, among others, which are obtained through food (Baião & Lara, 2005). In this study is possible to observe this pattern, since the lipids and sugars increase with the longer trips.

The reproductive success of a species depends on several factors, like being the resources that they can find while searching for food. If they can find food for themselves and to the chick, not losing too much energy reserves and managing to improve or just maintain their body condition. This happens when parents are in a poor condition (Ferrer *et al.*, 2013).

According to Weimerskirch (1999) the highest values of fatty acids or carbohydrates are obtained before incubating the egg, this is because parents need to prepare themselves physically and have the necessary reserves for the constraints in the next months, and the lowest values are observed at the time when who have to take care of the chick and feed it. During this time, the reserves decrease as well as the body mass. In smaller species it has already been mentioned in other works that this decrease in mass allows these species to transport more food without presenting a higher energy cost, but in the case of larger species such as Wandering albatrosses they have an extensive safety margin, and they can carry heavier food due to higher body reserves.

Over the months, there were no major differences in diet for both sexes. The values obtained were quite close in the plasma samples. It was only observed that the females presented a greater variation of fatty acids in relation to the males. Both sexes seem to have been searching for food in the same waters and for the same type of food. Only in the blood samples, more differences were observed in some of the months, during the autumn season. Since blood has a turnover lifetime higher than that of plasma, it may indicate that throughout all short-trips or during the long-trip, individuals showed greater variation in the amount of food they captured or that was richer in fatty acids and carbohydrates.

The last point we wanted to evaluate in this study the variation in the condition of both parents while they had to care for the young. What was observed was the females had less reserves in relation to the males as soon as the chick was born, but later managed to improve. In the case of males, they only seem to have benefited from longer trips by managing to have less energy expenditure and managing to recover their physical shape and energy reserves.

In other studies in which they analyzed the same individuals, they said that during this year in which the samples were collected, the oceanographic conditions were different compared to the other years, which may have caused constraints for reproduction and in the search for food, which may be more scarce or there is no food that was rich in nutrients. With this indication, and analyzing our data, it seems that in adverse conditions, both males and females strive for their only offspring to have healthy growth and the food they need. The difference between the sexes does not seem to be much in most months, and even with the stress of reproduction and all the constraints, the females not seem to be in worse condition than males. However, males as they are bigger and seem to have a little more reserves will be able to contribute more in the care of the young.

It would be interesting in the future to analyze the same data in more favorable conditions, to see if this behavior continues or if there are more differences between sexes. Also, with greater availability of food, it may give rise to a different response from parents.

In our study it was not possible to identify essential fatty acids, these are present mainly in fish as EPA and DHA. The fact that these fatty acids are not identified may indicate that the type of food that the individuals found would not be so nutritious. Another constraint we had in our study was the fact that the blood samples had clotted and therefore was added 70% of ethanol to dilute the samples. Future studies may evaluate the impact of these dilution on the accuracy of the experimental results.

Since a large part of the individuals' diet was fish and cephalopods, especially when they were close to the colony, and that the females often seek food from fishing vessels in this region, it is important to continue the conservation studies and carry out sampling of the food of these individuals and their interactions with fishing boats.

Other aspect that have been studied is the influence of changes in the winds due to climate changes in Wandering albatrosses. It was already observed that westerlies winds have shifted poleward and increase their intensity, especially in Subantarctic waters (Weimerskirch *et al.*, 2012). The males and females Wandering albatrosses try to search for food near the colony during chick rearing and later they can spend more time searching for food when the chick is more independent. That trips are important to gain more body mass because it is when individuals search for food to restore the body mass that lost. As it is described in the literature, males prefer colder waters more to the south, whereas females search for food more to the North in warmer waters (Ceia *et al.*, 2012; Pereira *et al.*, 2018). With the shift in the winds, the travel distances for both sexes do not experience any difference but it last shorter times since the intensity of the winds increased (Weimerskirch *et al.*, 2012; Cornioley *et al.*, 2016). This is an advantage since they can return to the chick with more energy since the parents can fly faster, have less energy expenditure and they can do more trips to search for food. This parameter is important for Wandering albatrosses since they need the wind to take-off and to help during the flight. The fact that climate changes increased the intensity of the winds it seems to benefit these species, so they can have a higher success with the only chick they rear. In terms of conservation, if these changes in the winds makes that females range moves more to south, it is easier to avoid the encounter with tuna longlines and decreased their mortality. Also, females have less body condition so with stronger winds they can travel quicker and saving more energy what it is important since they seem to be in worst condition than males. Wandering albatrosses seems to adapt to the changes that have been occurred but it is necessary to continue to analyze which effects this change will bring in long-term. Continuing to increase the intensity of the winds more towards the poles, this species, as well as other seabirds, could be directed to regions with less nutritious food. In the paper of Pereira *et al.* (2018), where the same data were analyzed, it is possible to observe that both males and females search for food in regions with more intense winds, more to the north of South Georgia, while in the south the winds were less intense and there was less primary production. As already mentioned, in our study both males and females seem to have managed to improve their body condition after the chick is born. However, if this species begins to move further south, it may no longer find so much food. The regions with the highest productivity are in the vicinity of the Subtropical front and Sub-Antarctic front.

5. REFERENCES

Arrigo KR & Thomas DN (2004). Large scale importance of sea ice biology in the Southern Ocean. *Antarctic Science*, *16*(4), 471-486.

Baião NC & Lara LJC (2005). Oil and fat in broiler nutrition. *Brazilian Journal of Poultry Science, 7*(3), 129-141.

Beardsley C, Moss S, Malfatti F & Azam F (2011). Quantitative role of shrimp fecal bacteria in organic matter fluxes in a recirculating shrimp aquaculture system. FEMS microbiology ecology, 77(1), 134-145.

Bergé JP & Barnathan G (2005). Fatty acids from lipids of marine organisms: molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects. In *Marine biotechnology I* (pp. 49-125). Springer, Berlin, Heidelberg.

Bergstrom DM & Chown SL (1999). Life at the front: history, ecology and change on Southern Ocean islands. *Trends in Ecology & Evolution*, *14*(12), 472-477.

Biddanda B & Benner R (1997). Carbon, nitrogen, and carbohydrate fluxes during the production of particulate and dissolved organic matter by marine phytoplankton. *Limnology and Oceanography*, *42*(3), 506-518.

Biggs CI, Bailey TL, Stubbs C, Fayter A & Gibson MI (2017). Polymer mimics of biomacromolecular antifreezes. *Nature communications*, *8*(1), 1546.

Brown DJ, Boyd IL, Cripps GC & Butler PJ (1999). Fatty acid signature analysis from the milk of Antarctic fur seals and Southern elephant seals from South Georgia: implications for diet determination. *Marine Ecology Progress Series, 187*, 251-263.

Ceia FR, Phillips RA, Ramos JA, Cherel Y, Vieira RP, Richard P & Xavier JC (2012). Short-and long-term consistency in the foraging niche of wandering albatrosses. *Marine biology*, *159*(7), 1581-1591.

Ceia FR, Ramos JA, Phillips RA, Cherel Y, Jones DC, Vieira RP & Xavier JC (2015). Analysis of stable isotope ratios in blood of tracked wandering albatrosses fails to distinguish a δ13C gradient within their winter foraging areas in the southwest Atlantic Ocean. *Rapid Communications in Mass Spectrometry*, *29*(24), 2328-2336.

Chang ES & O'Connor JD (1983). Metabolism and transport of carbohydrates and lipids. The biology of Crustacea, 5, 263-87.

Cherel Y, Robin JP & Maho YL (1988). Physiology and biochemistry of long-term fasting in birds. *Canadian Journal of Zoology*, *66*(1), 159-166.

Coimbra MA, Delgadillo I, Waldron KW & Selvendran RR (1996). Isolation and analysis of cell wall polymers from olive pulp. In *Plant cell wall analysis* (pp. 19-44). Springer, Berlin, Heidelberg.

Connan M, Mayzaud P, Boutoute M, Weimerskirch H & Cherel Y (2005). Lipid composition of stomach oil in a procellariiform seabird *Puffinus tenuirostris*: implications for food web studies. *Marine Ecology Progress Series*, 290, 277-290.

Connan M, Cherel Y & Mayzaud P (2007). Lipids from stomach oil of procellariiform seabirds document the importance of myctophid fish in the Southern Ocean. *Limnology and Oceanography*, *52*(6), 2445-2455.

Connan M, Mayzaud P, Duhamel G, Bonnevie BT & Cherel Y (2010). Fatty acid signature analysis documents the diet of five myctophid fish from the Southern Ocean. *Marine biology*, *157*(10), 2303-2316.

Connan M, McQuaid CD, Bonnevie BT, Smale MJ & Cherel Y (2014). Combined stomach content, lipid and stable isotope analyses reveal spatial and trophic partitioning among three sympatric albatrosses from the Southern Ocean. *Marine Ecology Progress Series*, 497, 259-272.

Cornioley T, Börger L, Ozgul A & Weimerskirch H (2016). Impact of changing wind conditions on foraging and incubation success in male and female wandering albatrosses. *Journal of Animal Ecology*, *85*(5), 1318-1327.

Cossa D, Heimbürger L-E, Lannuzel D, Rintoul SR, Butler EC, Bowie AR, Averty B, Watson RJ & Remenyi T (2011) Mercury in the Southern Ocean. Geochimica et Cosmochimica Acta 75:4037-4052

Cotonnec G, Brunet C, Sautour B & Thoumelin G (2001). Nutritive value and selection of food particles by copepods during a spring bloom of *Phaeocystis sp.* in the English Channel, as determined by pigment and fatty acid analyses. *Journal of Plankton Research*, *23*(7), 693-703.

Craig S, Helfrich LA, Kuhn D & Schwarz MH (2017). Understanding fish nutrition, feeds, and feeding.

Cripps GC, Watkins JL, Hill HJ & Atkinson A (1999). Fatty acid content of Antarctic krill *Euphausia superba* at South Georgia related to regional populations and variations in diet. *Marine Ecology Progress Series*, *181*, 177-188.

Cripps GC & Atkinson A (2000). Fatty acid composition as an indicator of carnivory in Antarctic krill, *Euphausia superba*. *Canadian Journal of Fisheries and Aquatic Sciences*, *57*(S3), 31-37.

Croxall JP, North AW & Prince PA (1988). Fish prey of the wandering albatross *Diomedea exulans* at South Georgia. *Polar Biology*, *9*(1), 9-16.

Croxall JP, Rothery P, Pickering SPC & Prince PA (1990). Reproductive performance, recruitment and survival of wandering albatrosses *Diomedea exulans* at Bird Island, South Georgia. *The Journal of Animal Ecology*, 775-796.

Cuzon G, Rosas C, Gaxiola G, Taboada G & Van Wormhoudt A (2000). Utilization of carbohydrates by shrimp. *Avances en nutrición acuícola V. Memorias del V simposium internacional de nutrición acuícola*, 19-22.

del Rio CM & McWilliams SR (2016). How essential fats affect bird performance and link aquatic ecosystems and terrestrial consumers. *Proceedings of the National Academy of Sciencies*, *113*(43), 11988-11990.

Danckwerts DK, McQuaid CD, Connan M, Smale MJ, Le Corre M, Humeau L, Kaehler S, Juhasz CC, Orlowski S, Tourmetz J & Jaquemet S (2016). Intra-annual variation in the foraging ecology of the endangered endemic Barau's Petrel (*Pterodroma baraui*) from Réunion Island, south-western Indian Ocean: insights from a multifaceted approach. *Marine biology*, *163*(1), 18.

El-Sheekh MM, Khairy HM & El-Shenody R (2012). Algal production of extra and intra-cellular polysaccharides as an adaptive response to the toxin crude extract of *Microcystis aeruginosa*. *Iranian journal of environmental health science & engineering*, *9*(1), 10.

Engel A, Thoms S, Riebesell U, Rochelle-Newall E & Zondervan I (2004). Polysaccharide aggregation as a potential sink of marine dissolved organic carbon. *Nature, 428*(6986), 929.

Ericson JA, Hellessey N, Nichols PD, Kawaguchi S, Nicol S, Hoem N & Virtue P (2018). Seasonal and interannual variations in the fatty acid composition of adult *Euphausia superba* Dana, 1850 (Euphausiacea) samples derived from the Scotia Sea krill fishery. *Journal of Crustacean Biology*, *38*(6), 662-672.

Fauchald P (2009). Spatial interaction between seabirds and prey: review and synthesis. *Marine Ecology Progress Series, 391*, 139-151.

Ferrer M, Belliure J, Vinuela J & Martin B (2013). Parental physiological condition and reproductive success in chinstrap penguins (*Pygoscelis antarctica*). *Polar biology, 36*(4), 529-535.

Ferrer M, Morandini V, Perry L & Bechard M (2017). Factors affecting plasma chemistry values of the black-browed albatross *Thalassarche melanophrys*. *Polar Biology, 40*(8), 1537-1544.

Filimonova V, Gonçalves F, Marques JC, De Troch M & Goncalves AM (2016a). Fatty acid profiling as bioindicator of chemical stress in marine organisms: a review. *Ecological indicators*, *67*, 657-672.

Filimonova V, Goncalves F, Marques JC, De Troch M & Goncalves AMM (2016b). Biochemical and toxicological effects of organic (herbicide Primextra® Gold TZ) and inorganic (copper) compounds on zooplankton and phytoplankton species. *Aquatic Toxicology*, *177*, 33-43.

Filimonova V, De Troch M, Gonçalves F, Marques JC, Marques SM, Gonçalves AM & De Laender F (2018a). Effects of a herbicide and copper mixture on the quality of marine plankton. *Ecotoxicology and environmental safety*, *156*, 9-17.

Filimonova V, Nys C, De Schamphelaere KA, Gonçalves F, Marques JC, Gonçalves AM & De Troch M (2018b). Ecotoxicological and biochemical mixture effects of an herbicide and a metal at the marine primary producer diatom *Thalassiosira weissflogii* and the primary consumer copepod *Acartia tonsa*. *Environmental Science and Pollution Research*, 1-16.

Geng JJ, Li H, Liu JP, Yang Y, Jin ZL, Zhang YN, Zhang ML, Chen LQ & Du ZY (2015). Nutrients and contaminants in tissues of five fish species obtained from Shanghai markets: risk–benefit evaluation from human health perspectives. *Science of the Total Environment*, *536*, 933-945.

Gonçalves AMM, Pardal MA, Marques SC, Mendes S, Fernández-Gómez MJ, Galindo-Villardón MP & Azeiteiro UM (2012a). Diel vertical behavior of Copepoda community (naupliar, copepodites and adults) at the boundary of a temperate estuary and coastal waters. *Estuarine, Coastal and Shelf Science*, *98*, 16-30.

Gonçalves AMM, Azeiteiro UM, Pardal MA & DeTroch M (2012b). Fatty acid profiling reveals seasonal and spatial shifts in zooplankton diet in a temperate estuary. Estuar. Coast. Mar. Sci. 109, 70–80.

Grémillet D, Prudor A, Le Maho Y & Weimerskirch H (2012). Vultures of the seas: hyperacidic stomachs in wandering albatrosses as an adaptation to dispersed food resources, including fishery wastes. *PloS one*, *7*(6), e37834.

Griffiths HJ (2010). Antarctic marine biodiversity–what do we know about the distribution of life in the Southern Ocean?. *PloS one*, *5*(8), e11683.

Gutiérrez IB, Mesquita AFC, Nunes C, Coimbra MA, Gonçalves FJM, Marques JC & Gonçalves AMM (2019). Impacts of S-metolachlor and terbuthylazine in fatty acid and carbohydrate composition of the benthic clam *Scrobicularia plana*. *Ecotoxicology and Environmental Safety*. Elsevier 173:293-304.

Gutt J, Bertler N, Bracegirdle TJ, Buschmann A, Comiso J, Hosie G, Isla E, Schloss IR, Smith CR, Tournadre J & Xavier JC (2015). The Southern Ocean ecosystem under multiple climate change stresses-an integrated circumpolar assessment. *Global Change Biology*, *21*(4), 1434-1453.

Helbert W (2017). Marine polysaccharide sulfatases. *Frontiers in Marine Science*, *4*, 6.

Hughes KA, Constable A, Frenot Y, López-Martínez J, Mclvor E, Njåstad B, Terauds A, Liggett D, Roldan G, Wilmotte A & Xavier JC (2018). Antarctic environmental protection: Strengthening the links between science and governance. Environmental Science & Policy 83:86-95.

Hulbert AJ & Abbott SK (2012). Nutritional ecology of essential fatty acids: an evolutionary perspective. *Australian Journal of Zoology, 59*(6), 369-379.

Jabbari H (2020). Synthesis of carbohydrate esters and investigation reaction of Phosphoryl chloride from anomeric position of Per-Acetylated Carbohydrates. *Archives of Pharmacy Practice, 1,* 71.

Jiao G, Yu G, Zhang J & Ewart H (2011). Chemical structures and bioactivities of sulfated polysaccharides from marine algae. *Marine drugs*, *9*(2), 196-223.

Kennicutt MC, Chown SL, Cassano JJ, Liggett D, Massom R, Peck LS, Rintoul SR, Storey J, W. V., Vaughn DG, Wilson TJ, Sutherland WJ, Allison I, Ayton J, Badhe R, Baeseman J, Barrett PJ, Bell RE, Bertler N, Bo S, Brandt A, Bromwich D, Cary C, Clark MS, Convey P, Costa ES, Cowan D, DeConto R, Dunbar R, Elfring C, Escutia C, Francis J, Fricker HA, Fukuchi M, Gilbert N, Gutt J, Havermans C, Hik D, Hosie G, Jones C, Kim Y, Le Maho Y, Lee S, Leppe M, Leichenkova G, Li X, Lipenkov V, Lochte K, López-Martínez J, Lüdecke C, Lyons WB, Marenssi S, Miller H, Morozova P, Naish T, Nayak S, Ravindra R, Retamales J, Ricci CA, Rogan-Finnemore M, Ropert-Coudert Y, Samah AA, Sanson L, Scambos T, Schloss I, Shiraishia K, Siegert MA, Simões J, Sparrow MD, Storey B, Wall DH, Walsh JC, Wilson G, Winter J-G, Xavier JC & Yang H (2014) Six priorities for Antarctic Science (and supplementary material). Nature 512:23-25.

Kirchman DL, Meon B, Ducklow HW, Carlson CA, Hansell DA & Steward GF (2001). Glucose fluxes and concentrations of dissolved combined neutral sugars (polysaccharides) in the Ross Sea and Polar Front Zone, Antarctica. *Deep Sea Research Part II: Topical Studies in Oceanography, 48*(19-20), 4179-4197.

Krogdahl Å, Hemre GI & Mommsen TP (2005). Carbohydrates in fish nutrition: digestion and absorption in postlarval stages. *Aquaculture nutrition*, *11*(2), 103-122.

Lahaye M & Ray B (1996). Cell-wall polysaccharides from the marine green alga *Ulva rigida* (Ulvales, Chlorophyta) - NMR analysis of ulvan oligosaccharides. *Carbohydrate research, 283,* 161-173.

Lea, M. A., Nichols, P. D. & Wilson, G. (2002). Fatty acid composition of lipid-rich myctophids and mackerel icefish (*Champsocephalus gunnari*)–Southern Ocean food-web implications. *Polar Biology*, *25*(11), 843-854.

Lecomte VJ, Sorci G, Cornet S, Jaeger A, Faivre B, Arnoux E, Gaillard M, Trouvé C, Besson D, Chastel O & Weimerskirch H (2010). Patterns of aging in the long-lived wandering albatross. *Proceedings of the National Academy of Sciences*, 200911181.

Lewis S, Nussey DH, Wood AG, Croxall JP & Phillips RA (2012). Intrinsic determinants of a population trend in timing of breeding in the wandering albatross. *Oikos*, *121*(12), 2061-2071.

Mackley EK, Phillips RA, Silk JR, Wakefield ED, Afanasyev V, Fox JW & Furness RW (2010). Free as a bird? Activity patterns of albatrosses during the nonbreeding period. *Marine Ecology Progress Series*, *406*, 291-303.

Mayzaud P (1997). Spatial and life-cycle changes in lipid and fatty acid structure of the Antarctic euphausiid *Euphausia superba*. *Antarctic communities: species, structure and survival. Cambridge University Press, Cambridge,* 284-294.

Michael Allaby "turnover time." A Dictionary of Plant Sciences. Retrieved February 19, 2021 from encyclopedia.com: https://www.encyclopedia.com/science/dictionaries-thesauruses-pictures-and-press-releases/turnover-time-1.

Moon SK, Kim IS, Hong SN & Jeong BY (2011). Food components of the muscle and liver of Patagonian toothfish *Dissostichus eleginoides*. *Korean Journal of Fisheries and Aquatic Sciences, 44*(5), 451-455.

Moura GC, Lucena Barbosa JE, Patrício J, Nery JF & Gonçalves AMM (2016). Seasonal and spatial shifts in copepod diets within tropical estuaries measured by fatty acid profiles. *Ecological indicators*, *69*, 284-294.

Mühlenbruch M, Grossart HP, Eigemann F & Voss M (2018). Mini-review: Phytoplankton-derived polysaccharides in the marine environment and their interactions with heterotrophic bacteria. *Environmental microbiology*, *20*(8), 2671-2685.

Neves M, Castro BB, Vidal T, Vieira R, Marques JC, Coutinho JAP, Gonçalves F & Gonçalves AMM (2015). Biochemical and populational responses of an aquatic bioindicator species, *Daphnia longispina*, to a commercial formulation of a herbicide (Primextra® Gold TZ) and its active ingredient (S-metolachlor). Ecological Indicators 53: 220-230.

Niizuma Y, Takahashi A, Sasaki N, Hayama SI, Tokita N & Watanuki Y (2001). Benefits of mass reduction for commuting flight with heavy food load in Leach’s storm-petrel, *Oceanodroma leucorhoa*. *Ecological research*, *16*(2), 197-203.

Parsons TR, Stephens K & Strickland JDH (1961). On the chemical composition of eleven species of marine phytoplankters. *Journal of the Fisheries Board of Canada*, *18*(6), 1001-1016.

Percival E & McDowell RH (1967). Chemistry and enzymology of marine algal polysaccharides. Academic Press: New York, NY, USA, 1967; p. 219.

Pereira JM, Paiva VH & Xavier JC (2017). Using seabirds to map the distribution of elusive pelagic cephalopod species. *Marine Ecology Progress Series*, *567*, 257-262.

Pereira JM, Paiva VH, Phillips RA & Xavier JC (2018) The devil is in the detail: small-scale sexual segregation despite large-scale spatial overlap in the wandering albatross. Marine Biology 165:55.

Phillips KL, Nichols PD & Jackson GD (2002). Lipid and fatty acid composition of the mantle and digestive gland of four Southern Ocean squid species: implications for food-web studies. *Antarctic Science*, *14*(3), 212-220.

Phillips RA, Bearhop S, Mcgill RA & Dawson DA (2009). Stable isotopes reveal individual variation in migration strategies and habitat preferences in a suite of seabirds during the nonbreeding period. *Oecologia*, *160*(4), 795-806.

Phillips RA, McGill RA, Dawson DA & Bearhop S (2011). Sexual segregation in distribution, diet and trophic level of seabirds: insights from stable isotope analysis. *Marine Biology*, *158*(10), 2199-2208.

Poncet S, Robertson G, Phillips RA, Lawton K, Phalan B, Trathan PN & Croxall JP (2006). Status and distribution of wandering, black-browed and grey-headed albatrosses breeding at South Georgia. *Polar Biology*, 29(9), 772-781.

Prince PA, Wood AG, Barton T & Croxall JP (1992). Satellite tracking of wandering albatrosses (*Diomedea exulans*) in the South Atlantic. *Antarctic science*, *4*(1), 31-36.

Raclot T, Groscolas R & Cherel Y (1998). Fatty acid evidence for the importance of myctophid fishes in the diet of King penguins, *Aptenodytes patagonicus*. *Marine Biology, 132*(3), 523-533.

Raposo MFDJ & Morais AM (2014). Bioactivity and applications of polysaccharides from marine microalgae.

Reinhardt SB & Van Vleet ES (1986). Lipid composition of twenty-two species of Antarctic midwater zooplankton and fish. *Marine Biology*, *91*(2), 149-159.

Richoux NB, Jaquemet S, Bonnevie BT, Cherel Y & McQuaid CD (2010). Trophic ecology of grey-headed albatrosses from Marion Island, Southern Ocean: insights from stomach contents and diet tracers. *Marine biology*, *157*(8), 1755-1766.

Rintoul SR (2018). The global influence of localized Dynamics in the Southern Ocean. Nature 558:209-218.

Rintoul SR, Chown SL, DeConto RM, England MH, Fricker HA, Masson-Delmotte V, Naish TR, Siegert MJ & Xavier JC (2018). Choosing the future of Antarctica. *Nature, 558*(7709), 233.

Sarmiento JL, Gruber N, Brzezinski MA & Dunne JP (2004) High-latitude controls of thermocline nutrients and low latitude biological productivity. Nature 427:56-60.

Schmidt-Nielsen K (1997). Animal physiology: adaptation and environment. Cambridge University Press.

Scully RT (1978). The Marine Living Resources of the Southern Ocean. *U. Miami L. Rev.*, *33*, 341.

Selmi S & Boulinier T (2001). Ecological biogeography of Southern Ocean islands: the importance of considering spatial issues. *The American Naturalist*, *158*(4), 426-437.

Stowasser G, Pond DW & Collins MA (2009). Using fatty acid analysis to elucidate the feeding habits of Southern Ocean mesopelagic fish. *Marine Biology*, *156*(11), 2289-2302.

Stowasser G, Pond DW & Collins MA (2012). Fatty acid trophic markers elucidate resource partitioning within the demersal fish community of South Georgia and Shag Rocks (Southern Ocean). *Marine biology*, *159*(10), 2299-2310.

Tickell WLN (1968). The biology of the great albatrosses, *Diomedea exulans* and *Diomedea Epomophora* (pp. 1-56). American Geophysical Union (AGU).

Villanueva R, Sykes AV, Vidal EA, Rosas C, Nabhitabhata J, Fuentes L & Iglesias J (2014). Current status and future challenges in cephalopod culture. In *Cephalopod Culture* (pp. 479-489). Springer, Dordrecht.

Virtue P, Nichols PD, Nicol S & Hosie G (1996). Reproductive trade-off in male Antarctic krill, *Euphausia superba*. *Marine Biology*, *126*(3), 521-527.

Xavier JC, Croxall JP, Trathan PN & Wood AG (2003) Feeding strategies and diets of breeding grey-headed and wandering albatrosses at South Georgia. Marine Biology 143:221-232.

Xavier JC, Trathan PN, Croxall JP, Wood AG, Podestá GP & Rodhouse PG (2004) Foraging ecology and interactions with fisheries of wandering albatrosses at South Georgia. Fisheries Oceanography 13:324-344.

Xavier JC & Croxall JP (2005). Sexual differences in foraging behaviour and diets: a case-study of wandering albatrosses. Sexual segregation in vertebrates: ecology of the two sexes. *Cambridge University Press, Cambridge,* 74-91.

Xavier JC, Geraint GA & Croxall JP (2006). Determining large scale distribution of pelagic cephalopods, fish and crustaceans in the South Atlantic from wandering albatross (*Diomedea exulans*) foraging data. Ecography 29:260-272.

Xavier JC (2009). Can we save the Wandering albatross?. Accessed 10 May 2019. https://cientistapolarjxavier.blogspot.com/search?q=wandering+albatross

Xavier JC & Peck LS (2015). Life Beyond the Ice. In: Liggett D, Storey B, Cook Y, Meduna V (eds). Exploring the last continent. Springer International Publishing.

Xavier JC, Cherel Y, Allcock L, Rosa R, Sabirov RM, Blicher ME & Golikov AV (2018) A review on the biodiversity, distribution and trophic role of cephalopods in the Arctic and Antarctic marine ecosystems under a changing ocean. Marine Biology 165:93

Xu XQ, Beardall J & Hallam ND (1998). Modification of fatty acid composition in halophilic Antarctic microalgae. *Phytochemistry*, *49*(5), 1249-1252.

Wang SW, Iverson SJ, Springer AM & Hatch SA (2009). Spatial and temporal diet segregation in northern fulmars *Fulmarus glacialis* breeding in Alaska: insights from fatty acid signatures. *Marine Ecology Progress Series*, 377, 299-307.

Wang X, Wang L, Zhang H, Liu R & Song L (2015). The carbohydrate metabolism of scallop *Chlamys farreri* in the immune response against acute challenge of Vibrio anguillarum. *Aquaculture international*, *23*(5), 1141-1155.

Weimerskirch H, Cherel Y, Cuenot-chaillet F & Ridoux V (1997) Alternative foraging strategies and resource allocation by male and female wandering albatrosses. Ecology 78:2051-2063.

Weimerskirch H (1999). The role of body condition on breeding and foraging decisions in albatrosses and petrels. In *Proc.Int. Ornithol. Congr.* (Vol. 22, pp. 1178-1189).

Weimerskirch H & Wilson RP (2000). Oceanic respite for wandering albatrosses. *Nature*, *406*(6799), 955.

Weimerskirch H, Åkesson S & Pinaud D (2006). Postnatal dispersal of wandering albatrosses *Diomedea exulans*: implications for the conservation of the species. *Journal of Avian Biology*, *37*(1), 23-28.

Weimerskirch H, Louzao M, De Grissac S & Delord K (2012). Changes in wind pattern alter albatross distribution and life-history traits. *Science 335*(6065), 211-214.

Wilson GA (2004). The lipid composition of Patagonian toothfish from the Macquarie Island region: ecological and dietary implications within a regional food web (Doctoral dissertation, University of Tasmania).

Zou E & Fingerman M (1999). Chitobiase activity in the epidermis and hepatopancreas of the fiddler crab *Uca pugilator* during the molting cycle. *Marine Biology*, *133*(1), 97-101.