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FATTY ACID PROFILE OF ZOOPLANKTON SPECIES OF THE ANTARCTIC SOUTHERN OCEAN

Dissertação no âmbito do Mestrado em Química – ramo de Controle de Qualidade e Ambiente, orientada pela Doutora Ana Marta dos Santos Mendes Gonçalves e pela professora Doutora Maria João Pedrosa Ferreira Moreno Silvestre e apresentada ao Departamento de Química da Faculdade de Ciências e Tecnologia da Universidade de Coimbra.

Dezembro de 2020

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Dissertação apresentada para provas de Mestrado em Química, Área de Especialização em Controle de Qualidade e Ambiente

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"Ora, lege, lege, lege, relege, labora et invenies"

Anónimo

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TABLE OF CONTENTS

INDEX OF FIGURES

INDEX OF TABLES

RESUMO

Os ecossistemas presentes na Antártida e no Oceano Antártico têm vindo a sofrer alterações ao longo dos últimos 30 anos, especialmente com o aumento de temperatura dos oceanos. Estas alterações não ocorrem de um forma homogénea, pelo que existem zonas mais afetadas do que outras, havendo, também por isso, espécies que são mais afetadas por estas alterações. Torna-se assim necessário perceber como as espécies lidam com estas alterações e como estas mudanças poderão alterar os sistemas marinhos globais. A Antártida é o habitat de numerosas espécies endémicas, tornando-se importante o estudo da sua cadeia trófica num contexto de alterações climáticas. Devido aos impactos ambientais, existe já evidência de uma alteração desta cadeia trófica, com o camarão da Antártida (Antarctic krill *Euphausia superba*) a diminuir o seu papel preponderante em algumas regiões, passando a outras espécies de zooplâncton esse papel. Assim, é de extrema importância o estudo na base desta cadeia trófica, de modo a analisar os possíveis efeitos de mudanças ambientais nas espécies que habitam na Antártida e com um papel chave para os níveis tróficos mais elevados. A análise do perfil de ácidos gordos permite verificar se as espécies estão em stress devido a mudanças ambientais, num contexto de ecologia trófica. No presente estudo, as espécies de zooplâncton *Euphausia superba, Euphausia triacantha* e *Themisto gaudichaudii* e o género *Thysanoessa* spp. foram recolhidas entre dezembro de 2016 e janeiro de 2017, em 3 locais com características diferentes: águas Antárticas, Intermédias e Sub-Antárticas. Após análise por GC-MS, foi possível verificar que as espécies *E. triacanta* e *T. gaudichaudii* e o género *Thysanoessa* spp. apresentavam uma melhor condição corporal do que *E. superba*. Além disso, também foi possível verificar que todas estas espécies se encontravam em melhores condições (maior abundância de ácidos gordos na sua composição e maior abundância de ácidos gordos essenciais) em águas com temperaturas mais elevadas (águas sub-Antárticas) do que em águas com temperaturas mais baixas, próximas das encontradas no Oceano Antártico. No entanto, *E. superba* mostrou um perfil de ácidos gordos distinto dos apresentados pelas outras espécies estudadas nesta tese (independentemente da sua localização) e do que é reportado na literatura (ausência de ácidos gordos altamente insaturados presentes na sua constituição e domínio de ácidos gordos saturados). Mais estudos para compreender as alterações que esta espécie vive nesta região são necessários para um melhor entendimento destes resultados.

Palavras-chave: Antártida, Oceano Antártico, zooplankton, ácidos gordos, alterações climáticas.

ABSTRACT

The Antarctica and Southern Ocean ecosystems have been suffering changes through the last 30 years, in particular raising of the ocean temperature. These changes occur in a non-circumpolar distribution, for which there are more affected zones than others and, consequently more species in particular regions. It is necessary to understand how these species deal with the environmental changes that are influencing its habitat and how these changes will alter the global marine ecosystems. As Antarctica is the habitat of numerous endemic species, such studies are urgently needed. Due to climate impacts, it is possible to observe evidence of change in the Antarctic food web, where Antarctic krill (*Euphausia superba*) has decreased its important role in some regions, allowing other zooplankton species to have that role. It is of extreme importance to study the lower trophic levels of this food web, so it is possible to verify the effects of climate change in the species that live in these waters that may help higher trophic levels. Fatty acid analysis allows to assess the response of species to environmental stressors and identify potential food sources, in a trophic ecology context. The zooplankton species of *Euphausia superba, Euphausia triacantha, Themisto gaudichaudii, Thysanoessa* spp were collected between December 2016 and January 2017, in three locations with distinct characteristics: Antarctic waters, Intermediate and Sub-Antarctic waters. After GC-MS analysis, it was possible to verify that the species *E. triacantha, T. gaudichaudii* and *Thysanoessa* spp. presented a better body condition than *E. superba*. Besides, it was also possible to observe that these three species all revealed better conditions in waters with a higher temperature (Sub-Antarctic waters) than in waters with lower temperatures, closer to the ones found in the Southern Ocean. However, *E. superba* exhibited a different fatty acid profile (characterized by the absence of highly unsaturated fatty acids in its constitution and dominance of saturated fatty acids) from what is reported in literature and to what it was found for the remaining species studied in this thesis. Further investigation is needed in order to understand how *E. superba* deals with climate changes for a better understanding of these results.

Keywords: Antarctica, Southern Ocean, zooplankton, fatty acid profile, climate change

1. Introduction

Aquatic environments can be divided into two groups: freshwater and marine ecosystems. Marine ecosystems reveal a high content in salt and represent the largest of the aquatic ecosystems, counting for 97% of the planet's water supply, while also representing over 70% of the surface of the earth's ecosystems (IPCC et al. 2019). Marine environments are among the most important habitats on the planet, ecologically and socio-economically (Cardoso et al. 2008).

Biodiversity in marine ecosystems includes the species richness and abundance present in these ecosystems, such as bacteria, phytoplankton, zooplankton, algae, invertebrates, fishes, seabirds and mammals (Tittensor et al. 2010). Marine ecosystems are biologically and physically diverse, although suffering from some species losses (Borja 2014). Worldwide, hundreds of millions of people are dependent on marine ecosystems for nutritional, economic, cultural and coastal protection benefits (Neeman et al. 2015, Selig et al. 2019). For this reason, the marine ecosystem is also one of the most exploited ecosystems on the planet, providing fish harvests, wild plant and animal resources, and also recreation and tourism activities, transportation, research opportunities, environmental control, pollution control, breeding and nursing habitats, along with many other benefits (Barbier 2017). Due to its ecological, economic and social importance, it is important to define what is at stake with the loss or invasions (which may be caused by habitat destruction, pollution and climate change or fishing) of marine ecosystems (Mar & Ecosystems 2006, Hiscock 2009, Barbier 2017)

In the Southern Ocean, fauna is diverse and cold-adapted, due to the past and present extreme conditions of it, with a range of general adaptations and life-history characteristics, which are due to several physical factors such as isolation, cold, ice and seasonality, and biological factors such as predation (Xavier & Peck 2015, Hawkins et al. 2018).

1.1. The importance of Antarctica and the Southern Ocean

Antarctica is the southernmost continent and is surrounded by the Southern Ocean (Tynan 1998). This continent represents 90% of the Earths' ice and 70% of Earth's freshwater (Kennicutt et al. 2014). Both the continent and the ocean show vast endemic biodiversity (Knox 2006). From a political perspective, the Antarctic Treaty governs the region. The Protocol on Environmental Protection to the Antarctic Treaty describes Antarctica as a "natural reserve, devoted to peace and science" (SAT 2014) and its isolation makes this continent a great laboratory for marine biodiversity and biogeography studies (di Prisco & Verde 2012) on issues relevant to the World, such as

climate change, ocean acidification, sea level rise and thermohaline ocean circulation (Kennicutt et al. 2019).

The Southern Ocean is defined as south of the Antarctic Polar Front and southern extremes of the Indian, Pacific and Atlantic Oceans, extending southwards to the Antarctic continent (Adler et al. 2016) (Figure 1). Within the Southern Ocean, the Antarctic Circumpolar Current (ACC) contains surface temperatures close to the freezing point closer to the continent (Marshall 2012, Maheshwari et al. 2013, Merino et al. 2016), allowing it to control heat, nutrients and properties which influence the world's climate (Rintoul et al. 2001). Indeed, the ACC allows exchange of the properties mentioned above between Atlantic, Pacific and Indian Oceans (Orsi et al. 1995, Adler et al. 2016, Rintoul et al. 2018). and corresponds to one of the most productive marine areas in the planet (Grant et al. 2006).

The Southern Ocean is characterized with a high nutrient and low chlorophyll system (Boyd et al. 2012) and contains several physical properties that vary seasonally. For instance, during winter, there are reductions in irradiance and water temperature and increases in mixed layer depth, nutrients and sea-ice extent (Constable et al. 2014). The Southern Ocean can also be divided into three different zones that will influence zooplankton distribution (Knox 2006): northern zone characterized by the ACC and low biomass and primary production; intermediate zone characterized by seasonal ice and for the most primary productivity in all the Southern Ocean; and, finally, southern zone characterized by permanent sea ice, with zooplankton and biomass abundance reduced (Knox 2006).

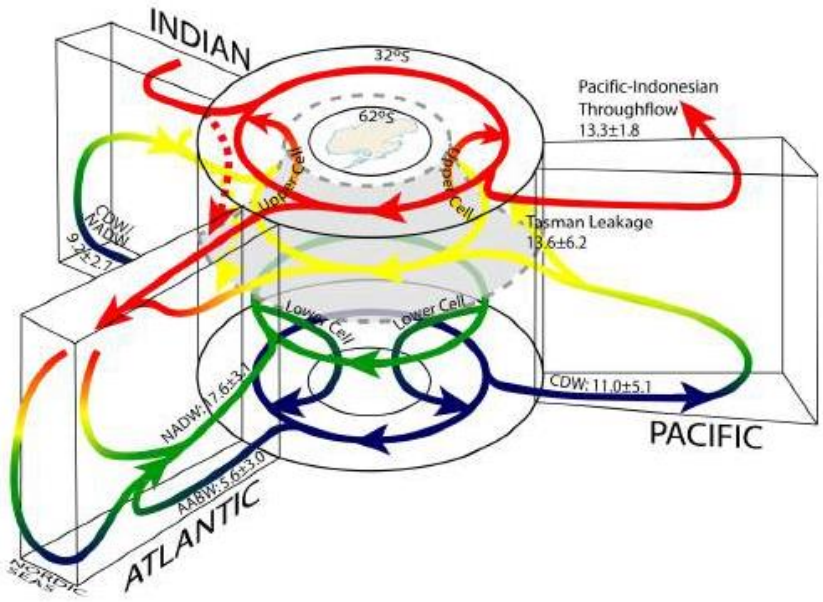

Figure 1 - Model of the global ocean circulation, emphasising the central role played by the Southern Ocean (Turner et al. 2009).

The upwelling of deep waters, demonstrated in figure 1, brings to the surface many nutrients and carbon dioxide, which allows the Southern Ocean to be the most biologically productive ocean (Turner et al. 2009).

Antarctic and Southern Ocean ecosystems have changed over the last 30 years (Turner et al. 2009), where the most obvious changes are rising of water temperatures, approximation of ocean currents to the poles and changes in extension and seasonality of sea-ice. This will have implications in marine ecosystems (Constable et al. 2014). In latitudes closer to the South pole (greater than 40ºS), the concentration of CO_2 in the ocean has increased at a faster rate than the one observed in the atmosphere. This fact allied with superficial waters mixed with bottom waters richer in CO_2 leads to the saturation of the reservoirs of carbon, limiting the ability of absorption of atmospheric CO_2 and lowers pH in the Southern Ocean (Turner et al. 2009). This increase in CO_2 taken in the Southern Ocean is prejudicial to species sensitive to pH alterations, such as species that depend on calcium carbonate for exoskeleton (Bednaršek et al. 2012, 2020, Constable et al. 2014). The acidification of the ocean will raise calcification energetic costs, change nutrient availability and affect cell physiology. However, it is difficult to predict how microorganisms will react to the acidification of the ocean waters due to other environmental stress factors interfering and indirect feedbacks within the food chain (Constable et al. 2014).

Since there are oceanic connections that link Southern Ocean to other oceans, leading to climate variations in the Southern Ocean and Antarctica, these variations will

change the polar atmosphere, ocean, ice sheet, sea ice and biosphere which will also influence the rest of the world climate system. Likewise Southern Ocean is influenced by the global system, Antarctica and the Southern Ocean will also influence the sea level, climate and marine ecosystems globally. Change in this region will have consequences for the planet and mankind, such as affecting the global overturning circulation (affecting the energy budget of the planet), the amount of CO_2 in the atmosphere and the availability of nutrients to support marine life (Rintoul et al. 2018). The Southern Ocean ventilates global oceans and accumulates heat, fresh water, oxygen and carbon dioxide from the atmosphere (Turner et al. 2009)

It is also evident that in the last 50 years, the Antarctic Oscillation[1] became more positive, leading to increasingly strong westerly winds around Antarctica (15 to 20% increase) (Adler et al. 2016). With stronger winds, a greater shift to the south pole is expected, impacting temperature and sea ice in western coastal zones of Antarctica and a reduction in size of the Southern Ocean. The Antarctic Oscillation was altered due to the greater amount of greenhouse gases and the development of the Ozone depletion, the latter with greater importance to these alterations (Turner et al. 2009). In the last 50 years, the marine ecosystem was affected by climate changes, specifically Western Peninsula, with a decrease in sea-ice extent (Turner et al. 2009).

Temperature has been rising over the Antarctic Peninsula since the 1950's (Turner et al. 2009, Xavier & Peck 2015, Adler et al. 2016), with a greater increase in western Peninsula (Turner et al. 2009, Xavier & Peck 2015). Although it is difficult to determine if increased temperature will have an impact in microbial taxa, it is possible it will have a negative impact in *E. superba* around South Georgia, once temperature has risen in this region before. For myctophid and icefish species, it could result in extinction of island shelves isolated populations, while toothfish may be more resilient to change, once they are able to descend to more favourable conditions. In this region, also the populations of Adélie penguins (*Pygoscelis adeliae*) have been significantly declining (Constable et al. 2014).

With climate change, changes in marine environments will influence marine and terrestrial habitats and make organisms vulnerable to these changes at all biological levels, pressuring scientists to investigate biodiversity at high latitudes and understand the past and current changes and its influence in the future for species living in these

[1] A circumpolar pattern of atmospheric mass displacement in which intensity and location of the gradient of air pressure between mid-latitudes and the Antarctic coast changes in a non-periodic way over a wide range of time scales.

areas (di Prisco & Verde 2012). The dynamics of ecosystem are dominated by seasonal growth, extent and retreat of sea ice and their variations (Ducklow et al. 2007).

Antarctic species show low rates of growing and high levels of endemism, which may lead to an establishment of invader species, due to warming of the oceans and expansion of touristic and scientific activities. However, due to the extreme conditions of the Southern Ocean, these invaders may stay confined in a particular area (Turner et al. 2009).

Within the resources that are exploited in the Antarctic today, the Antarctic krill *Euphausia superba* fishery is one of the few fisheries that is over-exploited, which translates to a possible extension in the next few years, with new products using *E. superba* and other crustaceans surging in the markets. To monitor *E. superba*'s fisheries, CCAMLR (Comission for the Conservation of Antarctic Marine Living Resources) created CEMP (Ecosystem Monitoring Programme), which monitors *E. superba*'s fishery and its effects in predators (Kock et al. 2007). This species, as well as most Antarctic marine species, is characterized by high levels of endemism (Turner et al. 2009), slow growth, longevity and late maturity (di Prisco & Verde 2012), with trophic chains characterized by few trophic levels and dependence on a small number of key-species, as it is common in polar trophic chains (Corsolini & Sarà 2017). Variations in the biomass, distribution and species composition of microalgae are driven by hydrodynamic processes because it affects both availability of nutrients and light, also influencing the distribution of microalgae (Dalsgaard et al. 2003). These variations lead to changes in the basic fatty acid pattern (Dalsgaard et al. 2003). Zooplankton, including *E. superba*, is important to ecotoxicological studies due to their position in the trophic chain (Filimonova et al. 2016), which provides a link between primary producers and predators in the Southern Ocean (Ducklow et al. 2007).

It is of extreme importance to understand *E. superba*'s ecology to better understand the ecosystem dynamics and advise fisheries (Everson 2000). Furthermore to improve the responsiveness of the ecosystem-based management approach adopted by the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR), critical knowledge gaps need to be filled (Flores et al. 2012) under a climate change context.

1.2. The role of Antarctic Zooplankton within the Southern Ocean food web

Antarctic zooplankton can be divided into 4 groups: cold-water species, warm-water species, neritic species and widespread species, with its study stimulated by *E. superba* exploitation in the mid-1960s (Knox 2006). Antarctic Zooplankton can be characterized by rarity of invertebrates larval forms in depth, superficial layers which tend to be poor in

species but rich in individuals, with the number of species increasing in depth, annual vertical migration shown by the dominant zooplankton species, and reproduction coincident to extremely seasonal pulse of primary production (Knox 2006). Euphausiids constitute around 50% of the total zooplankton population, with *E. superba* being the most studied species of the Antarctic zooplankton due to the enormous biomass, ecological relevance and resource potential (Knox 2006). In the beginning of the 21st century, studies on Antarctic fauna were mostly terrestrial, with little information about zooplankton (Razouls et al. 2000).

The Southern Ocean contains a range of species of zooplankton, with copepods and euphausiids being the main components of Antarctic zooplankton (Razouls et al. 2000, Knox 2006, Ducklow et al. 2007), accounting for over 50% of the zooplankton populations (Ducklow et al. 2007). They are the main prey of numerous predators, such as fish, albatrosses, penguins, seals and whales (Tynan 1998, Knox 2006). Salps are omnivorous zooplankton, feeding through filters and co-exist with *E. superba* (Constable et al. 2014).

Trophic chains in Antartica are characterized by few trophic levels and dependence on a small number of key-species, as previously mentioned, so it becomes of great importance to study these species under a context of environmental change. Within Antarctic zooplankton, Antarctic krill *Euphausia superba, Euphausia triacantha, Themisto gaudichaudii* and *Thysanoessa* spp. are important components (Knox, 2006), in which, there is already information that *E. superba* has declined its abundance in certain regions of the Southern Ocean (Atkinson et al. 2004; see below).

1.2.1. Antarctic krill, *Euphausia superba*

Antarctic krill is the dominant herbivore of the Southern Ocean and major route of carbon transference from primary producers to higher trophic-levels (Ducklow et al. 2012, Constable et al. 2014). It has a circumpolar distribution south of the Antarctic Polar Front (Figure 2), related to the ACC (being also distributed a bit more north of the ACC only in the Atlantic sector) (Jarvis et al. 2010) and it is dominant near South Georgia, the intermediate zone of the Southern Ocean, the Scotia Sea and the Weddel Sea (Knox 2006). Its life cycle is intimately connected to the seasonality of Antarctic environment, such as duration and sea ice characteristics, photoperiod, and temperature (Constable et al. 2014). *E. superba* is positively dependent on sea ice, depending on it to reproduce, survive and recruit. It also tends to co-exist with its major source of food, which are species of phytoplankton (diatoms, flagellates) and zooplankton (copepods) (Cuzin-Roudy et al. 2014). Some *E. superba* predators are penguins, albatrosses, petrels and seals (Kock et al. 2007).

Different life stages of *E. superba oc*cur in different physical environment, since every life stage has a different habitat (Constable et al. 2014). Eggs and early larvae experience diverse physical conditions while descend to deep waters just to rise in the water column. Mid-larvae and juveniles depend on the sea-ice environment for growth and survivorship. Sub-adults and adults live in areas where juveniles are less frequent (Knox 2006, Meyer 2012). Thus, *E. superba's* life cycle is about 5 to 7 years (Xavier & Peck 2015).

E. superba has massive importance due to its stock size (from 200 to 600 million specimens) and it is believed that it can be an explored resource (Ikeda 1985). The latitudinal extent of their range depends on the tolerance of warming oceans and changes of productivity (Constable et al. 2014). But, since the 1980's, *E. superba* densities have been declining by 30% (Atkinson et al. 2004) since winters and sea ice extent have also diminished (Constable et al. 2014). Between the 1920s and 2010s, there was a southward contraction which became more proeminent since the 1970s of about 440 km in krill densities (Atkinson et al. 2019).

The reduction of sea-ice leads to the decline of algae and, consequently, the decline of *E. superba* population (Atkinson et al. 2004) which will affect the "Antarctic krill-based" food web (Kawaguchi et al. 2007). These changes, coupled with rise of temperatures around South Georgia, may lead to metabolic costs which may be unsustainable (Constable et al. 2014). This species may respond to warmer conditions, increasing its metabolic rate: around Antarctica Peninsula, the warmer temperatures may promote *E. superba* production while around South Georgia the temperatures have already risen and currently is the warmest habitat where krill is abundant (Mackey et al. 2012). This property reveals great interest in our project since krill's adaptation potential is yet unknow (Constable et al. 2014). Kawaguchi and his peers have also studied the impacts of the acidification of the Southern Ocean and concluded that, while it does not affect adults, it affects embryos in a negative light – more acidification leads to less embryos (Kawaguchi et al. 2011).

The abundance of *E. superba* shows large interannual variability and it has been reported that in years where *E. superba* abundance is lower, many predators feed on other crustaceans like *Themisto gaudichaudii* (Collins et al. 2008). It has also been shown that *E. superba* abundance is linked to water temperatures – during summer 2009, one of the warmest at South Georgia, indices of *E. superba* abundance lowered greatly (Mackey et al. 2012) and part of the krill population was found at depth, in cooler waters (Schmidt et al. 2011); and is also directly proportional to the extent of sea ice (Kock et al. 2007).

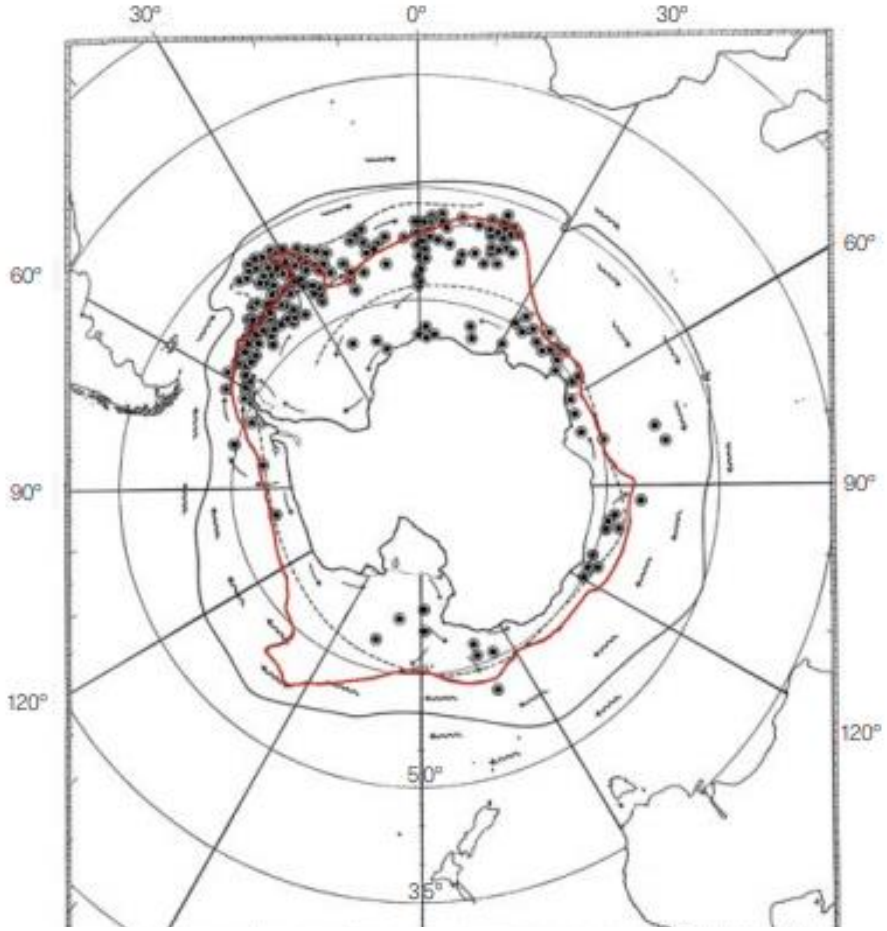

Figure 2 - Antarctic Krill, *Euphausia superba*, distribution in the Southern Ocean. The red line represents the ACC border and the arrows represent the direction in which the ACC flows (Tynan 1998).

1.2.2. Euphausia triacantha

Euphausia triacantha is an amphipod (Hosie et al. 2003) and occurs circumpolar around the Antarctic continent in Antarctic waters, up to near the Antarctic Polar Front (Kittel & StĘpnik 1983, Cuzin-Roudy et al. 2014) (Figure 3). The larvae of this species occur at low latitudes, such as waters of the Antartic Circumpolar Current and it is not encountered in the southern periphery neither near the Antarctic Peninsula. It also breeds over all of its habitat (Schnack 1983). *E. triacantha* is only found in the Scotia Sea, in areas not influenced by the Weddel Sea (Knox 2006), with a life span of over 2 years and it feeds on mesozooplankton. The predators of *E. triacantha* include birds, fish and squid (Cuzin-Roudy et al. 2014).

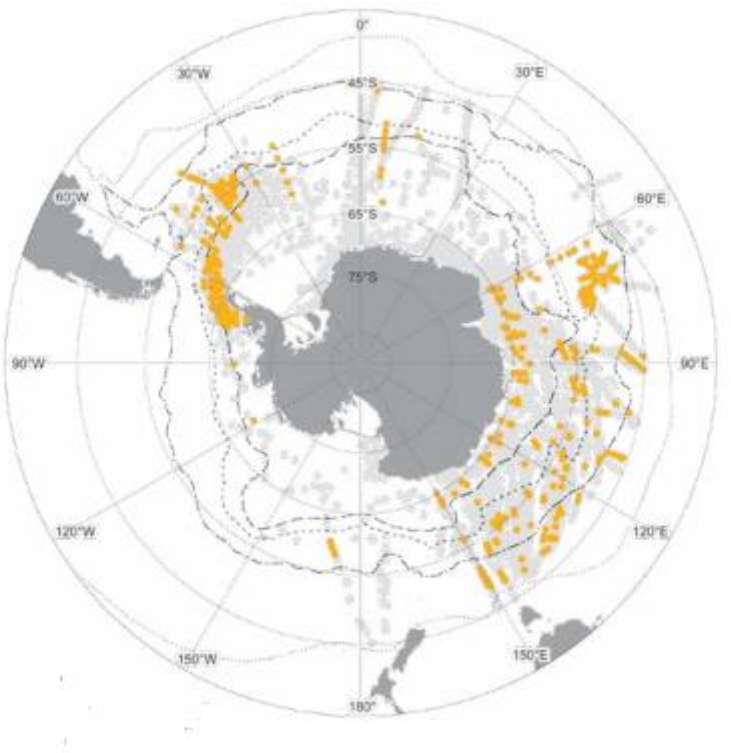

Figure 3 - Distribution map of *Euphausia triacantha*. The yellow dots represent the species distribution while the grey dots represent all records of euphausiacea in the Southern Ocean (Cuzin-Roudy et al. 2014).

1.2.3. Themisto gaudichaudii

T. gaudichaudii is also a major component of the zooplankton of the Southern Ocean community since it is one of the most abundant hyperiid amphipods of the southern hemisphere (Watts & Tarling 2012) and the most common species of hyperiidean in the Southern Ocean (Griffiths et al. 2014) with distribution in Antarctic and sub-Antarctic waters, particularly around islands (specifically the Falkland islands (Griffiths et al. 2014)) and coastal Sub-Antarctic and North Antarctic regions (Padovani et al. 2012, Zeidler & De Broyer 2014) (Figure 4). It is very common to find this species around the Sub-Antarctic and the Antarctic Peninsula and south of New Zealand, to the Ross Sea (Zeidler & De Broyer 2014). Closer to the northern region of the Southern Ocean, this species can reproduce year-round, but closer to the south, its reproduction becomes more seasonal (Watts & Tarling 2012). It is a very abundant species at water surface at night but descends to about 25-50 m during daytime (Griffiths et al. 2014, Zeidler & De Broyer 2014). Their rates of growth and maturation depend on temperature (temperate waters are related to a life-cycle shorter than 1 year). Antarctic waters represent greater longevity and food availability for this species (Watts & Tarling 2012). This species can maintain rich oils reserves, which allows to survive more time without food (Gibbons et al. 1992), which makes them energy riched food for the higher trophic

levels. Therefore, *T. gaudichaudii*, as zooplankton in general, is an important link between primary producers and top predators (Watts & Tarling 2012). Besides, higher trophic level predators feed in *Themisto gaudichaudii*, a species that can survive at greater temperatures than krill. *T. gaudichaudii* can be found in the northern zone of the southern Ocean, where it is a common species and in the Scotia Sea (Knox 2006).

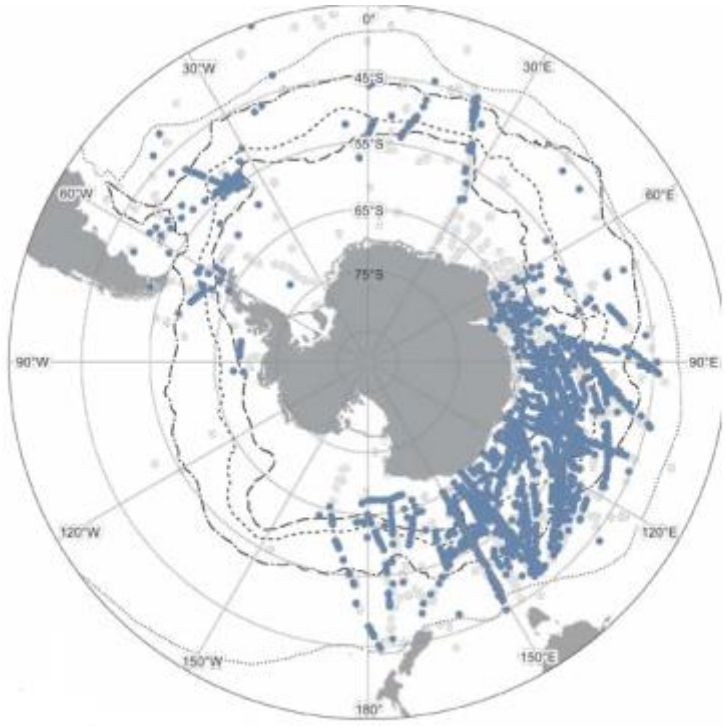

Figure 4 - Distribution of *T. gaudichaudii* in the Southern Ocean (Zeidler & De Broyer 2014). The blue dots represent the occurences of this species in the Southern Ocean.

1.2.4. *Thysanoessa* spp.

In this thesis, *Thysanoessa* spp. comprises two copepod species: *Thysanoessa macrura* and *Thysanoessa vicina*. These two species are very difficult to distinguish (Nemoto 1966, Cuzin-Roudy et al. 2014) due to the difficulty experienced when observing specific characteristics on these fragile species, characteristics usually damaged by net capture (Cuzin-Roudy et al. 2014). Thus, in the present work, they will be considered as *Thysanoessa* spp..

Thysanoessa macrura mostly lives in shelf regions (Hosie 1991) and it is found in several high predators feeding, like *T. vicina* (Hosie 1991). *T. macrura* is the most common and abundant copepod species in the Southern Ocean, with a circumpolar distribution and can be found up into the Sub-Tropical Front (Figure 5). *T. macrura* is more present in regions with lower concentrations of oxygen. *T. macrura*'s life span is 2 years and its diet consists of mesozooplankton. Its predators include whales, birds, fish and seals (Cuzin-Roudy et al. 2014).

Larvae of *T. macrura* show a very wide distribution and breeds over all of its habitat. Its maturation depends on the interaction between the inshore water and the ACC and annual melting (Schnack 1983).

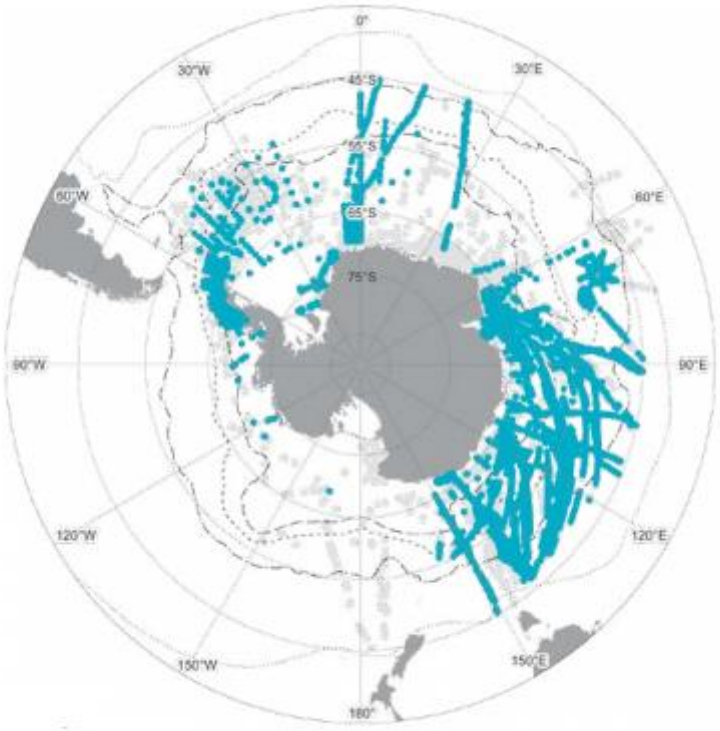

Figure 5 - Distribution of *Thysanoessa macrura* in the Southern Ocean. The blue dots represent the occurences of this species in the Southern Ocean (Cuzin-Roudy et al. 2014).

Thysanoessa vicina is found and more abundant at east of Falkland Islands, south of New Zealand and its distribution is believed to not be circumpolar (Figure 6). Its abundance raises questions about its importance in the diet of top predators and for fisheries (Cuzin-Roudy et al. 2014). This species can live up from 5 to 7 years and its diet is composed of microzooplankton, with predators being whales, birds, fish and seals (Cuzin-Roudy et al. 2014).

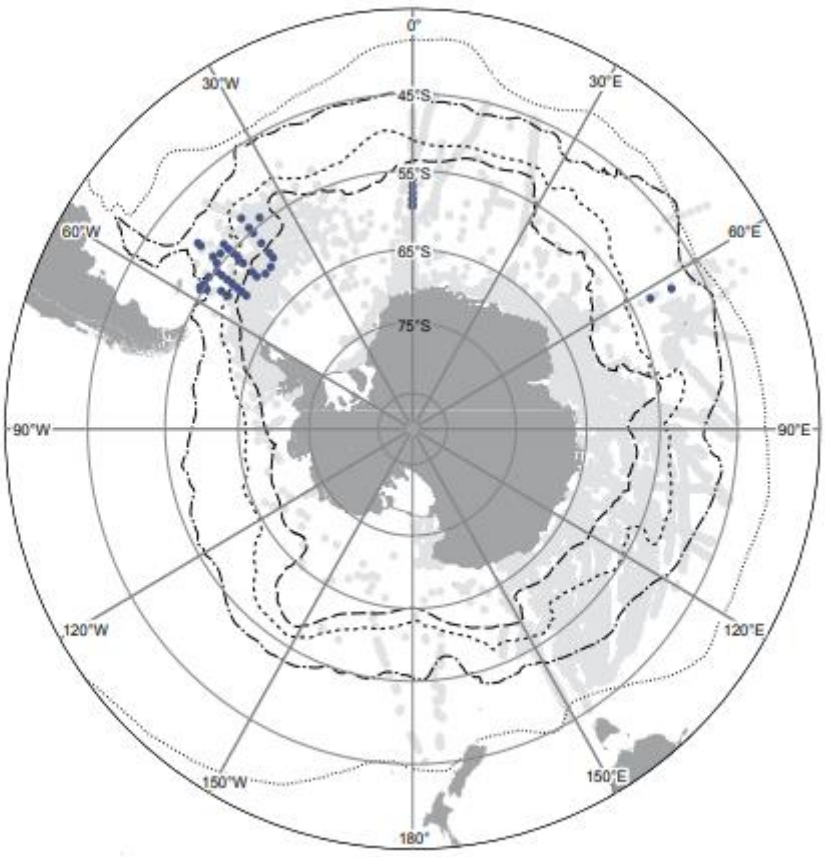

Figure 6 - Distribution of *Thysanoessa vicina* in the Southern Ocean. The blue dots represent the occurences of this species in the Southern Ocean (Cuzin-Roudy et al. 2014).

Thysanoessa spp. is common in the north zone of the Southern Ocean, in the Scotia Sea and in the Weddel Sea (Knox 2006). All the euphausiids species previously mentioned can be found in the south of the Antarctic Polar Front (Cuzin-Roudy et al. 2014).

Stressors affect processes at the biochemical and cellular levels directly. The study of fatty acids profile can help to answer several questions including how these organisms respond to environmental changes (Filimonova et al. 2016) and may help with the interpretation of trophic interactions (Cripps & Atkinson 2000).The main causers of stress for marine biota are the increase of ice-loading and coastal concentrations of large icebergs, the increase in coastal sedimentation associated to ice melting, stratification of the water column caused by cooling of superficial waters (the surface waters reaching coastal seas are cooled by contact with ice) and thermal events such as the ones associated with El Niño (Turner et al. 2009).

1.3. The method of Fatty Acids Analyses to assess food web trophic interactions

Fatty acids (FA's) are aliphatic monocarboxylic acids derived from or contained in esterified form, in the form of fat, oil or wax and consist of an hydrocarbon chain (-CH_2-CH_2-) complemented with a carboxyl group at one end of the molecule and a methyl group at the other end. In figure 7, it is possible to observe examples of each of the types of fatty acids: saturated fatty acids (SFA, e.g. stearic acid), monounsaturated fatty acids (MUFA, e.g. oleic acid), polyunsaturated fatty acids (PUFA, e.g. linoleic acid and α-linoleic acid).

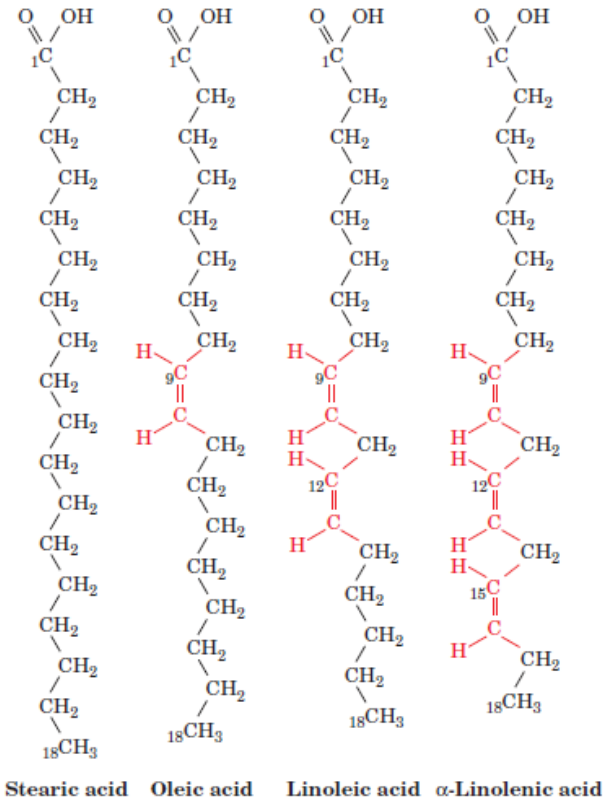

Figure 7 – Examples of fatty acids (Voet & Voet 2011)

Its length can vary from 4 to 36 carbons (C_4 to C_{36}) and it can be divided in saturated (no double bond) or unsaturated (with double bonds) fatty acids (FA). Examples of these fatty acids are α-linolenic acid (ALA , C18:3ω-3) and linoleic acid (LA, C18:2Δ-6) (Vedtofte et al. 2012). There are four common conventions for fatty acid nomenclature: the trivial system, the systematic method, the structural system and the n- and ω-systems (Davidson & Cantrill 1985). The trivial system consists in trivial names for fatty acids, for example, α-linolenic acid and linoleic acid (described before), while the structural systematic system is named based on the number of carbons and unsaturations present in fatty acids, with specification of location of the double bonds. The structural system is based in the number of carbons and unsaturations present in the fatty acids, with no specification on location of the double bonds. On a final note, the n- and ω- systems are very similar and relate to the ending carbon of the chain (carbon ω, while carbon α corresponds to the carbon of the beginning of the chain, at the carboxyl group) (Davidson & Cantrill 1985). This system allows the possibility of distinguishing between *cis* and *trans* configuration, once it is stated by *c* or *t* in the fatty acid nomenclature. If nothing is stated in the nomenclature, is assumed that the double bond(s) present in the described fatty acid appear in *cis* configuration (Gunstone 1996). Due to its importance in human feeding, another form to identify fatty acids is according to the unsaturation present in the carbons 3 and 4 from the end of the chain emerged. This new convention determines that fatty acids with a double bond between these carbons are named ω-3. This also happens to fatty acids containing a double bond between carbons 6 and 7, nominated ω-6. The physical properties of these molecules are dependent of the length of the carbon chain and of the number of double bonds (Boyle 2005).

The unsaturated fatty acids can also be divided into monounsaturated (MUFA), with one double bond, polyunsaturated (PUFA), with 2 double bonds, or highly unsaturated (HUFA) fatty acids, with 3 or more double bonds. HUFA and some PUFA are also called essential FA's (EFAs), since animals do not have the ability to synthesize them or sinthesize in very low amounts with high energetic costs. However, all organisms are completely capable of saturated fatty acids synthesis. PUFAs can be divided into, at least, four different families that will lead to the formation of EFAs: a) ω-3, α-linolenic acid derivatives (ALA 18:3 ω-3); b) ω-6, derived from linoleic acid (LA 18:2 ω-6); c) ω-9, derived from oleic acid (OA 18:1 ω-9) and d) ω-7, derived from palmitoleic acid (PA 16:1) (Das 2011). MUFAs and PUFAs may occur in both cis and trans conformations (Joris & Mensink 2016, Fattore & Massa 2018), although most fatty acid double bonds occur in *cis* conformation (Voet & Voet 2011). They can be found in lipids, which provides several functions in cellular life (German & Dillard 2010), and they provide physiological and

structural functions (Vara-Messler et al. 2015) while they also storage energy (Fattore & Massa 2018). They also have important roles in inflammation, metabolism and regulation of intracellular signalling processes and gene expression (Azrad et al. 2013, Vara-Messler et al. 2015). Fatty acids, especially PUFA's, with a focus in EFAs, also reduces the risk of cardiovascular (Innes & Calder 2018) and autoimmune diseases (Simopoulos 2002), and act as cancer preventive (Rose 1999). Essential fatty acids play a key role in patients who suffer from dry eye syndrome (Bhargava et al. 2013).

The most common fatty acids occur with a pair number of carbons and a chain length between 12 to 24 carbons and, in monounsaturated fatty acids, most of the double bonds occur between C-9 and C-10 (Δ9), whereas in polyunsaturated, occur at Δ12 and Δ15 (Boyle 2005).

Nonetheless, when it comes to ω-3 and ω-6, only plants are capable of biosynthesizing this type of fatty acid in higher amount, which are essential to heterotrophic organisms (Dalsgaard et al. 2003).

The consumption of fish or fish-oil could protect against events associated with coronary artery disease, which is related to the intake of long-chain polyunsaturated fatty acids like eicosapentaenoic acid (EPA, C20:5ω-3) and docosahexaenoic acid (DHA, C22:6ω-3), present in fish products. EPA functions are related to platelet aggregation, vasodilation, antiproliferation, plaque-stabilisations and reduction in lipid action (Yokoyama et al. 2007). EPA and DHA are part of a group nominated omega-3 or ω-3 polyunsaturated fatty acids, which also include seafood derived, long-chain ω-3 PUFA and plant-derived alpha-linolenic acid (ALA, C18:3ω-3) (Vedtofte et al. 2012, Kromhout & de Goede 2014, Maki et al. 2018). These PUFA are essential constituents of heterotrophic organisms, pointing the central position of algae in marine food webs (Dalsgaard et al. 2003).

Although there are inconsistent data about dietary intake of ω-3 PUFA's and its correlation with reduced risks of colon inflammations, colon cancer and carcinogenesis (Azrad et al. 2013, Vara-Messler et al. 2015), the various health-promoting claims led to a popularity in ω-3 PUFA's dietary supplements and food ingredients (Wang et al. 2017). However, certain studies support the idea that populations that consume ω-6 to ω-3 FAs in the ratio of 1:1 have fewer chronic diseases (Lee et al. 2018).

For more than thirty years, fatty acids have been studied as qualitative markers to trace trophic relationships in marine environments (Dalsgaard et al. 2003). The FATMs (fatty acid trophic markers) concept is based on the examination on marine primary producers fatty acid patterns which may be transferred to primary consumers, keeping its properties untouched and hence, be recognized in primary consumers (Dalsgaard et al. 2003).One of the issues for ecologists is to solve and predict the impacts of global

change on ecosystem dynamics. FA are considered good trophic markers since they are incorporated into consumers in a conservative manner, providing information on predator-prey relations (Dalsgaard et al. 2003). FA also provide information on dietary intake and food constituents over a longer period of time than the traditional gut content analysis (Dalsgaard et al. 2003). The concept of FATMs has especially been applied for herbivorous zooplankton that represent a link between primary producers and higher trophic levels (Dalsgaard et al. 2003). FA profiles can also contribute to answer some questions such as how species structurally respond to environmental changes, since alteration in FA composition is a sensitive early warning of stress (Gonçalves et al. 2012, Filimonova et al. 2016).

The FA pattern in marine food webs is laid down by primary producers (diatoms, dinoflagellates and prymnesiophyceae). This pattern is influenced by temperature, light and nutrient availability, with these factors affecting the FA pattern of the local community (Dalsgaard et al. 2003).

1.4. Objectives of the thesis

The objectives of my thesis, using four zooplankton species (*Euphausia superba, Euphausia triacantha, Themisto gaudichaudii* and *Thysanoessa* spp.) from three different zones of the Southern Ocean (Antarctic, Antarctic Polar Front Zone and Sub-Antarctic waters), were to:

1. Assess their nutritional value by determining their fatty acid profile,
2. Evaluate and compare the species' biochemical composition at the different zones of the Southern Ocean against environmental conditions measured and,
3. Determine fatty acid trophic markers to identify and characterise the food sources of the four zooplankton studied species.

The present study also intended to highlight the potential impacts of environmental changes of the four zooplankton studied species from the Antarctic Ocean, through the analyses of fatty acid profiles and inferences into species' body condition, and thus predict potential changes in food quality of these zooplankton species and consequences up the Southern Ocean trophic web.

2. Materials and Methods

2.1. Study areas

All samples were collected in the Southern Ocean, on board of the RRS James Clark ship, which rumed to the Southern Ocean on the 8th of December 2016 and finished its course on the 17th of January 2017. The total information about the investigations performed on board of the RRS James Clark can be found in the ship's log book.

Samples were collected between two different islands in the Southern Ocean: the Falkland Islands (51° 45' S 59° 00' W) and the South Georgia Island (54°17′S 36°30'W) (Figure 8).

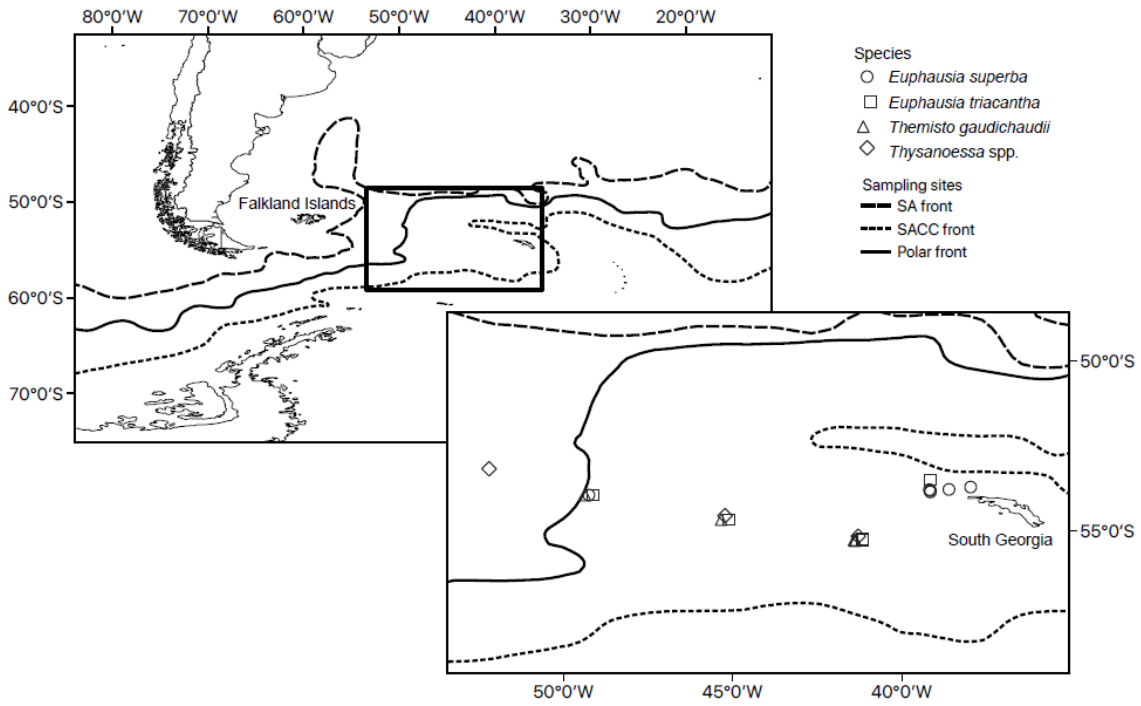

Figure 8 – Map of distribution of collected specimens in the Southern Ocean (SA-Subantarctic; SACCF – South Antarctic Circumpolar Current Front)

The Falkland Islands are an archipelago in the South Atlantic Ocean and are also part of an eastward extension of the Patagonian shelf (Agnew 2002). The Patagonian shelf is influenced by two branchs of the Antarctic Circumpolar Current, one of them being a jet known as the Malvinas Current. This current is the western boundary current of the subpolar South Atlantic (Sabatini et al. 2004).

On the other hand, South Georgia is a mountainous island in the Southern Ocean characterised by high biomass and productivity of zooplankton. It lies south of the Polar Front and zooplankton is high around the island and the region is important for commercial fisheries for *E. superba*. It is an example of productive, cold water ecosystem

(Atkinson et al. 2001).Between these regions, the oceanography is very dynamic with considerable changes in water temperatures (Figure 9).

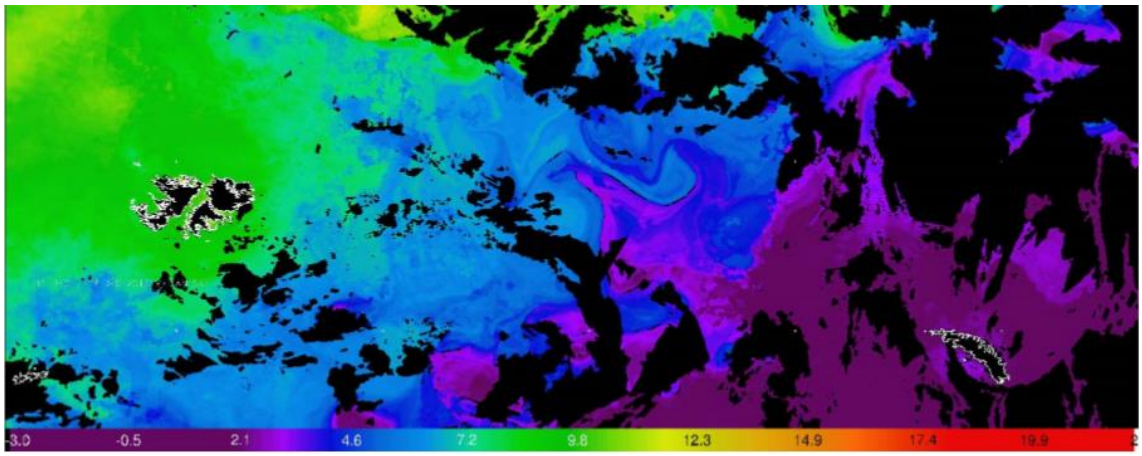

Figure 9 - Water temperatures between Falkland Islands and South Georgia registered during cruise.

2.2. Sampling Collection

The species of this thesis were caught from the water column using RTM8 (Rectangular Midwater Trawl of 8m2), RMT25 (Rectangular Midwater Trawl of 25 m2) and MOCNESS (*Multiple Opening/Closing Net and Environmental Sensing System*) nets, as described in the log book for the RRS James Clark expedition ('JR16003 Cruise Report Western Core Box' 2017). All the nets are remotely opened and closed at different depths and were equipped with a flow meter, temperature and salinity sensors. The fishing with rectangular midwater trawls nets occurred during the nighttime, while the MOCNESSS net was used during daytime or close to sunset. All species were collected between 0 m and 1000 m depth. All samples were stored in plastic bags and at -80ºC for subsequent analyses.

2.3. Biochemical Analysis

The methodology used for total lipids extraction and methylation to fatty acid methyl esters was as described in Gonçalves et al. (2012).

For most species, 3 replicates containing 1 individual each were prepared. Other species had replicates with a different number of individuals per replicate, according to the amount and quality of individuals available (Table 1, observations). At the fatty acid (FA) quantification the number of organisms were taken into consideration, with the results expressed in mg/ ind. The extraction of total lipids of zooplankton species and methylation to fatty acid methyl esters (FAMEs) for fatty acid analysis was achieved by a modified one-step derivatisation method following (Abdulkadir & Tsuchiya 2008), with

Table 1 - Studied samples, Latitude – South; Longitude (West); RMT – Rectangular Midwater Trawl; MOCNESS – Multiple Opening/Closing Net and Environmental Sensing System

Event ID	Latitude (South)	Longitude (West)	Type	Type of Sample	Species	Number	Frezzer	Sex/Maturity	Observations
39	-53.50226	-39.25351	RMT8	Zooplankton	*Euphausia triacantha*	100	-80	Random	3 replicas, 1 individual per replica
40	-53.79133	-39.1749	Unknown	Zooplankton	*Euphasia superba*	100	-80	Random	3 replicas, 1 individual per replica
41	-53.8587	-39.14853	RMT8	Zooplankton	*Euphausia superba*	100	-80	Random	3 replicas, 3 individuals per replica
42	-53.8209	-39.14515	RMT8	Zooplankton	*Euphausia superba*	100	-80	Random	3 replicas, 1 individual per replica
51	-53.71417	-37.95603	RMT8	Zooplankton	*Euphausia superba*	100	-80	Random	3 replicas, 1 individual per replica
59	-53.78494	-38.59711	RMT8	Zooplankton	*Euphausia superba*	100	-80	Random	3 replicas, 1 individual per replica
95	-55.28826	-41.25545	MOCNESS	Zooplankton	*Themisto gaudichaudii*	100	-80	Random	3 replicas, 4 individuals per replica
95	-55.28826	-41.25545	MOCNESS	Zooplankton	*Euphausia triacantha*	20	-80	Random	Specimens in 24 hours in cold room (2 degrees Celcius) before frozen. 3 replicas, 1 individual per replica
95	-55.28826	-41.25545	MOCNESS	Zooplankton	*Themisto gaudichaudii*	27	-80	Random	Specimens in 48 hours in cold room (2 degrees Celcius) before frozen. 3 replicas, 1 individual per replica
98	-55.28107	-41.28104	MOCNESS	Zooplankton	*Thysanoessa* spp.	100	-80	Random	3 replicas, 4 individuals per replica
98	-55.28107	-41.28104	MOCNESS	Zooplankton	*Euphausia triacantha*	33	-80	Random	3 replicas, 1 individual per replica

98	-55.28107	-41.28104	MOCNESS	Zooplankton	*Themisto gaudichaudii*	100	-80	Random	3 replicas, 4 individuals per replica
98	-55.28107	-41.28104	MOCNESS	Zooplankton	*Themisto gaudichaudii*	47	-80	Random	Specimens in 36 hours in cold room (2 degrees Celcius) before frozen. 3 replicas, 2 individuals per replica
112	-55.25898	-41.26033	RMT25	Zooplankton	*Euphausia triacantha*	100	-80	Random	3 replicas, 1 individual per replica
129	-54.67554	-45.21932	RMT25	Zooplankton	*Themisto gaudichaudii*	21	-80	Random	3 replicas, 1 individual per replica
129	-54.67554	-45.21932	RMT25	Zooplankton	*Thysanoessa* spp.	20	-80	Random	3 replicas, 1 individual per replica
129	-54.67554	-45.21932	RMT25	Zooplankton	*Euphausia triacantha*	100	-80	Random	3 replicas, 1 individual per replica
147	-53.94909	-49.24781	RMT25	Zooplankton	*Euphausia superba*	100	-80	Random	3 replicas, 1 individual per replica
147	-53.94909	-49.24781	RMT25	Zooplankton	*Euphausia triacantha*	100	-80	Random	3 replicas, 1 individual per replica
147	-53.94909	-49.24781	RMT25	Zooplankton	*Themisto gaudichaudii*	85	-80	Random	3 replicas, 1 individual per replica
164	-53.28735	-52.1965	RMT25	Zooplankton	*Thysanoessa* spp.	23	-80	Random	3 replicas, 1 individual per replica

a difference in reagents used for extraction and esterification of fatty acids. While Abdulkadir and his peers used boron trifluoride-methanol (BF3-methanol) as a reagent for the extraction and esterification, it was replaced by H_2SO_4-methanol solution since BF3-methanol can cause artefacts or loss of PUFAs (Eder 1995). C19:0 was added as an internal standard for the quantification (Fluka 74208).

The FAMEs obtained were analysed using a GC-MS. The quantification function of each FAME was obtained by linear regression applied to the chromatographic peak areas and corresponding known concentrations of the standards.

2.3.1. Fatty Acid Trophic Markers (FATM's)

Based on El-Sabaawi et al. (2009), fatty acid ratios were calculated and used as biomarkers to analyse food sources, therefore reflecting their trophic position and dietary quality.

Carnivorous zooplankton shows higher quantities of polar lipids (amphiphilic lipids with a hydrophilic head and a hydrophobic tail, (Zheng et al. 2019), rich in PUFA than herbivorous crustaceans. Thus, the ratio PUFA (sum of all polyunsaturated fatty acids) / SFA (sum of all saturated fatty acids) can be used to detect carnivory of *E. superba* and other zooplankton (Cripps & Atkinson 2000). Another ratio that allows to determine the degree of carnivory is the ratio DHA/EPA (docosahexaenoic acid to eicosapentaenoic acid, 22:6ω-3/20:5ω-3) (Dalsgaard et al. 2003). DHA is highly conserved in food webs, as it is an important component of polar lipids (Scott et al. 2002). Therefore, the ratio DHA/EPA should increase towards higher trophic levels. Because DHA is often dominant in dinoflagellates, while EPA is mainly found in diatoms, this ratio may also reflect the proportion of dinoflagellates and diatom in the diets of omnivorous and herbivorous organisms (Dalsgaard et al. 2003).

The proportion of all diatom markers (D = 16PUFA + 16:1Δ7 + 20:5ω-3) to all flagellate markers (F = 18PUFA + 18:2ω-6, + 22:6ω-3), D/F, is used to distinguish between diatom and dinoflagellate-based diet (El-Sabaawi et al. 2009). High proportions of C18:2ω-6 denote the presence of terrestrial detritus or green algae in the zooplankton diet (Dalsgaard et al. 2003). For further details about trophic and dietary tracers used in this study, see Table 1 in El-Sabaawi et al. (2009).

2.4. Statistical Analysis

A multivariate statistical analysis with the Primer-5 software was performed to examine the fatty acid profiles for discriminatory information about spatial variations. Non-metric multidimensional scaling (nMDS) plots were conducted to address the variations and the groups formed according to the species fatty acid composition.

Biochemical data was converted into similarity matrices, using a Bray-Curtis coefficient, and tested with a one-way analysis of similarity (ANOSIM), taking into consideration the species and the studied regions. The similarities and dissimilarities were verified through a similarity percentage analysis routine (SIMPER).

3. Results

3.1. Analytical analysis

For the analysis of fatty acids, it was obtained to each sample a chromatogram where it was identified the methyl-esters based on the standard 37 component Fatty Acids Methyl-Esters (FAME) Mix (figure 10). According to the time retention of the FAMEs and those described in literature, it was possible to understand the fatty acid methyl esther (FAME) present in the samples and, consequently, the corresponding fatty acid.

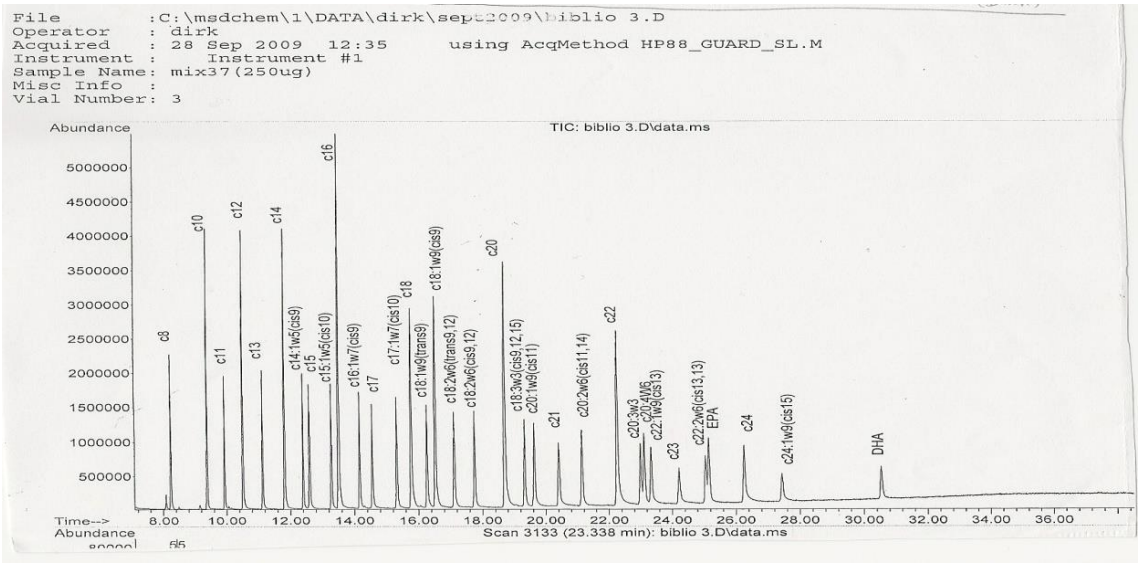

Fig. 10 - Chromatogram of the 37 component Fatty Acids Methyl-Esters (FAME) Mix (From: Ir. Dirk Van Gansbeke)

With the information of the area of the peaks and time retentions obtained for the collected samples for this study, it is possible to calculate the abundance of each fatty acid in the samples. Some examples of obtained peaks and times are described in table 2 and peak areas with corresponding fatty acids are described through tables 20 to 26, in Supplementary Information.

Table 2 - Obtained peaks of fatty acids for the replica 19.1, of sample WEt19

PeaRet	Time	Type	Width	AreaStart	TiEnd	Time
1	6.166	M	0.029	615574	6.141	6.201
2	6.492	M	0.016	1021811	6.478	6.523
3	6.794	M	0.013	627500	6.779	6.812
4	6.859	M	0.017	906578	6.838	6.883
5	6.942	VB	0.031	12806439	6.902	7.107
6	7.138	M	0.025	449975	7.121	7.175
7	7.295	BV	0.048	130404474	7.198	7.427
8	7.458	M	0.03	1103425	7.431	7.514
9	7.601	VV	0.018	2970196	7.574	7.636
10	7.674	M	0.019	962835	7.646	7.699
11	7.962	M	0.028	1387525	7.914	7.976
12	8.014	M	0.035	544591	7.997	8.074
13	8.228	BB	0.041	3554398	8.175	8.294
14	8.598	BV	0.088	561269141	8.432	8.691
15	8.713	VV	0.065	218684117	8.691	8.809
16	9.003	M	0.029	2174975	8.962	9.034
17	9.129	BV	0.037	4618869	9.1	9.193
18	9.247	PV	0.031	12189639	9.193	9.319
19	9.356	M	0.048	4173703	9.322	9.406
20	9.701	VV	0.032	5861831	9.651	9.767
21	9.848	PV	0.037	11004681	9.767	9.913
22	9.972	VV	0.038	5829039	9.913	10.063
23	10.112	VV	0.033	9598323	10.063	10.141
24	10.171	VV	0.04	10808064	10.141	10.253
25	10.297	VV	0.04	10527839	10.253	10.415
26	11.062	BV	0.063	166749020	10.778	11.112
27	11.184	VV	0.095	242125671	11.112	11.326
28	11.423	VV	0.073	29197372	11.326	11.478
29	11.55	VV	0.054	14094499	11.478	11.583
30	11.648	VB	0.09	60259487	11.583	11.836
31	12.012	M	0.073	7738836	11.938	12.122
32	12.659	BV	0.087	148123437	12.455	12.789
33	13.036	BB	0.082	113983257	12.942	13.166
34	13.544	M	0.065	3253967	13.469	13.639
35	13.915	BB	0.054	12899868	13.838	14.033
36	14.36	M	0.065	1447540	14.292	14.456
37	14.72	M	0.081	7242660	14.646	14.816
38	14.899	M	0.073	1987451	14.836	15.047
39	15.435	M	0.049	2014831	15.384	15.494
40	15.916	M	0.068	2446490	15.85	16.052
41	16.235	BB	0.065	17832367	16.14	16.417
42	17.563	VB	0.201	895812125	17.398	18.171
43	19.856	BB	0.179	44169557	19.696	20.192
44	20.61	BB	0.076	25555494	20.421	20.773
45	23.77	BB	0.287	512885541	23.514	24.954
46	32.941	M	0.08	3076342	32.912	33.086
47	33.633	M	0.156	5117784	33.527	33.852
48	35.678	M	0.063	1768623	35.616	35.79
49	40.378	M	0.197	7936099	40.257	40.675

3.2. Fatty acid composition

Fatty acid profiles were described in terms of biochemical abundance for each species sampled in the Southern Ocean, in cold waters (Table 2 and Figure 11), intermediate waters (Table 3 and Figure 12) and warmer waters (Table 4 and Figure 13).

The stearic fatty acid, C18:0, was the most common and abundant fatty acid for all species combined. In some (8) of the collected samples, it represents over 50% of its fatty acid composition.

Samples collected in cold waters revealed a higher diversity of FA (N=17-23) than the samples collected in intermediate (N=11-21) and warmer waters (N=14-20), with these samples presenting a higher range on FA diversity among samples. *Euphausia triacantha* revealed the greatest abundance and variety of fatty acids between the samples collected in cold and transitional waters. In warmer waters, *Themisto gaudichaudii* revealed the greatest abundance and variety of fatty acids, followed by *E. triacantha*.

In cold regions (Table 2, Figure 11), the most abundant SFA was C18:0, while for samples collected in transitional waters (Table 3, Figure 12), the most common SFAs was not only C18:0, but also C14:0 and C20:0. For samples collected in warmer regions (Table 4, Figure 13), C12:0, C14:0, C16:0, C17:0 and C18:0 were the most abundant SFAs. At lower temperatures, the most abundant MUFAs in the samples collected were C14:1Δ9, C15:1Δ10 and C17:1Δ10. C18:1Δ9 also has an important role in some of the collected samples in a colder environment. C14:1Δ9, C15:1Δ10 and C17:1Δ10 were also the most common MUFAs for samples collected in warmer waters, while for samples collected in intermediate waters, the most abundant MUFAs were C14:1Δ9, C15:1Δ10 and C18:1Δ9. Samples collected in cold regions reveal very low amounts of PUFAs, and C20:3Δ7,10,13 is the most abundant PUFA present in these samples. In terms of PUFAs, samples collected in transitional and warmer waters can be characterized by the presence of C20:3Δ7,10,13 and C18:2Δ9,12 (linoleic acid or LA). HUFAs were not present in the samples collected for *Euphausia superba* in cold waters, but they were present in the sample collected for *E. triacantha,* where it was possible to find EPA (Eicosapentaenoic acid**)**, DHA (Docosahexaenoic acid) and ARA (Arachidonic acid). In intermediate waters, it is possible to find DHA in several collected samples of *T.* gaudichaudii and *E. triacantha*. Other species did not reveal the presence of this fatty acid. In warmer regions, the majority of the collected samples revealed HUFAs in their composition, with DHA being the most common. ARA and EPA were also present in some samples, although they were present at a smaller extent. In cold regions, *E. triacantha* reveals a better body condition, containing large quantities of essential fatty acids, like EPA and DHA. *E. superba* revealed a profile of less nutritional importance, without the presence of HUFAs. In intermediate waters, *T. gaudichaudii* and *E. triacantha* revealed an overall body condition similar to one another, with samples for *T. gaudichaudii* revealing the best (ITg9) and the worst (ITg12) body condition found in these waters. In warmer waters, *E. superba* reveals a lower body condition than those

presented by the other samples collected in these waters. *T. gaudichaudii* reveals the best body condition, with the presence of EPA, DHA and ARA.

Table 3 - Abundance (in µg/g) of fatty acids of the zooplankton species collected in cold regions of the Southern Ocean.

Species / FA (µg/g)	*E. superba* (CEs1)	*E. triacantha* (CEt3)	*E. superba* (CEs4)	*E. superba* (CEs5)	*E. superba* (CEs22)	*FA range*
C10:0	-	-	8 (0.3%)	-	-	
C11:0	30 (0.8%)	72 (0.8%)	12 (0.4%)	3 (0.2%)	1 (0.1%)	1 – 72
C12:0	9 (0.2%)	-	4 (0.1%)	6 (0.3%)	2 (0.1%)	2 – 9
C13:0	75 (1.9%)	28 (0.3%)	28 (0.9%)	34 (1.7%)	28 (1.5%)	28 – 75
C14:0	-	443 (4.8%)	10 (0.3%)	8 (0.4%)	5 (0.3%)	5 – 443
C15:0	5 (0.1%)	200 (2.1%)	4 (0.1%)	3 (0.1%)	2 (0.1%)	2 – 200
C16:0	201 (5.2%)	763 (8.2%)	-	-	-	201-763
C17:0	98 (2.5%)	119 (1.3%)	69 (2.1%)	44 (2.2%)	67 (3.6%)	44 – 119
C18:0	2374 (61.7%)	2265 (24.3%)	2712 (82.6%)	1650 (82.3%)	1000 (52.8%)	1000 – 2712
C20:0	8 (0.2%)	330 (3.5%)	-	6 (0.3%)	3 (0.2%)	3 – 330
C21:0	9 (0.2%)	1 (0.0%)	3 (0.1%)	3 (0.2%)	3 (0.2%)	1 – 9
C22:0	-	66 (0.7%)	158 (4.8%)	5 (0.2%)	133 (7.0%)	5 – 158
C23:0	12 (0.3%)	-	-	-	-	-
Total SFA	2822 (73.3%)	4287 (46.1%)	3008 (91.6%)	1761 (87.9%)	1244 (65.7%)	1244 – 4287
C14:1Δ9	29 (0.9%)	57 (0.6%)	20 (0.6%)	13 (0.6%)	15 (0.8%)	13 – 57
C15:1Δ10	36 (0.9%)	171 (1.8%)	2 (0.0%)	13 (0.7%)	19 (1.0%)	2 – 171
C16:1Δ9	45 (1.2%)	-	7 (0.2%)	2 (0.1%)	38 (2.0%)	2 – 45
C17:1Δ10	210 (5.5%)	308 (3.3%)	113 (3.4%)	88 (4.4%)	59 (3.1%)	59 – 308
C18:1Δ9	138 (3.6%)	886 (9.5%)	-	-	-	138 – 886
C20:1Δ11	12 (0.3%)	108 (1.2%)	20 (0.6%)	13 (0.7%)	20 (1.1%)	12 – 108
Total MUFA	470 (12.2%)	1530 (16.4%)	161 (4.9%)	129 (6.5%)	151 (8.0%)	129 – 1530
C18:2Δ9,12 (LA)	-	125 (1.3%)	70 (2.1%)	8 (0.4%)	-	8 – 125
C20:2Δ11,13	-	57 (0.6%)	28 (0.8%)	9 (0.5%)	-	9 – 57
C22:2Δ13,16	-	39 (0.4%)	14 (0.4%)	15 (0.7%)	-	14 – 39
C18:3Δ6,9,12	-	-	3 (0.1%)	1 (0.0%)	-	1 – 3
C18:3Δ9,12,15 (ALA)	354 (9.2%)	13 (0.1%)	-	-	413 (21.8%)	13 – 413
C20:3Δ7,10,13	202 (5.2%)	232 (2.5%)	-	81 (4.0%)	86 (4.5%)	81 – 232
Total PUFA	556 (14.4%)	466 (5.0%)	114 (3.5%)	114 (5.7%)	499 (26.3%)	114 – 556
C20:4Δ5,8,11,14 (ARA)	-	81 (0.9%)	-	-	-	-
C20:5Δ5,8,11,14,17 (EPA)	-	1554 (16.7%)	-	-	-	-
C22:6Δ4,7,10,13,16,19 (DHA)	-	1390 (14.9%)	-	-	-	-
Total HUFA	-	3025 (32.5%)	-	-	-	-
Total FA	3848	9308	3283	2005	1893	1893 – 9308
N	*18*	*23*	*19*	*20*	*17*	*17 – 23*

Table 4 - Abundance (*in* µ*g/g)* of fatty acids of the zooplankton species *T. gaudichaudii*, *Thysanoessa* spp. and *E. triacantha* in the Southern Ocean, in intermediate regions.

Species / FA (µg/g)	*T. gaudichaudii* (ITg6)	*Thysanoessa* spp. (ITspp7)	*E. triacantha* (IEt8)	*T. gaudichaudii* (ITg9)	*E. triacantha* (IEt10)	*E. triacantha* (IEt11)	*T. gaudichaudii* (ITg12)	*T. gaudichaudii* (ITg13)	*FA range*
C11:0	54 (1.1%)	209 (5.9%)	17 (0.5%)	-	11 (0.2%)	16 (0.5%)	109 (5.4%)	51 (1.9%)	11 – 209
C12:0	25 (0.5%)	-	8 (0.3%)	-	15 (0.2%)	-	92 (4.6%)	11 (0.4%)	8 – 25
C13:0	6 (0.1%)	-	-	-	34 (0.5%)	-	114 (5.7%)	4 (0. 2%)	4 – 114
C14:0	2 (0.0%)	235 (6.6%)	25 (0.8%)	583 (6.5%)	219 (3.5%)	12 (0.4%)	284 (14.2%)	229 (8.5%)	2 – 284
C15:0	5 (0.1%)	-	-	-	29 (0.5%)	-	73 (3.6%)	81 (3.0%)	5 - 81
C16:0	439 (9.1%)	188 (5.3%)	-	1030 (11.4%)	436 (6.9%)	-	-	459 (17.0%)	188 – 1030
C17:0	30 (0.6%)	222 (6.2%)	118 (3.6%)	70 (0.8%)	179 (2.8%)	154 (5.1%)	18 (0.9%)	69 (2.6%)	18 - 222
C18:0	2309 (47.7%)	1561 (43.7%)	1849 (56.1%)	1555 (17.3%)	1539 (24.3%)	1587 (53.0%)	753 (37.5%)	855 (31.6%)	753 - 2309
C20:0	182 (3.8%)	117 (3.3%)	81 (2.5%)	482 (5.4%)	166 (2.6%)	163 (5.4%)	152 (7.6%)	43 (1.6%)	43 - 482
C21:0	-	6 (0.2%)	4 (0.1%)	-	2 (0.0%)	-	2 (0.1%)	5 (0.2%)	2 - 5
C22:0	10 (0.2%)	65 (1.8%)	100 (3.0%)	69 (0.8%)	118 (1.9%)	-	120 (6.0%)	128 (4.7%)	10 - 128
Total SFA	3062 (63.2%)	2604 (72.9%)	2203 (66.8%)	3790 (42.1%)	2749 (43.3%)	1932 (64.5%)	1716 (85.5%)	1937 (71.5%)	1716 - 3062
C14:1Δ9	4 (0.1%)	93 (2.6%)	48 (1.5%)	30 (0.3%)	47 (0.7%)	50 (1.7%)	4 (0.2%)	11 (0.4%)	4 – 93
C15:1Δ10	52 (1.1%)	63 (1.8%)	68 (2.1%)	75 (0.8%)	125 (2.0%)	65 (2.2%)	43 (2.2%)	34 (1.2%)	34 – 125
C17:1Δ10	52 (1.1%)	278 (7.8%)	-	-	53 (0.8%)	-	-	163 (6.0%)	52 - 278
C18:1Δ9	-	118 (3.3%)	720 (21.8%)	596 (6.6%)	592 (9.3%)	719 (24.0%)	77 (3.8%)	245 (9.0%)	118 - 720
C20:1Δ11	-	-	-	-	26 (0.4%)	-	-	-	
Total MUFA	108 (2.2%)	552 (15.5%)	835 (25.4%)	701 (7.8%)	843 (13.3%)	833 (27.8%)	124 (6.2%)	452 (16.7%)	108 - 843
C18:2Δ9,12	243 (5.0%)	-	-	332 (3.7%)	50 (0.8%)	46 (1.5%)	56 (2.8%)	129 (4.8%)	46 – 332
C20:2Δ11,13	-	-	-	-	29 (0.5%)	-	-	18 (0.7%)	18 – 29
C22:2Δ13,16	12 (0.2%)	-	37 (1.1%)	-	24 (0.4%)	-	13 (0.7%)	-	12 - 37
C18:3Δ6,9,12	42 (0.9%)	-	-	-	-	-	-	-	-
C18:3Δ9,12,15	1 (0.0%)	-	-	-	-	-	-	-	-
C20:3Δ7,10,13	9 (0.2%)	414 (11.6%)	220 (6.7%)	-	197 (3.1%)	183 (6.1%)	96 (4.8%)	172 (6.4%)	9 - 414
Total PUFA	306 (6.3%)	414 (11.6%)	257 (7.8%)	332 (3.7%)	300 (4.7%)	229 (7.6%)	166 (8.3%)	320 (11.8%)	166 - 414
C22:6Δ4,7,10,13,16,19 (DHA)	1366 (28.2%)	-	-	4185 (46.5%)	2452 (38.7%)	-	-	-	1366 - 4185
Total HUFA	1366 (28.2%)	-	-	4185 (46.5%)	2452 (38.7%)	-	-	-	1366 - 4185
Total FA	4841	3570	3295	9008	6344	2994	2006	2709	2006 - 9008
N	*19*	*13*	*13*	*11*	*21*	*10*	*16*	*18*	*10 - 21*

Table 5 - Abundance (in µg/g) of fatty acids of the zooplankton species *T. gaudichaudii*, *Thysanoessa spp.*, *E. triacantha* and *E. superba* in the Southern Ocean, in warmer regions.

Species / FA (µg/g)	*T. gaudichaudii* (WTg14)	*Thysanoessa* spp. (WTspp15)	*E. triacantha* (WEt16)	*E. superba* (WEs18)	*E. triacantha* (WEt19)	*T. gaudichaudii* (WTg20)	*Thysanoessa* spp. (WTspp21)	*FA range*
C10:0	-	-	-	2 (0.2%)	-	-	-	-
C11:0	-	-	16 (0.5%)	2 (0.2%)	18 (0.2%)	-	-	2 – 18
C12:0	26 (0.5%)	15 (0.2%)	8 (0.2%)	2 (0.3%)	16 (0.2%)	27 (0.0%)	8 (0.2%)	2 - 27
C13:0	-	15 (0.2%)	4 (0.1%)	3 (0.4%)	17 (0.2%)	4 (0.0%)	-	3 - 17
C14:0	194 (3.7%)	456 (7.7%)	30 (0.9%)	3 (0.3%)	6 (0.1%)	3511 (5.8%)	16 (0.4%)	3 - 3511
C15:0	-	47 (0.8%)	80 (2.3%)	6 (0.6%)	140 (1.5%)	443 (0.7%)	8 (0.2%)	6 - 443
C16:0	269 (5.1%)	538 (9.0%)	-	-	621 (6.5%)	9578 (15.8%)	1125 (26.4%)	269 - 9578
C17:0	73 (1.4%)	165 (2.8%)	56 (1.6%)	27 (2.9%)	105 (1.1%)	407 (0.7%)	72 (1.7%)	27 - 407
C18:0	1910 (36.2%)	1801 (30.2%)	2501 (71.4%)	712 (76.4%)	2140 (22.3%)	841 (1.4%)	1220 (28.7%)	712 - 2501
C20:0	150 (2.8%)	346 (5.8%)	31 (0.9%)	-	252 (2.6%)	87 (0.1%)	118 (2.8%)	31 - 346
C21:0	2 (0.0%)	4 (0.1%)	5 (0.1%)	3 (0.3%)	-	-	-	2 - 5
C22:0	10 (0.2%)	-	6 (0.2%)	47 (5.1%)	-	31 (0.1%)	388 (9.1%)	6 - 388
C23:0	-	-	-	-	6 (0.1%)	-	-	-
Total SFA	2635 (49.9%)	3387 (56.9%)	2736 (78.1%)	806 (86.6%)	3321 (34.6%)	14930 (24.7%)	2957 (69.5%)	806 - 14930
C14:1Δ9	-	62 (1.0%)	46 (1.3%)	15 (1.7%)	41 (0.4%)	-	22 (0.5%)	15 - 62
C15:1Δ10	54 (1.0%)	25 (0.4%)	52 (1.5%)	2 (0.2%)	124 (1.3%)	229 (0.4%)	12 (0.3%)	2 - 229
C16:1Δ9	211 (4.0%)	-	-	-	-	-	-	-
C17:1Δ10	8 (0.1%)	97 (1.6%)	153 (4.4%)	27 (2.9%)	-	139 (0.2%)	-	8 - 153
C18:1Δ9	-	297 (5.0%)	-	-	1427 (14.9%)	9501 (15.7%)	-	297 - 9501
C20:1Δ11	59 (1.1%)	41 (0.7%)	-	10 (1.1%)	-	1010 (1.7%)	-	10 - 1010
C22:1Δ13	-	-	-	-	53 (0.6%)	-	-	-
Total MUFA	333 (6.3%)	522 (8.8%)	251 (7.2%)	54 (5.8%)	1645 (17.1%)	10879 (18.0%)	34 (0.8%)	34 - 10879
C18:2Δ9,12 (LA)	137 (2.6%)	254 (4.3%)	-	-	121 (1.3%)	99 (0.2%)	93 (2.2%)	93 - 254
C20:2Δ11,13	-	-	8 (0.2%)	60 (6.4%)	17 (0.2%)	-	201 (4.7%)	8 - 201
C22:2Δ13,16	-	-	56 (1.6%)	11 (1.2%)	-	20 (0.0%)	-	11 - 56
C18:3Δ6,9,12	52 (1.0%)	-	3 (0.1%)	-	18 (0.2%)	37 (0.1%)	-	3 - 52
C18:3Δ9,12,15 (ALA)	-	-	8 (0.2%)	-	-	-	-	-
C20:3Δ7,10,13	51 (1.0%)	163 (2.7%)	443 (12.6%)	-	115 (1.2%)	53 (0.1%)	204 (4.8%)	51 - 443
Total PUFA	240 (4.5%)	417 (7.0%)	518 (14.8%)	71 (7.6%)	271 (2.8%)	208 (0.3%)	499 (11.7%)	71 - 499

Table 5 (cont.) - Abundance (in µg/g) of fatty acids of the zooplankton species *T. gaudichaudii*, *Thysanoessa* spp, *E. triacantha* and *E. superba* in the Southern Ocean, in warmer regions.

Species / FA (µg/g)	*T. gaudichaudii* (WTg14)	*Thysanoessa* spp. (WTspp15)	*E. triacantha* (WEt16)	*E. superba* (WEs18)	*E. triacantha* (WEt19)	*T. gaudichaudii* (WTg20)	*Thysanoessa* spp. (WTspp21)	*FA range*
C20:4Δ5,8,11,14 (ARA)	-	71 (1.2%)	-	-	-	517 (0.9%)	-	71 – 517
C20:5Δ5,8,11,14,17 (EPA)	-	-	-	-	-	16144 (26.7%)	-	-
C22:6Δ4,7,10,13,16,19 (DHA)	2069 (39.2%)	1558 (26.2%)	-	-	4359 (45.4%)	17864 (29.5%)	767 (18.0%)	767 – 17864
Total HUFA	2069 (39.2%)	1629 (27.4%)	-	-	4359 (45.4%)	34525 (57.0%)	767 (18.0%)	767 - 34525
Total FA	5276	5954	3505	932	9596	60542	4256	932 - 60542
N	*16*	*18*	*18*	*16*	*19*	*20*	*14*	*14 - 20*

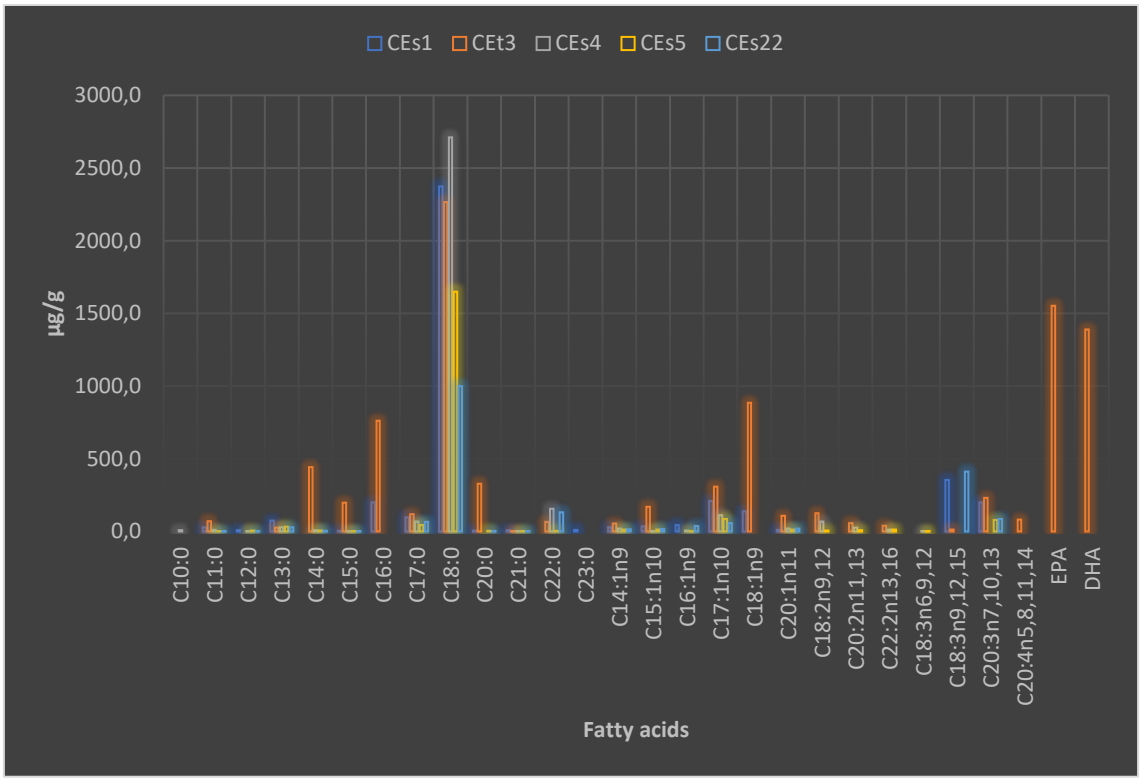

Figure 11 – Graphic representing the present fatty acids in the samples collected in cold waters (CEs – *E. superba* collected in cold waters; CEt – *E. triacantha* collected in cold waters).

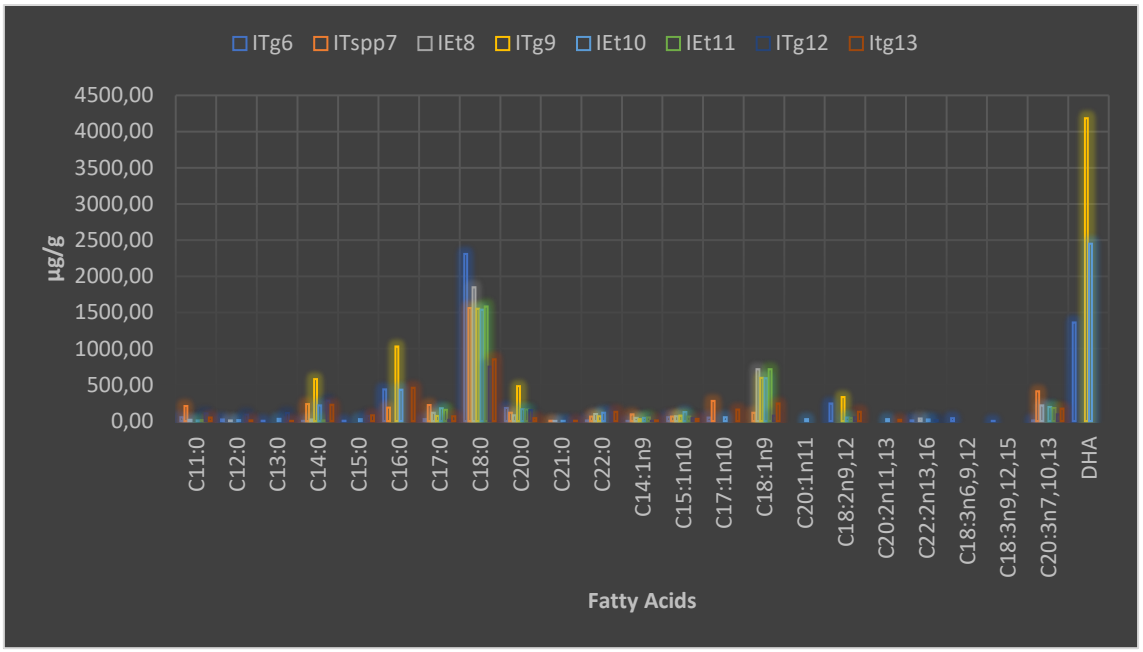

Figure. 12 – Graphic representing the fatty acids present in the samples collected in intermediate waters (ITg – *T. gaudichaudii* collected in intermediate waters; ITspp – *Thysanoessa* spp collected in intermediate waters; IEt – *E. triacantha* collected in intermediate waters).

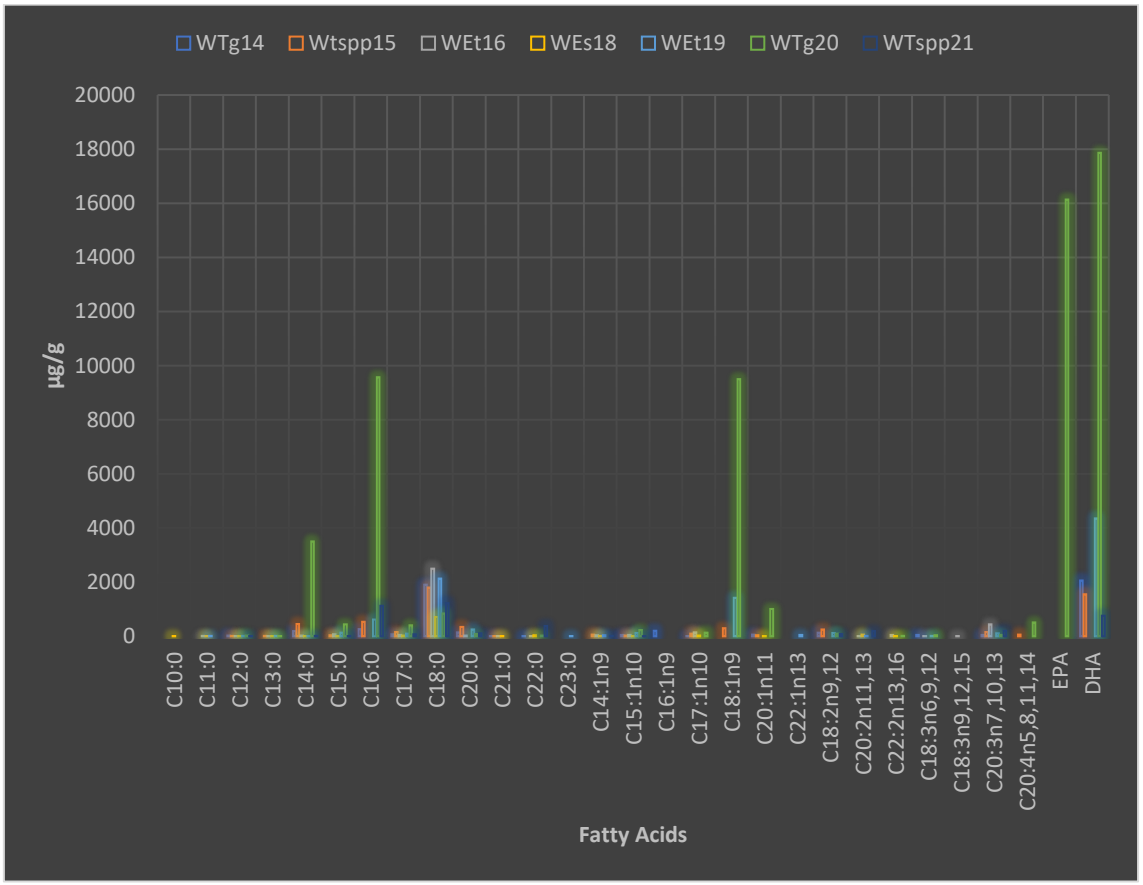

Figure. 13 - Graphic representing the fatty acids present in the samples collected in warmer waters (WTg – *T. gaudichaudii* collected in warmer waters; WTspp – *Thysanoessa* spp collected in warmer waters; WEt – *E. triacantha* collected in warmer waters; WEs – *E. superba* collected in warmer waters)

Fatty acid profiles were also described based in the species collected and not the location, *Euphausia superba* (Table 5), *Euphausia triacantha* (Table 6), *Themisto gaudichaudii* (Table 7) and *Thysanoessa* spp (Table 8).

Euphausia superba (Table 5) was collected in cold and warmer waters, corresponding to four samples collected in cold water (samples CEs1, CEs4, CEs5 and CEs22) and one collected in warmer water (sample WEs18). For this species, samples collected in cold waters showed a higher abundance of FA when compared to the sample collected in warm waters (Table 5). The values of total FA in cold waters were 3848 µg/g for sample CEs1, 3283 µg/g for sample CEs4, 2005 µg/g for sample CEs5 and 1893 µg/g for sample CEs22. For the sample collected in warmer waters, WEs18, the total amount of FA present was 932 µg/g. The FA profiles of *E. superba* in the studied areas were constituted by a considerable portion of SFAs. Sample CEs1 showed a percentage of 73.3% of SFAs in its composition; sample CEs4 revealed a value of 91.6%, the highest of the samples collected for this species. Sample CEs5 exhibited a value of 87.9% of SFAs in its constitution and sample CEs22 revealed the lowest amount of SFAs in samples collected of *E. superba*, 65.7%. The sample WEs18 showed a value of 86.6% of SFAs in its composition. It is possible to observe that all samples were mainly constituted by SFAs. The SFAs which showed to have contributed more largely to these values are C17:0, C18:0 and C22:0. PUFAs were the following contributors; however, its contribution is much smaller than the contribution of SFAs. PUFAs were found in all samples. Two of the samples collected in cold waters exhibited percentages of PUFAs above 10%, while the other two samples exhibited percentages below 10%. The sample collected in warmer waters exhibited a percentage of PUFAs contribution below 10%. Samples CEs1 and CEs22 showed a bigger amount of PUFAs in their composition (14.4% and 26.3%, respectively) and samples CEs4 and CEs5 showed smaller amounts of PUFAs in their compositions (3.5% and 5.7%, correspondingly). Sample WEs18 exhibited a value of 7.6% to describe the contribution of PUFAs in its composition. The PUFA that contributed the most for the percentages of PUFAs found in these samples was C18:3Δ9,12,15. MUFAs were the third contributor for the composition of samples of the species *E. superba*. Its contribution was even smaller than the one present in PUFAs, with only one sample revealing a percentage of MUFAs superior to 10%. Samples collected in cold water revealed percentages of MUFAs below 10%, except the sample CEs1. Samples CEs4, CEs5 and CEs22 all revealed low values of MUFAs, under 10% (4.9%, 6.5% and 8.0%, respectively), while sample CEs1 exhibited a value of 12.2% of MUFAs contribution. The sample collected in warmer water (WEs18) also exhibited a value for the contribution of MUFAs below to 10% (5.8%). The MUFA that most

contributed to these percentages was C17n1:10. HUFA's were not present in any of the samples collected either in cold or warmer waters.

Table 6 - Abundance (in µg/g) of fatty acids of the zooplankton species *E. superba* in the Southern Ocean, in cold and warmer regions.

FA (µg/g) \ Species	*E. superba* (CEs1)	*E. superba* (CEs4)	*E. superba* (CEs5)	*E. superba* (CEs22)	*E. superba* (WEs18)	*FA range*
C10:0	-	8 (0.3%)	-	-	2 (0.2%)	-
C11:0	30 (0.8%)	12 (0.4%)	3 (0.2%)	1 (0.1%)	2 (0.2%)	1 – 30
C12:0	9 (0.2%)	4 (0.1%)	6 (0.3%)	2 (0.1%)	2 (0.3%)	2 – 9
C13:0	75 (1.9%)	28 (0.9%)	34 (1.7%)	28 (1.5%)	3 (0.4%)	3 – 75
C14:0	-	10 (0.3%)	8 (0.4%)	5 (0.3%)	3 (0.3%)	3 - 10
C15:0	5 (0.1%)	4 (0.1%)	3 (0.1%)	2 (0.1%)	6 (0.6%)	2 – 6
C16:0	201 (5.2%)	-	-	-	-	-
C17:0	98 (2.5%)	69 (2.1%)	44 (2.2%)	67 (3.6%)	27 (2.9%)	44 – 98
C18:0	2374 (61.7%)	2712 (82.6%)	1650 (82.3%)	1000 (52.8%)	712 (76.4%)	712 – 2712
C20:0	8 (0.2%)	-	6 (0.3%)	3 (0.2%)	-	3 – 8
C21:0	9 (0.2%)	3 (0.1%)	3 (0.2%)	3 (0.2%)	3 (0.3%)	3 – 9
C22:0	-	158 (4.8%)	5 (0.2%)	133 (7.0%)	47 (5.1%)	5 – 158
C23:0	12 (0.3%)	-	-	-	-	-
Total SFA	2822 (73.3%)	3008 (91.6%)	1761 (87.9%)	1244 (65.7%)	806 (86.6%)	806 - 3008
C14:1Δ9	29 (0.9%)	20 (0.6%)	13 (0.6%)	15 (0.8%)	15 (1.7%)	13 – 29
C15:1Δ10	36 (0.9%)	2 (0.0%)	13 (0.7%)	19 (1.0%)	2 (0.2%)	2 – 36
C16:1Δ9	45 (1.2%)	7 (0.2%)	2 (0.1%)	38 (2.0%)	-	2 – 45
C17:1Δ10	210 (5.5%)	113 (3.4%)	88 (4.4%)	59 (3.1%)	27 (2.9%)	27 - 210
C18:1Δ9	138 (3.6%)	-	-	-	-	-
C20:1Δ11	12 (0.3%)	20 (0.6%)	13 (0.7%)	20 (1.1%)	10 (1.1%)	10 - 20
Total MUFA	470 (12.2%)	161 (4.9%)	129 (6.5%)	151 (8.0%)	54 (5.8%)	54 - 470
C18:2Δ9,12 (LA)	-	70 (2.1%)	8 (0.4%)	-		8 – 70
C20:2Δ11,13	-	28 (0.8%)	9 (0.5%)	-	-	9 – 28
C22:2Δ13,16	-	14 (0.4%)	15 (0.7%)	-	60 (6.4%)	14 – 60
C18:3Δ6,9,12	-	3 (0.1%)	1 (0.0%)	-	11 (1.2%)	1 – 11
C18:3Δ9,12, 15 (ALA)	354 (9.2%)	-	-	413 (21.8%)	-	354 – 413
C20:3Δ7,10, 13	202 (5.2%)	-	81 (4.0%)	86 (4.5%)	-	81 - 202
Total PUFA	556 (14.4%)	114 (3.5%)	114 (5.7%)	499 (26.3%)	71 (7.6%)	71 - 556
Total FA	3848	3283	2005	1893	932	932 - 3848
N	*18*	*19*	*20*	*17*	*16*	*16-20*

Euphausia triacantha was collected in cold, intermediate and warmer waters: one sample collected in cold waters (CEt3), three samples collected in intermediate waters (IEt8, IEt10 and IEt11) and two samples collected in warmer waters (WEt16 and WEt19). This species revealed a higher FA abundance in cold waters than in intermediate waters (Table 7). Nonetheless, the two samples collected in warmer waters revealed distinct abundances. The sample collected in cold waters (CEt3) revealed a total amount of FA of 9308 µg/g and the two samples collected in warmer waters showed values of FA abundance of 3505 µg/g (WEt16) and 9596 µg/g (WEt19). The three samples collected in transitional waters showed values of total FA abundance of 3295 µg/g (IEt8), 6344 µg/g (IEt10) and 2994 µg/g (IEt11). The FA profiles of *E. triacantha* in the studied areas were constituted by a considerable portion of SFAs. The sample collected in cold waters

revealed a percentage of 46.1% of SFAs in its composition, while samples collected in intermediate waters revealed variable value for SFAs portion in their composition: the samples IEt8 and IEt11 reveal a percentage above 60% (66.8% and 64.5%, respectively), while the sample IEt10 revealed a percentage of 43.3%, a value nearer to the one exhibited in cold waters. Samples collected in warmer waters showed very different results, with the sample WEt16 exhibiting a value of 78.1% for the contribution of SFAs and the sample WEt19 exhibiting a value of 34.6% for the same group of fatty acids. The value obtained for WEt19 showed the lowest abundance of SFAs exhibited by this species. The most abundant SFAs in this species, in general, were C14:0, C16:0, C17:0, C18:0 and C20:0. MUFAs were the following contributors to the total abundance of fatty acids. The sample CEt3 revealed a value of 16.4% of MUFAs in its composition. Samples collected in transitional waters revealed, once again, difference in its composition, with the samples IEt8 and IEt11 revealing values for MUFAs contribution closer to each other (25.4% and 27.8%, correspondingly) and the sample IEt10 exhibiting a value closer to the one showed by the sample CEt3 than to the others collected in cold areas. IEt10 exhibited a value of 13.3% of MUFAs contribution. Both samples collected in warm waters revealed a tendency to have a smaller percentage of MUFAs in their composition, with the sample WEt16 presenting a percentage of 7.2% and the sample WEt19 exhibiting a percentage of 17.1%. The most common MUFAs in this species were C15n1:10 and C18:1Δ9. The contribution of MUFAs is followed by the contribution of PUFAs, in which the sample collected in cold water, CEt3, presented a value of 5.0%. All three samples collected in transitional waters, IEt8, IEt10 and IEt11, revealed percentages of PUFAs below 10% (7.8%, 4.7% and 7.6%, correspondingly). The contribution of PUFAs in samples collected in warm waters vary with the sample WEt16 revealing a percentage of 14.8% and sample WEt19 exhibiting a percentage of 2.8%. The PUFA that most contributed to these results was C20:3Δ7,10,13. Lastly, the contribution of HUFAs was not uniform in this species. The sample CEt3, collected in cold water, exhibited a percentage of HUFAs contribution of 32.5%. However, in the samples collected in intermediate waters, only IEt10 exhibited the presence of HUFAs (38.7%) and the same is exhibited in both samples collected in warm waters (only the sample WEt19 reveals the presence of HUFAs, with 45.4% of its FA composition belonging to this group of fatty acids). The HUFA that most contributed to these values was DHA.

Table 7 - Abundance (in µg/g) of fatty acids of the zooplankton species *E. triacantha* in the Southern Ocean, in cold, intermediate and warmer regions.

Species / FA (µg/g)	*E. triacantha* (CEt3)	*E. triacantha* (IEt8)	*E. triacantha* (IEt10)	*E. triacantha* (IEt11)	*E. triacantha* (WEt16)	*E. triacantha* (WEt19)	*FA range*
C10:0	-	17 (0.5%)	-	-	-	-	11 - 17
C11:0	72 (0.8%)	8 (0.3%)	11 (0.2%)	16 (0.5%)	16 (0.5%)	18 (0.2%)	8 - 72
C12:0	-	-	15 (0.2%)	-	8 (0.2%)	16 (0.2%)	8 - 16
C13:0	28 (0.3%)		34 (0.5%)	-	4 (0.1%)	17 (0.2%)	4 - 34
C14:0	443 (4.8%)	25 (0.8%)	219 (3.5%)		30 (0.9%)	6 (0.1%)	6 - 443
C15:0	200 (2.1%)	-	29 (0.5%)	12 (0.4%)	80 (2.3%)	140 (1.5%)	29 - 200
C16:0	763 (8.2%)	-	436 (6.9%)	-	-	621 (6.5%)	436 - 763
C17:0	119 (1.3%)	118 (3.6%)	179 (2.8%)	-	56 (1.6%)	105 (1.1%)	56 - 179
C18:0	2265 (24.3%)	1849 (56.1%)	1539 (24.3%)	154 (5.1%)	2501 (71.4%)	2140 (22.3%)	154 - 2501
C20:0	330 (3.5%)	81 (2.5%)	166 (2.6%)	1587 (53.0%)	31 (0.9%)	252 (2.6%)	31 - 1587
C21:0	1 (0.0%)	4 (0.1%)	2 (0.0%)	163 (5.4%)	5 (0.1%)	-	1 - 163
C22:0	66 (0.7%)	100 (3.0%)	118 (1.9%)	-	6 (0.2%)	-	6 - 118
C23:0	-	-	-	-	-	6 (0.1%)	-
Total SFA	4287 (46.1%)	2203 (66.8%)	2749 (43.3%)	1932 (64.5%)	2736 (78.1%)	3321 (34.6%)	1932 - 4287
C14:1Δ9	57 (0.6%)	48 (1.5%)	47 (0.7%)	50 (1.7%)	46 (1.3%)	41 (0.4%)	41 - 57
C15:1Δ10	171 (1.8%)	68 (2.1%)	125 (2.0%)	65 (2.2%)	52 (1.5%)	124 (1.3%)	52 - 171
C17:1Δ10	308 (3.3%)	-	53 (0.8%)	-	153 (4.4%)	-	53 - 308
C18:1Δ9	886 (9.5%)	720 (21.8%)	592 (9.3%)	719 (24.0%)	-	1427 (14.9%)	592 - 1427
C20:1Δ11	108 (1.2%)	-	26 (0.4%)	-	-	-	26 - 108
C22:1Δ13						53 (0.6%)	-
Total MUFA	1530 (16.4%)	835 (25.4%)	843 (13.3%)	833 (27.8%)	251 (7.2%)	1645 (17.1%)	251 - 1645
C18:2Δ9,12 (LA)	125 (1.3%)	-	50 (0.8%)	46 (1.5%)		121 (1.3%)	46 - 125
C20:2Δ11,13	57 (0.6%)	-	29 (0.5%)	-	8 (0.2%)	17 (0.2%)	8 - 57
C22:2Δ13,16	39 (0.4%)	37 (1.1%)	24 (0.4%)	-	56 (1.6%)	-	24 - 56
C18:3Δ6,9,12	-	-	-	-	3 (0.1%)	18 (0.2%)	3 - 18
C18:3Δ9,12,15 (ALA)	13 (0.1%)	-	-	-	8 (0.2%)	-	8 - 13
C20:3Δ7,10,13	232 (2.5%)	220 (6.7%)	197 (3.1%)	183 (6.1%)	443 (12.6%)	115 (1.2%)	115 - 443
Total PUFA	466 (5.0%)	257 (7.8%)	300 (4.7%)	229 (7.6%)	518 (14.8%)	271 (2.8%)	229 - 518
C20:4Δ5,8,11,14 (ARA)	81 (0.9%)	-	-	-	-	-	-
C20:5Δ5,8,11,14,17 (EPA)	1554 (16.7%)	-	-	-	-	-	-
C22:6Δ4,7,10,13,16,19 (DHA)	1390 (14.9%)	-	2452 (38.7%)	-	-	4359 (45.4%)	1390 - 4359
Total HUFA	3025 (32.5%)	-	2452 (38.7%)	-	-	4359 (45.4%)	1390 - 4359
Total FA	9308	3295	6344	2994	3505	9596	2994 - 9596
N	*23*	*13*	*21*	*10*	*18*	*19*	*10 - 21*

Themisto gaudichaudii was collected in intermediate and warmer waters: four samples collected in intermediate waters (ITg6, ITg9, ITg12 and ITg13) and two samples

collected in warmer waters (WTg14 and WTg20). This species showed higher FA abundance in warmer waters than in intermediate waters (Table 8). The values of total FA in warmer waters were 5276 µg/g and 60542 µg/g for the samples collected in warmer waters, while the values of total FA for each sample collected in transitional waters were 4841 µg/g, 9008 µg/g, 2006 µg/g and 2709 µg/g, respectively. The FA profiles of *T. gaudichaudii* in the studied areas were constituted by a considerable portion of SFA's in all samples excluding samples 9 and 20. Sample ITg6 showed a percentage of 63.24% of SFAs present in its composition, sample ITg9 exhibited a value of 42.07%, and the samples ITg12 and ITg13 revealed a value upper to 70%, in both samples (85.5% and 71.5%, respectively). Both samples collected in warmer waters registered a value of SFAs in their composition lower than 50% (WTg14 registered 49.9% and WTg20 exhibited a value of 24.7%). The SFAs that were most present in these profiles were C16:0 and C18:0. After SFAs, MUFAs contributed the most for the FA profiles in samples for *T. gaudichaudii*. The samples ITg6, ITg9 and ITg12 all exhibited a percentage of MUFAs present in their composition lower than 10% (2.2%, 7.8%, 6.2%, respectively) and the sample ITg13 showed a value of 16.7% for MUFAs. Samples collected in warmer waters also revealed different values for MUFAs in their compositions, with WTg14 presenting a value of 6.3% and WTg20 a value of 18.0%. The most common MUFAs were C18n1:19 and C20n1:11.The contribution of MUFAs is followed by the contribution of PUFAs, to which each sample contributed less than 10%, excluding the sample ITg13 (11.8%). The samples ITg6, ITg9 and ITg12, collected in intermediate waters, exhibited values of PUFAs of 6.3%, 3.7% and 8.3%, respectively, while ITg13, also collected in transitional waters, exhibited a value of PUFAs of 11.8% (as previously mentioned), becoming, of all the studied samples for *Themisto gaudichaudii*, the one with greater amount of PUFAs in its composition. Samples collected in warmer waters, WTg14 and WTg20, all revealed a contribution of PUFAs inferior to 5% in both samples; sample WTg14 revealed a percentage of 4.5% of PUFA contribution and WTg20, a contribution of 0.3%. The most representative PUFA of all samples was C18:2Δ9,12. Lastly, the contribution of HUFAs was minor in almost every sample collected of this species, except the sample WTg20, due to its high abundance of DHA and EPA. In the samples collected in intermediate waters, the contribution of HUFAs varied, with the samples ITg6 and ITg9 showing a high percentage of HUFAs (28.2% and 46.5%, respectively) and the samples ITg12 and ITg13 not showing HUFAs. Both samples collected in warmer waters (WTg14 and WTg20) showed high abundances of HUFAs (39.2% and 57.0%, correspondingly). In all samples, the abundance of HUFAs depended mostly in DHA, except in the sample WTg20, in which the contribution of both DHA and EPA had a big impact. The sample

WTg20 had the highest abundance of FAs of all the collected samples, differentiating from other samples due to its large quantities of FAs.

Table 8 - Abundance (in µg/g) of fatty acids of the zooplankton species *T. gaudichaudii* in the Southern Ocean, in intermediate and warmer regions.

Species / FA (µg/g)	*T. gaudichaudii* (ITg6)	*T. gaudichaudii* (ITg9)	*T. gaudichaudii* (ITg12)	*T. gaudichaudii* (ITg13)	*T. gaudichaudii* (WTg14)	*T. gaudichaudii* (WTg20)	*FA range*
C11:0	54 (1.1%)	-	109 (5.4%)	51 (1.9%)	-	-	51 - 109
C12:0	25 (0.5%)	-	92 (4.6%)	11 (0.4%)	26 (0.5%)	27 (0.0%)	11 - 92
C13:0	6 (0.1%)	-	114 (5.7%)	4 (0.2%)	-	4 (0.0%)	4 - 114
C14:0	2 (0.0%)	583 (6.5%)	284 (14.2%)	229 (8.5%)	194 (3.7%)	3511 (5.8%)	2 - 3511
C15:0	5 (0.1%)	-	73 (3.6%)	81 (3.0%)	-	443 (0.7%)	5 - 443
C16:0	439 (9.1%)	1030 (11.4%)	-	459 (17.0%)	269 (5.1%)	9578 (15.8%)	269 - 9578
C17:0	30 (0.6%)	70 (0.8%)	18 (0.9%)	69 (2.6%)	73 (1.4%)	407 (0.7%)	18 - 407
C18:0	2309 (47.7%)	1555 (17.3%)	753 (37.5%)	855 (31.6%)	1910 (36.2%)	841 (1.4%)	753 - 2309
C20:0	182 (3.8%)	482 (5.4%)	152 (7.6%)	43 (1.6%)	150 (2.8%)	87 (0.1%)	43 - 482
C21:0	-	-	2 (0.1%)	5 (0.2%)	2 (0.0%)	-	2 - 5
C22:0	10 (0.2%)	69 (0.8%)	120 (6.0%)	128 (4.7%)	10 (0.2%)	31 (0.1%)	10 - 128
Total SFA	3062 (63.2%)	3790 (42.1%)	1716 (85.5%)	1937 (71.5%)	2635 (49.9%)	14930 (24.7%)	1716 - 14930
C14:1Δ9	4 (0.1%)	30 (0.3%)	4 (0.2%)	11 (0.4%)	-	-	4 - 30
C15:1Δ10	52 (1.1%)	75 (0.8%)	43 (2.2%)	34 (1.2%)	54 (1.0%)	229 (0.4%)	34 - 229
C16:1Δ9					211 (4.0%)	-	-
C17:1Δ10	52 (1.1%)	-	-	163 (6.0%)	8 (0.1%)	139 (0.2%)	8 - 163
C18:1Δ9	-	596 (6.6%)	77 (3.8%)	245 (9.0%)	-	9501 (15.7%)	77 - 9501
C20:1Δ11	-	-	-	-	59 (1.1%)	1010 (1.7%)	59 - 1010
Total MUFA	108 (2.2%)	701 (7.8%)	124 (6.2%)	452 (16.7%)	333 (6.3%)	10879 (18.0%)	108 - 10879
C18:2Δ9,12	243 (5.0%)	332 (3.7%)	56 (2.8%)	129 (4.8%)	137 (2.6%)	99 (0.2%)	56 - 332
C20:2Δ11,13	-	-	-	18 (0.7%)	-	-	
C22:2Δ13,16	12 (0.2%)	-	13 (0.7%)	-	-	20 (0.0%)	12 - 20
C18:3Δ6,9,12	42 (0.9%)	-	-	-	52 (1.0%)	37 (0.1%)	37 - 52
C18:3Δ9,12,15	1 (0.0%)	-	-	-	-	-	-
C20:3Δ7,10,13	9 (0.2%)	-	96 (4.8%)	172 (6.4%)	51 (1.0%)	53 (0.1%)	9 - 172
Total PUFA	306 (6.3%)	332 (3.7%)	166 (8.3%)	320 (11.8%)	240 (4.5%)	208 (0.3%)	166 - 332
C20:4Δ5,8,11,14 (ARA)	-	-	-	-	-	517 (0.9%)	-
C20:5Δ5,8,11,14,17 (EPA)	-	-	-	-	-	16144 (26.7%)	-
C22:6Δ4,7,10,13,16,19 (DHA)	1366 (28.2%)	4185 (46.5%)	-	-	2069 (39.2%)	17864 (29.5%)	1366 – 17864
Total HUFA	1366 (28.2%)	4185 (46.5%)	-	-	2069 (39.2%)	34525 (57.0%)	1366 - 34525
Total FA	4841	9008	2006	2709	5276	60542	932 - 60542
N	*19*	*11*	*16*	*18*	*16*	*20*	*11 - 20*

Thysanoessa spp. was collected in intermediate and warmer water: one sample collected in intermediate water (ITspp7) and two samples collected in warmer water (WTspp15 and WTspp21). *Thysanoessa* spp. showed higher FA abundance in warmer waters than in intermediate waters (Table 9). For the sample collected in transitional waters, the total amount of fatty acids was 3570 µg/g, while for both samples collected in warmer waters (WTspp15 and WTspp21) the amount of fatty acids present in their constitution were 5954 µg/g and 4256 µg/g, respectively. The FA profiles of *Thysanoessa* spp. in the studied areas were constituted by a considerable portion of SFAs. The sample collected in intermediate waters, ITspp7, revealed a percentage of 72.9% for the contribution of SFAs in its composition. The samples collected in warmer waters, WTspp15 and WTspp21, exhibited percentages of 56.9% and 69.5% fo the contribution of SFAs, correspondingly. The most present SFAs in the fatty acid composition of the samples for this species were C14:0, C16:0,C17:0, C18:0 and C20:0. PUFAs were the following contributors in this species composition. ITspp7, the only sample collected in transitional waters, exhibited a percentage of 11.6% of PUFAs in its constitution, while samples WTspp15 and WTspp21, both collected in warm waters, reveal a percentage of 7.0% and 11.7%, respectively. The main contributors for the abundance of PUFAs in these samples were C18:2Δ9,12 and C20:3Δ7,10,13. PUFAs are followed by MUFAs, with varying values for each sample. The sample collected in transitional waters, ITspp7, had the highest abundance of MUFAs in its composition, when compared to the other samples collected for this species, with a value of 15.5%. Samples WTspp15 revealed a lower percentage, at 8.8%, while sample WTspp21 exhibited the lowest percentage between these samples, at 0.8%. MUFAs contribution was mainly represented by C17:1Δ10 and C18:1Δ9. HUFAs were not present in all samples collected, being completely absent in the sample collected in intermediate waters, ITspp7. HUFAs were present in samples WTspp15 and WTspp21, both collected in warmer waters, representing 27.36% and 18.01%, correspondingly. The HUFA that most contributed to these values was DHA.

Table 9 - Abundance (in µg/g) of fatty acids of the zooplankton species *Thysanoessa* spp in the Southern Ocean, in intermediate and warmer regions.

FA (µg/g) \ Species	Thysanoessa spp. (ITspp7)	*Thysanoessa* spp. (WTspp15)	*Thysanoessa* spp. (WTspp21)
C11:0	209 (5.9%)	-	-
C12:0	-	15 (0.2%)	8 (0.2%)
C13:0	-	15 (0.2%)	-
C14:0	235 (6.6%)	456 (7.7%)	16 (0.4%)
C15:0	-	47 (0.8%)	8 (0.2%)
C16:0	188 (5.3%)	538 (9.0%)	1125 (26.4%)
C17:0	222 (6.2%)	165 (2.8%)	72 (1.7%)
C18:0	1561 (43.7%)	1801 (30.2%)	1220 (28.7%)
C20:0	117 (3.3%)	346 (5.8%)	118 (2.8%)
C21:0	6 (0.2%)	4 (0.1%)	-
C22:0	65 (1.8%)	-	388 (9.1%)
Total SFA	2604 (72.9%)	3387 (56.9%)	2957 (69.5%)
C14:1Δ9	93 (2.6%)	62 (1.0%)	22 (0.5%)
C15:1Δ10	63 (1.8%)	25 (0.4%)	12 (0.3%)
C17:1Δ10	278 (7.8%)	97 (1.6%)	-
C18:1Δ9	118 (3.3%)	297 (5.0%)	-
C20:1Δ11	-	41 (0.7%)	-
Total MUFA	552 (15.5%)	522 (8.8%)	34 (0.8%)
C18:2Δ9,12 (LA)	-	254 (4.3%)	93 (2.2%)
C20:2Δ11,13	-	-	201 (4.7%)
C22:2Δ13,16	-	-	-
C18:3Δ6,9,12	-	-	-
C18:3Δ9,12,15 (ALA)	-	-	-
C20:3Δ7,10,13	414 (11.6%)	163 (2.7%)	204 (4.8%)
Total PUFA	414 (11.6%)	417 (7.0%)	499 (11.7%)
C20:4Δ5,8,11,14 (ARA)	-	71 (1.2%)	-
C20:5Δ5,8,11,14,17 (EPA)	-	-	-
C22:6Δ4,7,10,13,16,19 (DHA)	-	1558 (26.2%)	767 (18.0%)
Total HUFA	-	1629 (27.4%)	767 (18.0%)
Total FA	3570	5954	4256
N	*13*	*18*	*14*

The two-dimensional n-MDS plot showed a separation of samples based on fatty acid concentration and composition (stress=0.09). Four groups can be defined (Figure 14). Group A present the less diversified and the lowest abundance in FA, including *Euphausia superba* from cold waters and *Euphausia triacantha* and *E. superba* from warmer waters. Group B comprised the species with more diversed and slightly higher abundance in FA, including *Thysanoessa* spp., *Euphausia triacantha* and *Themisto gaudichaudii*, all collected in transitional waters. Group C included the species that presented the highest abundance in FA from all groups formed, including *E. triacantha* collected in cold waters, *T. gaudichaudii* and *E. triacantha* collected in transitional waters and *T. gaudichaudii, E. tricantha* and *Thysanoessa* spp. in warmer waters. Sample 20, *E. triacantha*, collected in warm water represents the 4th group, isolated from the other samples due to higher values of abundance of fatty acids when compared to the groups formed.

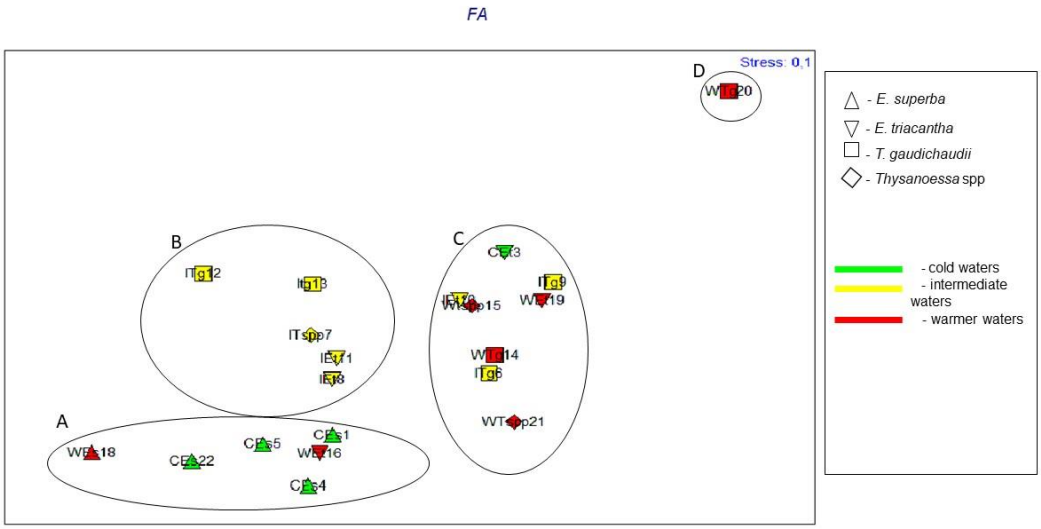

Figure 14 - Two dimensional non-metric multidimensional scaling (n-MDS) ordination plot of fatty acid composition of the zooplankton species sampled at the Southern Ocean, in cold, intermediate and warm temperatures

The ANOSIM analysis showed a clear separation of the groups defined by n-MDS (R=0.851, p=0.001). When comparing pair wise tests, groups A, B and C were significantly different ($p \leq 0.05$), but group D showed p values higher to 0.05 (due to the low number of permutations allowed by group D, which consists of one single sample) and presented high R values, showing good segregation (A/B: R = 0.48, p=0.002 ;A/C: R= 0.947, p= 0.001; A/D: R= 1, p=0.143; B/C: R= 0.889, p= 0.001; C/D: R=1, p=0.111; B/D: R=1, p=0.167).

SIMPER analysis (similarities described in Table 10) showed that at group A the fatty acids that explained 62.42% of the group similarity were, in decreasing order, C18:0; C17:1Δ10; C17:0; C20:3Δ7,10,13; and C22:0; at group B the fatty acids that explained 62.79% of the group similarity, in decreasing order of importance were C18:0; C18:1Δ9; C20:3Δ7,10,13; C20:0; C14:0; C17:0; C22:0 and C15:1Δ10; at group C the fatty acids that explained 68.79% of the group similarity, in decreasing order of importance, were C18:0; DHA; C16:0; C20:0; C18:1Δ9; C18:2Δ9,12 and C14:0 (Table 5). In general, the fatty acids that most contributed for the similarities within each group were C18:0; C18:1Δ9; C18:2Δ9,12; C14:0 and C20:3Δ7,10,13. Group D shows no similarity in this analysis because there are less than two samples present in this group.

Table 10 - Results of SIMPER analysis of fatty acid abundance showing average similarity among the species inside each group according to non-metric multidimensional scaling (n-MDS) analysis.

Group	Average Similarity	Fatty Acids	Av. Abundance	Av. Sim.	Sim/SD	Contrib%	Cum. %
A	62.42	C18:0	1.82	47.23	4.95	75.67	75.67
		C17:1Δ10	0.11	3.63	2.54	5.81	81.48
		C17:0	0.06	2.58	4.38	4.13	85.61
		C20:3Δ7,10,13	0.14	2.02	0.75	3.24	88.85
		C22:0	0.06	1.16	0.54	1.86	90.71
B	62.79	C18:0	1.32	30.83	4.82	49.10	49.10
		C18:1Δ9	0.38	7.19	1.10	11.45	60.55
		C20:3Δ7,10,13	0.22	6.08	4.21	9.68	70.23
		C20:0	0.11	3.48	1.96	5.54	75.78
		C14:0	0.16	3.34	0.81	5.33	81.10
		C17:0	0.12	2.75	1.46	4.39	85.49
		C22:0	0.08	2.24	1.04	3.57	89.06
		C15:1Δ10	0.05	1.98	3.87	3.16	92.23
C	68.79	C18:0	1.84	22.32	5.80	32.44	32.44
		DHA	2.27	20.47	3.86	29.76	62.20
		C16:0	0.65	8.81	3.52	12.81	75.00
		C20:0	0.25	3.75	4.19	5.44	80.45
		C18:1Δ9	0.47	2.81	0.70	4.09	84.54
		C18:2Δ9,12	0.17	2.49	1.99	3.63	88.17
		C14:0	0.24	1.94	0.76	2.82	90.98

In terms of dissimilarities between groups, described in Table 11, the fatty acids that contributed, in decreased order of importance for i) 46.16% of the dissimilarity among groups A/B were C18:0; C18:1Δ9; C20:3Δ7,10,13; C14:0; C16:0; C18:3Δ9,12,15; C20:0; C17:1Δ10; C17:0; C11:0; C22:0; C18:2Δ9,12; C13:0 and C15:1Δ10, ii) 60.24% of the dissimilarity among groups A/C were DHA; C16:0; C18:1Δ9; C18:0; C20:0; C14:0; C18:2Δ9,12; C20:3Δ7,10,13; C18:3Δ9,12,15; C22:0; EPA; C17:1Δ10 and C15:1Δ10, iii) 50.7% of the dissimilarity between groups B and C were DHA; C16:0; C18:1Δ9; C18:0; C14:0; C18:2Δ9,12; C20:0; C20:3Δ7,10,13; C17:1Δ10; C22:0; EPA; C11:0 and C17:0, iv) 88.79% of the dissimilarity among groups A/D were DHA; EPA; C18:1Δ9; C16:0; C14:0; C20:1Δ11; ARA and C18:0, v) 65.05% of the dissimilarity among groups C/D were EPA; C18:1Δ9; C16:0; DHA; C14:0; C20:1Δ11; C18:0; ARA and C15:0, vi) 81.60% of the dissimilarity among groups B/D were DHA; EPA; C16:0; C18:1Δ9; C14:0; C20:1Δ11; ARA and C15:0.

Table 11 - Results of SIMPER analysis of fatty acid abundance showing average dissimilarity between sample groups according to non-metric multidimensional scaling (n-MDS) analysis.

Group	Average Dissimilarity	Fatty acids	Av. Abund		Av. Diss	Diss/ SD	Contrib .%	Cum. %
A/B	46.16	C18:0	1.82	1.32	8.48	1.5	18.37	18.37
		C18:1Δ9	0.02	0.38	7.43	1.28	16.1	34.47
		C20:3Δ7,10,13	0.14	0.22	3.68	1.49	7.96	42.43
		C14:0	0.01	0.16	3.53	1.21	7.66	50.09
		C16:0	0.03	0.13	2.9	0.77	6.29	56.37
		C18:3Δ9,12,15	0.13	0.00	2.64	0.68	5.71	62.09
		C20:0	0.01	0.11	2.6	1.88	5.63	67.72
		C17:1Δ10	0.11	0.09	2.58	1.85	5.58	73.3
		C17:0	0.06	0.12	1.75	1.39	3.78	77.08
		C11:0	0.01	0.08	1.72	1.04	3.72	80.8
		C22:0	0.06	0.08	1.7	1.54	3.68	84.49
		C18:2Δ9,12	0.01	0.05	1.18	1.05	2.55	87.04
		C13:0	0.03	0.02	1,00	1.03	2.17	89.21
		C15:1Δ10	0.02	0.05	0.94	1.61	2.04	91.25
A/C	60.24	C22:6Δ4,7,10,13,16, 19 (DHA)	0,00	2.27	18.67	3.43	30.99	30.99
		C16:0	0.03	0.65	7.84	2.13	13.01	44.01
		C18:1Δ9	0.02	0.47	4.98	1.13	8.26	52.27
		C18:0	1.82	1.84	4.87	1.34	8.09	60.36
		C20:0	0.01	0.25	3.5	2.79	5.81	66.17
		C14:0	0.01	0.24	3.05	1.19	5.07	71.24
		C18:2Δ9,12	0.01	0.17	2.44	1.72	4.05	75.28
		C20:3Δ7,10,13	0.14	0.12	1.99	1.29	3.31	78.59
		C18:3Δ9,12,15	0.13	0,00	1.79	0.69	2.97	81.56
		C22:0	0.06	0.08	1.54	0.84	2.56	84.12
		C20:5Δ5,8,11,14,17 (EPA)	0,00	0.19	1.46	0.37	2.42	86.54
		C17:1Δ10	0.11	0.06	1.44	1.65	2.39	88.93
		C15:1Δ10	0.02	0.08	0.95	1.45	1.57	90.51

Table 11 (cont.) - Results of SIMPER analysis of fatty acid abundance showing average dissimilarity between sample groups according to non-metric multidimensional scaling (n-MDS) analysis.

Group	Average Dissimilarity	Fatty acids	Av. Abund		Av. Diss	Diss/SD	Contrib. %	Cum. %
C/B	50.7	C22:6Δ4,7,10,13,16,19 (DHA)	2.27	0.00	16.96	3.57	33.45	33.45
		C16:0	0.65	0.13	6	1.68	11.84	45.28
		C18:1Δ9	0.47	0.38	4.73	1.42	9.34	54.62
		C18:0	1.84	1.32	4.02	1.25	7.93	62.56
		C14:0	0.24	0.16	2.54	1.42	5.02	67.58
		C18:2Δ9,12	0.17	0.05	1.78	1.45	3.52	71.09
		C20:0	0.25	0.11	1.73	1.37	3.41	74.51
		C20:3Δ7,10,13	0.12	0.22	1.69	1.21	3.33	77.83
		C17:1Δ10	0.06	0.09	1.44	1.12	2.84	80.67
		C22:0	0.08	0.08	1.4	0.98	2.76	83.43
		C20:5Δ5,8,11,14,17 (EPA)	0.19	0.00	1.36	0.37	2.68	86.11
		C11:0	0.02	0.08	1.02	1.09	2.02	88.13
		C17:0	0.1	0.12	0.99	1.34	1.96	90.09
A/D	88.79	C22:6Δ4,7,10,13,16,19 (DHA)	0.00	17.86	17.50	26.01	19.71	19.71
		C20:5Δ5,8,11,14,17 (EPA)	0.00	16.14	16.93	26.01	19.07	38.78
		C18:1Δ9	0.02	9.50	13.89	18.13	15.64	54.42
		C16:0	0.03	9.58	13.88	15.86	15.63	70.05
		C14:0	0.01	3.51	8.92	24.37	10.05	80.10
		C20:1Δ11	0.01	1.01	4.08	26.35	4.60	84.70
		C20:4Δ5,8,11,14	0.00	0.52	2.48	26.01	2.80	87.50
		C18:0	1.82	0.84	2.41	1.48	2.71	90.21
C/D	65.05	C20:5Δ5,8,11,14,17 (EPA)	0.19	16.14	14.10	6.38	21.67	21.67
		C18:1Δ9	0.47	9.50	10.44	4.64	16.06	37.73
		C16:0	0.65	9.58	9.65	7.91	14.84	52.56
		C22:6Δ4,7,10,13,16,19 (DHA)	2.27	17.86	9.43	4.08	14.50	67.06
		C14:0	0.24	3.51	6.76	5.58	10.39	77.45
		C20:1Δ11	0.03	1.01	3.46	10.53	5.31	82.77
		C18:0	1.84	0.84	2.18	3.08	3.35	86.12
		C20:4Δ5,8,11,14	0.02	0.52	2.06	8.13	3.17	89.29
		C15:0	0.05	0.44	1.65	3.94	2.53	91.82
B/D	81.60	C22:6Δ4,7,10,13,16,19 (DHA)	0.00	17.86	16.94	47.79	20.75	20.75
		C20:5Δ5,8,11,14,17 (EPA)	0.00	16.14	16.38	47.79	20.08	40.83
		C16:0	0.13	9.58	12.97	11.64	15.90	56.73
		C18:1Δ9	0.38	9.50	11.84	8.73	14.51	71.24
		C14:0	0.16	3.51	7.87	11.86	9.65	80.89
		C20:1Δ11	0.00	1.01	4.03	47.79	4.93	85.82
		C20:4Δ5,8,11,14	0.00	0.52	2.40	47.79	2.95	88.77
		C15:0	0.03	0.44	1.94	9.02	2.38	91.15

3.3. Fatty Acid Trophic Markers

Fatty Acid Trophic Markers (FATMs) and diet sources were determined for each zooplankton species sampled in cold, intermediate and warmer waters (Tables 12, 13, 14 and 15).

Euphausia superba doesn't reveal differences between locations in its FATMs (Table 12). *E. superba* collected in cold waters, exhibits the presence of dinoflagellates in its diet, as it is shown by the C16:1Δ9/C16:0 ratio. All samples of *E. superba* collected in cold waters, have the presence of C16:1Δ9, indicating diatoms consumption by the ratio C16:1Δ9/C16:0. In general, *E. superba* exhibits little consumption on bacteria (C15:0 and C17:0) and small traces of carnivorism, due to the presence of C20:1Δ11 in these samples. One sample (CEs1) collected in cold waters also exhibit the presence of C18:1Δ9, related to carnivory, and two other samples (CEs4 and CEs5) exhibit the presence of C18:2ω6, related to green algae or detritus consumption. *E. superba* collected in warmer waters reveals a profile alike that described for *E. superba* collected in cold waters, with a small ratio of PUFAs/HUFAs and the evidence of bacteria consumption, through the C15:0 + C17:0 ratio. It was also possible to note small traces of carnivory, due to the presence of C20:1Δ11.

Based on the FATMs of *Euphausia triacantha* (described in Table 13), it is possible to assume an omnivorous behaviour, due to the low ratio PUFAs/SFAs in all samples collected at the three different temperatures. *E. triacantha* collected in cold waters shows a ratio of DHA/EPA of 0.89, which shows a bigger consumption of diatoms than in dinoflagellates. Nonetheless, some samples of *E. triacantha* (IEt10, collected in transitional waters, and WEt19, collected in warmer waters), exhibit high concentrations of DHA (DHA=2.45 and 4.36, correspondingly), which indicates a consumption on dinoflagellates. All samples collected for *E. triacantha* show little consumption of bacteria and small traces of carnivorism (small concentrations of C18:1Δ9 and C20:1Δ11). In one of the samples collected in warmer waters (WEt19), it is possible to observe a great concentration of C18:1Δ-9 (C18:1Δ-9 = 1.43), which is significant of a high intake of zooplankton. Some other samples collected for this species and in different environments (CEt3, collected in cold waters, IEt10, collected in transitional waters, and Wet19, collected in warmer waters) also show some green algae or detritus consumption at a small extent (small amounts of C18:2Δ-6 were found in these 3 samples).

Themisto gaudichaudii did not exhibit a carnivorous behaviour for the majority of the collected samples, both in intermediate and in warmer waters (ITg6, ITg9, ITg12, ITg13, in intermediate waters, and WTg14 collected in warmer waters), since they did not present high amounts of FATMs characteristic for this behaviour (Table 14). However, sample WTg20, *T. gaudichaudii* collected in warmer waters, showed high concentrations

of C18:1Δ-9, characteristic of carnivory, C20:1Δ-9, also characteristic of carnivory, and DHA, characteristic of dinoflagellates consumption, but its PUFAs/SFAs ratio is very low, which indicates an omnivorous behaviour. In two samples collected in intermediate waters (ITg6 and ITg9) it is possible to observe an intake of dinoflagellates, due to the concentration of C16:0. This fatty acid is also present in the samples collected in warmer waters for this species (WTg14 and WTg20). In one of the samples collected in intermediate waters (ITg9) and in one of the samples collected in warmer waters (WTg14), there is also a high concentration of DHA, which relates to the intake of dinofalgellates. Sample WTg20 showed the highest concentration of DHA, but also high concentrations of EPA, which confirms the intake of dinoflagellates (by the presence of DHA) and diatoms (by the presence of EPA), with a higher consumption of dinoflagellates than of diatoms. All samples collected for this species exhibited trace amounts of trophic markers for bacteria (C15:0 +C17:0) and green algae or detritus (C18:2ω-6) consumption.

Dietary sources, reflected by the FATMs, indicated that the samples collected for *Thysanoessa* spp. exhibited a non-carnivorous behaviour (Table 15). Both of the samples collected in warmer waters (WTspp15 and WTspp21) reflected an intake of dinoflagellates, shown by the concentration of DHA. The concentration of C16:0 and the lack of C16:1Δ9 also suggests dinoflagellates consumption. The only sample of *Thysanoessa* spp. collected in intermediate waters, ITspp7 also shows a small amount of C16:0 and complete lack of C16:1Δ9, also revealing dinoflagellates intake and no diatoms intake for this sample. All samples showed trace amounts of bacteria (C15:0+C17:0) trophic marker and green algae or detritus (C18:2ω-6) consumption.

Table 12 - Fatty acid trophic markers of samples collected for *Euphausia superba*.

FATM's / Species	CEs1	CEs4	CEs5	CEs22	WEs18
PUFA's/SFA's	0.20	0.04	0.06	0.40	0.09
DHA					
EPA					
DHA/EPA					
C16:1Δ9/C16:0	0.22				
C15:0 + C17:0	0.10	0.07	0.05	0.07	0.03
C18:1Δ9	0.14				
C20:1Δ11	0.01	0.02	0.01	0.02	0.01
C18:2ω-6		0.07	0.01		

Table 13 - Fatty acid trophic markers of the samples collected for *Euphausia triacantha*

FATM's / Species	CEt3	IEt8	IEt10	IEt11	WEt16	WEt19
PUFA's/SFA's	0.11	0.12	0.11	0.12	0.19	0.08
DHA	1.39		2.45			4.36
EPA	1.55					
DHA/EPA	0.89					
C16:1Δ9/C16:0						
C15:0 + C17:0	0.32	0.12	0.21	0.15	0.14	0.24
C18:1Δ9	0.89	0.72	0.59	0.72		1.43
C20:1Δ11	0.11		0.03	0.00		
C18:2ω-6	0.13		0.05	0.05		0.12

Table 14 – Fatty acid trophic markers of the samples collected for *Themisto gaudichaudii*.

FATM's / Species	ITg6	ITg9	ITg12	Itg13	WTg14	WTg20
PUFA's/SFA's	0.10	0.09	0.10	0.17	0.09	0.01
DHA	1.37	4.18			2.07	17.86
EPA						16.14
DHA/EPA						1.11
C16:1Δ7/C16:0					0.79	
C15:0 + C17:0	0.03	0.07	0.09	0.15	0.07	0.85
C18:1Δ9		0.60	0.08	0.25		9.50
C20:1Δ11					0.06	1.01
C18:2ω-6	0.24	0.33	0.06	0.13	0.14	0.10

Table 15 - Fatty acid trophic markers of the samples collected for *Thysanoessa* spp.

FATM's / Species	ITspp7	Wtspp15	WTspp21
PUFA's/SFA's	0.16	0.12	0.17
DHA		1.56	0.77
EPA			
DHA/EPA			
C16:1Δ7/C16:0			
C15:0 + C17:0	0.22	0.21	0.08
C18:1Δ9	0.12	0.30	
C20:1Δ11		0.04	
C18:2ω-6		0.25	0.09

4. Discussion

While *Themisto gaudichaudii*, *Euphausia triacantha* and *Thysanoessa* spp reveal a fatty acid profile alike the ones described for these species in literature, *Euphausia superba* revealed a low body condition detected by the fatty acid profile, without the presence of EFAs in its composition, raising questions about the adaptability of this species to climate change, especially rise of the temperature. Different zones with different temperatures also exhibited some differences in fatty acid composition for the collected species, with samples of *T. gaudichaudii, E. triacantha* and *Thysanoessa* spp. revealing a better body condition in warmer waters than in intermediate or cold waters. *E. superba*, on the other hand, revealed a lower body condition in warmer waters than in cold waters (it was not possible to study this species in intermediate waters); nonetheless, even in cold waters, these samples did not exhibit a great body condition, raising questions about the lower trophic chains in the Southern Ocean and its future. Most species (*T. gaudichaudii, E. triacantha* and *Thysanoessa* spp.) reveal the ingestion of flagellates, and *T. gaudichaudii* and *E. triacantha* also reveal the ingestion of diatoms (in a smaller extent). *E. superba* does not reveal the intake of any of these phytoplankton groups.

4.1. Fatty acid profile of Antarctic zooplankton and their nutritional value

The zooplankton species studied in the present work revealed diverse fatty acid composition, as it was expected for species exposed to different environmental and oceanographic conditions (Gonçalves et al. 2012).

In all studied areas, several fatty acids remained abundant among the FA profiles for the species collected. This was the case for the most abundant SFAs – myristic acid (C14:0), heptadecanoic acid (C17:0), octadecanoic acid (C18:0) and arachidic acid (C20:0) -, MUFAs – myristoleic acid (C14:1 ω-5), 10-pentadecenoic acid (C15:1 ω-5) - , and PUFAs – linoleic acid (C18:2 ω-6 or LA) and 7,10,13 – eicosatrienoic acid (C20:3ω-7). HUFAs were the most uncommon fatty acids present in the collected species; nonetheless, the most common HUFA was docosahexaenoic acid (C22;6 ω-3 or DHA). SFAs and MUFAs were the main contributors to the FA profiles of the studied zooplankton species, followed by PUFAs and HUFAs, where it is possible to insert the essential fatty acids (EFA).

For *Euphausia superba*, the general fatty acid profile can be described as rich in SFAs, with some presence of PUFAs and MUFAs and no presence of HUFAs in any of the collected samples, independent on location of capture. It also revealed a low body-condition, with samples ranging from 932 to 3848 µg/g of fatty acids present in the collected samples. These results complete those obtained by Ericson et al. (2018),

where it is described that FA amounts were decreasing through autumn of 2014 until spring of 2016, right before these samples were caught (the samples for this report were collected from December 8th, 2016, to January 17th, 2017, during the austral summer). However, the order of magnitude decreased greatly from spring 2016 to summer 2017, when the samples were collected for this study. In table 16 is present a comparison of the samples obtained for *E. superba* during four consecutive summers. It is possible to observe some differences between the samples collected at this study, between South Georgia and the Falkland Islands, and those collected by Ericson and colleagues, in a different location (i.e. samples were collected by fisheries, predominantly in cold waters West Antarctica Peninsula (WAP) and South Orkney Islands (SOI) (Ericson et al. 2018)). When comparing the obtained results with those obtained by Ericson and colleagues (Ericson et al. 2018), it is possible to understand some changes in the fatty acid profiles: in my thesis, *E. superba* exhibited a significant portion of SFAs, while *E. superba* collected by Ericson et al. (2018) in colder waters exhibited a profile richer in PUFAs, especially EPA and DHA, which are non-existing in *E. superba* collected in 2017. In terms of SFAs, the most abundant fatty acid present in the collected samples for this study was stearic acid (C18:0), with an average of 71.2% of the total fatty acid composition in the samples. For samples collected in 2014, 2015 and 2016 the most common SFA was palmitic acid (C16:0), with percentages ranging from 17.7% (2014) to 41.1% (2015) (Ericson et al. 2018). Myristic acid (C14:0) is also somewhat common in the collected samples by Ericson et al. (2018), ranging from 5.2% (2014) to 8.8% (2015). MUFAs representation was higher in samples collected by Ericson et al. (2018), representing from 26.2% (2014) to 30.4% (2015) of the total fatty acids, where the most common MUFA was oleic acid (C18:1Δ9). For the samples collected in 2017, MUFAs represented 7.5% of the total fatty acid composition, with particular emphasis in heptadecenoic acid (C17:1Δ10), corresponding to 3.9% of the total fatty acids present in the samples for this year. PUFAs were the main contributors of the fatty acid composition of the samples collected by Ericson et al. (2018), ranging from 37.7% (2015) to 47.9% (2014), with the main PUFAs contributing to these values being eicosapentaenoic acid (EPA, 20:5ω-3), docosahexaenoic acid (DHA, C22:6ω-3) and stearidonic acid (C18:4ω-3). However, in samples collected in 2017 there was no presence of these fatty acids and the PUFA that mainly contributed to the presence of PUFAs in their composition was ω-3 α-linolenic acid (C18:3ω-3). The amount of PUFAs present in these samples was 11.5% of the total fatty acid composition.

Table 16 - Comparison between collected samples and samples collected through previous years of *Euphausia superba*, during summer.

Fatty Acids	Summer 2014[1]	Summer 2015[1]	Summer 2016[1]	Summer 2017[2]
C10:0	0.0%	0.0%	0.0%	0.1%
C11:0	0.0%	0.0%	0.0%	0.3%
C12:0	0.0%	0.0%	0.0%	0.2%
C13:0	0.0%	0.0%	0.0%	1.3%
C14:0	5.2%	8.8%	8.5%	0.2%
C15:0	0.0%	0.0%	0.0%	0.2%
C16:0	17.7%	41.1%	20.7%	1.0%
C17:0	0.0%	0.0%	0.0%	2.6%
C18:0	1.1%	1.4%	1.6%	71.2%
C20:0	0.0%	0.0%	0.0%	0.1%
C21:0	0.0%	0.0%	0.0%	0.2%
C22:0	0.0%	0.0%	0.0%	3.4%
C23:0	0.0%	0.0%	0.0%	0.1%
C14:1Δ9	0.0%	0.0%	0.0%	0.9%
C15:1Δ10	0.0%	0.0%	0.0%	0.6%
C16:1Δ9	4.4%	6.9%	6.9%	0.7%
C17:1Δ10	0.0%	0.0%	0.0%	3.9%
C18:1Δ9	10.2%	12.5%	12.3%	0.7%
C18:1Δ-7	7.4%	7.0%	6.7%	0.0%
C20:1Δ11	0.6%	0.8%	0.8%	0.8%
C22:1Δ13	0.5%	0.6%	0.7%	0.0%
C18:2Δ9,12	2.2%	1.6%	1.2%	0.5%
C20:2Δ11,13	0.0%	0.0%	0.0%	1.5%
C22:2Δ13,16	0.0%	0.0%	0.0%	0.5%
C18:3Δ6,9,12	0.0%	0.0%	0.0%	0.0%
C18:3Δ9,12,15	1.6%	1.3%	1.1%	6.2%
C18:4ω-3	2.6%	3.4%	3.6%	0.0%
C20:3Δ7,10,13	0.0%	0.0%	0.0%	2.8%
C20:4Δ5,8,11,14	0.0%	0.0%	0.0%	0.0%
C16:4ω-1	0.5%	1.1%	1.2%	0.0%
EPA	20.7%	18.0%	19.1%	0.0%
C21:5ω-3	0.6%	0.7%	0.8%	0.0%
DHA	17.9%	9.8%	9.5%	0.0%
∑SFA	25.6%	31.8%	31.8%	81.0%
∑ MUFA	26.2%	30.4%	29.7%	7.5%
∑ PUFA	47.9%	37.7%	38.5%	11.5%
1 – Samples collected by Ericson et al. (2018); 2 – Samples collected for this thesis				

The results obtained in my thesis reveal relevant differences from those described by Ericson et al. (2018), with results which have never been found around South Georgia. Several hypothesis can be equated for these results. The austral summer of 2016/17 was one of the hottest in the Southern Ocean, without the influence of El Niño (NCDC 2017). The rise of temperatures may be causing some inbalance in the phytoplankton community, as it is described by McLeod et al. (2012) and Antoni et al. (2020). McLeod and his peers stated that climate change is influencing phytoplankton migration in the Australian Antarctic plate, with species of phytoplankton, endemic to the southern border of Australia migrating south of the ACC, compromising phytoplankton communities and diets of higher trophic levels. Although it is not possible to corroborate this sentence with data collected for this study, it should be equationed that

phytoplankton communities are suffering changes, with the introduction of new species migrating due to global warming and inbalancing the phytoplankton communities in the Southern Ocean. Proving that phytoplankton communities are changing along Antarctic waters would justify the obtained results in this thesis with particular emphasis for *E. superba*, which was the species with the most different fatty acid content when compared to what is present in literature (Fricke et al. 1984, Cripps et al. 1999, Hagen et al. 2001, Ericson et al. 2018). Further studies are necessary to assess the phytoplankton community dynamics in this region of the Southern Ocean.

The phytoplankton characterization around the studied areas described in my thesis was not developed during this cruise and there was no collection of these type of samples for further investigation. However, it is possible to assess the characterization of the phytoplankton community through other studies over the years. In South Georgia, phytoplankton community is characterized by a strong presence of diatoms (Nunes et al. 2019), and the phytoplankton blooms are favoured by relatively higher temperatures around this island (Korb et al. 2004). However, the results obtained during this work exhibit a very different feeding behavior that should be further investigated, with sampling of phytoplankton communities. Even though it is possible for us to understand that it would be possible for *E. superba* to obtain the necessary EFAs for its survival, due to the presence of several EFAs in the fatty acid profile of *E. triacantha* collected in cold waters (around South Georgia) and *E. triacantha, Themisto gaudichaudii* and *Thysanoessa* spp., all collected in warmer waters. It is necessary to understand the reason behind *E. superba* not intaking or, at least, not retaining any of the essential fatty acids. Phytoplankton alterations, like suggested before, may be to blame, but it is also possible that raises in temperature may have had not only an effect on phytoplankton community but in *E. superba* itself. This species is characterized to be an Antarctic species, while *E. triacantha* is characterized to be sub-Antarctic, translating to a better adaptation of warmer environments than *E. superba*. With the results obtained in this work, it is possible to speculate that, continuing temperature raises will lead to a switch of the Southern Ocean's main zooplankton from *E. superba* to *E. triacantha*. Once again, further study is necessary to evaluate the situation around South Georgia's phytoplankton and zooplankton communities. A new characterization of these communities is necessary for this geographical region.

One of the main differences found in this study was that zooplankton was not feeding in diatoms, but mainly in flagellates. According to Constable et al. (2014), small flagellates dominate the nutrient - low regions, such as open ocean (Mengesha et al. 1998), while diatoms are more abundant in nutrient-rich regions. In this study, samples that revealed a HUFA profile, all revealed a consumption of flagellates, which indicates

that these samples were collected in a poor-nutrient region of the Southern Ocean, which is contradictory to the High Nutrient – Low Chlorofil (HNLC) system described for the Southern Ocean by other studies (Venables & Moore 2010). These blooms are caused by the increase of chlorophyll-*a* concentration, which are a direct response to warmer temperatures. In 2014, there was a large increase in chlorophyll-*a* concentration, which was strongly correlated to DHA mass (Hellessey et al. 2020). According to this study, this fact suggests that *E. superba* was predominantly feeding on flagellates during the summer of 2014, due to a flagellate bloom, related to the increase of chlorophyll-*a* concentration, caused by the increase of temperature. The same may have happened during the 2017 summer, when our samples were collected, according to Jena & Pillai (2020), who describes abnormally high levels of chlorophyll-*a* concentration for the Maud Rise polynya, south of the ACC, for the year of 2017. However, it was important to assess phytoplankton community composition and also be necessary to assess the chlorophyll-*a* levels present in the collected areas during the collection of samples for these species to confirm our suggestions.

Themisto gaudichaudii is an opportunistic predator, feeding on available preys (Hopkins 1985, Havermans et al. 2019), with its fatty acid composition revealing this behaviour. In table 17, it is possible to compare the obtained results with two studies where the fatty acid composition for this species was defined (Nelson et al. 2001, Richoux 2011, Mayzaud & Boutoute 2015) The samples for Nelson et al. (2001) were collected during January and February 1997 and the samples for Mayzaud & Boutoute (2015) were collected during summer 1996-97, while samples for Richoux (2011) were collected from the 17th to the 27th of April, 2007. The obtained results are somewhat concordant to those obtained in the literature (Nelson et al. 2001, Richoux 2011, Mayzaud & Boutoute 2015). The collected samples for this study revealed a profile richer in SFAs than the other samples described in other studies, with a percentage of 56.2%, contrasting to an average of 24.9% (21.9%-26.5%). The SFAs that most contributed to the SFAs abundance were myristic acid (C14:0) and palmitic acid (C16:0), in collected samples and literature (Nelson et al. 2001, Richoux 2011, Mayzaud & Boutoute 2015). In the collected samples for this study, there is a clear proeminence of stearic acid (C18:0), being the most abundant saturated fatty acid present in the collected samples, while in literature C18:0 reveals a low abundance amongst the collected samples (Nelson et al. 2001, Richoux 2011, Mayzaud & Boutoute 2015). MUFAs reveal discrepancy even within literature: while Mayzaud & Boutoute (2015) and Nelson et al. (2001) reveal a profile with 26.3% – 26.5% presence of MUFAs. The obtained results in this work are closer to the ones revealed by Richoux (2011). The most abundant MUFA

in all of the samples, collected and reported in literature, was oleic acid (C18:1Δ9). PUFAs and HUFAs was the most abundant group of fatty acids in the samples described in the literature, even though the most abundant PUFA/HUFA is not conterminous. In the samples collected by Mayzaud & Boutoute (2015) and Nelson et al. (2001), the most abundant PUFA/HUFA was eicosapentaenoic acid (EPA, 20:5ω-3), while for the present study and for Richoux (2011), the most abundant PUFA/HUFA was docosahexaenoic acid (DHA, C22:6ω-3).

Table 17 - Comparison between collected samples and samples collected through previous years of *Themisto gaudichaudii*.

Fatty Acids	Summer 1996-1997[1]	Summer 1997[2]	Autumn, 2007[3]	Summer 2017[4]
C11:0	0.0%	0.0%	0.0%	1.4%
C12:0	0.0%	<1%	0.0%	1.0%
C13:0	0.0%	<1%	0.0%	1.0%
C14:0	8.4%	4.0%	1.0%	6.4%
C15:0	0.1%	1.0%	0.7%	1.2%
C16:0	16.0%	17.6%	13.0%	9.7%
C17:0	0.2%	1.9%	1.2%	1.2%
C18:0	0.8%	1.8%	2.5%	28.6%
C20:0	0.0%	<1%	2.1%	3.5%
C22:0	0.0%	<1%	0.0%	2.0%
C14:1Δ9	0.0%	<1%	0.0%	0.2%
C15:1Δ10	0.0%	<1%	0.0%	1.1%
C16:1Δ9	8.3%	<1%	0.3%	0.7%
C16:1Δ11	0.7%	5.0%	1.4%	0.0%
C17:1Δ10	0.0%	<1%	0.4%	1.2%
C18:1Δ9	19.8%	12.5%	5.4%	5.9%
C18:1Δ11	2.4%	5.4%	2.1%	0.0%
C18:1Δ13	1.3%	0.7%	0.2%	0.0%
C20:1Δ11	0.9%	0.3%	1.1%	0.5%
C20:1Δ13	0.1%	0.0%	0.0%	0.0%
C22:1Δ13	0.1%	<1%	0.0%	0.0%
C24:1Δ15	0.0%	0.0%	0.0%	0.0%
C16:2ω-4	0.6%	0.0%	0.0%	0.0%
C18:2Δ9,12	1.3%	0.0%	4.3%	3.2%
C20:2Δ11,13	0.0%	0.0%	0.0%	0.1%
C20:2Δ-6	0.0%	0.6%	0.2%	0.0%
C22:2Δ13,16	0.0%	0.0%	0.0%	0.2%
C16:3ω-6	0.2%	0.0%	0.0%	0.0%
C16:3ω-4	0.4%	0.0%	0.2%	0.0%
C18:3Δ6,9,12	0.0%	<1%	0.4%	0.3%
C18:3Δ9,12,15	1.2%	<1%	0.3%	0.0%
C20:3Δ7,10,13	0.0%	<1%	0.0%	2.1%
C20:4Δ5,8,11,14	0.3%	<1%	2.3%	0.1%
C20:4ω-3	0.4%	0.0%	0.5%	0.0%
EPA	13.9%	19.2%	16.7%	4.4%
C21:5ω-3	0.5%	0.0%	0.0%	0.0%
C22:5ω-3	0.3%	0.6%	0.5%	0.0%
C22:5ω-6	0.0%	0.0%	0.8%	0.0%
DHA	11.1%	14.9%	38.7%	23.9%
∑ SFAs	26.5%	26.3%	21.9%	56.2%
∑ MUFAs	35.1%	26.3%	11.6%	9.5%
∑ PUFAs + HUFAs	38.4%	42.3%	66.5%	34.3%

1 - (Mayzaud & Boutoute 2015); 2 - (Nelson et al. 2001); 3 - (Richoux 2011); 4 – samples collected for this study

Nonetheless, EPA and DHA were abundant in the collected samples and in the reported values from literature. The obtained results are concordant to what literature describes for this species and its behavior, allowing to conclude that, despite changes in

the Southern Ocean ecosystem, this species is still capable of adaptation, with the results obtained for this study similar to those obtained by other studies in previous years.

Thysanoessa spp. was the species with less collected samples (n=3). Nonetheless, the obtained results are concordant to those obtained in literature (Attwood & Hearshaw 1992, Stübing & Hagen 2003, Mayzaud et al. 2003). The comparison between the obtained results and the results described in literature can be found in table 18. Mayzaud et al. (2003) and Stübing & Hagen (2003) described the fatty acid profile of *Thysanoessa macrura* and Attwood & Hearshaw (1992) described the fatty acid profile of *Thysanoesa vicina*. The samples for Attwood & Hearshaw (1992) were collected from March 30th, 1989 to May 9th, 1989, samples for Mayzaud et al. (2003) were collected in February 1981 and samples for Stübing & Hearshaw were collected in two separate occasions: from April 14th to April 20th 1999 and from April 18th to May 1st 2001. Saturated fatty acids were the most abundant fatty acids amongst the collected samples for this study and for Stübing & Hagen (2003). Although it was not the most abundant group of fatty acids for Mayzaud et al. (2003), it was a close second to the most abundant fatty acids group for this species (PUFA/HUFA). The most abundant SFAs were myristic acid (C14:0) and palmitic acid (C16:0). Nonetheless, stearic acid (C18:0) was the most abundant SFA for the collected samples for this study, but had low representation in the samples depicted in literature. MUFAs had relatively low representation within the collected samples and described in literature. The most common MUFA for the samples for this study were concordant to the one described in literature: oleic acid (C18:1Δ9). PUFAs/HUFAs were also relevant to the fatty acid profile for the collected samples and in literature. In the collected samples for this study, EPA was not present, while DHA contributed the most to the PUFAs/HUFAs abundance. Although in the samples collected for Mayzaud et al. (2014) DHA also reveals to be the most abundant HUFA, EPA is also very abundant for these samples. In the samples collected for Stübing & Hagen (2003), the most abundant PUFA/HUFA was EPA followed by DHA.

For the genus *Thysanoessa*, it is possible to verify a raise in the amount of SFAs, with a decrease in MUFAs and in PUFAs/HUFAs. This decrease, especially in PUFAs/HUFAs, where EFAs are included, is a direct consequence of the climate change observed in this region (namely raise in temperature) (Gille 2002, Constable et al. 2014). The main difference, as stated previously, is the absence of EPA in these samples, revealing a dietary source of only flagellates and no diatoms, which has been discussed previously. Nonetheless, based on the obtained results, *Thysanoessa* reveals an ability to adapt to higher temperatures, which is revealed by the presence of EFAs. Nonetheless, these fatty acids appear at lower amounts as to what is described in

literature, so, although it is still capable of adaptation, further study should be developed to assess until when will *Thysanoessa* spp. be able to adapt.

Table 18 - Comparison between collected samples and samples collected through previous years of *Thysanoessa* spp.

FA	Winter 1981[1]	Autumns 1999 and 2001[2]	Summer 2017[3]
C11:0	0.0%	0.0%	2.0%
C12:0	0.0%	0.0%	0.1%
C13:0	0.0%	0.0%	0.1%
C14:0	17.5%	24.1%	4.9%
C15:0	1.2%	0.0%	0.3%
C16:0	22.0%	24.0%	13.6%
C17:0	0.0%	0.0%	3.6%
C18:0	0.6%	0.7%	34.2%
C20:0	0.0%	0.0%	4.0%
C21:0	0.0%	0.0%	0.1%
C22:0	0.0%	0.0%	3.7%
C14:1Δ9	0.0%	0.0%	1.4%
C15:1Δ10	0.0%	0.0%	0.8%
C16:1Δ7	0.5%	0.0%	0.0%
C16:1Δ9	2.6%	4.8%	0.0%
C17:1Δ10	0.0%	0.0%	3.1%
C18:1Δ9	7.6%	10.6%	2.8%
C18:1Δ11	5.2%	4.1%	0.0%
C20:1Δ11	0.0%	1.2%	0.2%
C18:2Δ9,12	4.0%	1.5%	2.2%
C20:2Δ11,13	0.0%	0.0%	1.6%
C18:3Δ9,12,15	0.8%	0.0%	0.0%
C20:3Δ7,10,13	0.0%	0.0%	6.4%
16:4ω-1	0.0%	0.2%	0.0%
18:4ω-3	0.5%	1.1%	0.0%
C20:4Δ5,8,11,14	2.1%	0.0%	0.4%
EPA	17.5%	18.7%	0.0%
DHA	17.8%	9.1%	14.7%
∑SFA	41.3%	48.8%	66.4%
∑ MUFA	15.9%	20.7%	8.3%
∑ PUFA/HUFA	42.7%	30.6%	25.2%
1 - (Mayzaud et al. 2003), 2 - (Stübing & Hagen 2003), 3 – samples collected for this thesis			

Euphausia triacantha was the only species present in this study that has representativity in all sampled regions (cold, transitional and warmer waters). The obtained results in this study were not concordant with those obtained in Stübing & Hagen (2003) and Phleger et al. (1998), though, and are presented in table 19. The samples for Stübing & Hagen (2003), as previously mentioned, were collected in two separate occasions: from April 14th to April 20th 1999 and from April 18th to May 1st 2001. Samples for Phleger et al. (1998) were collected during January and February 1996. Due to its northernmost distribution (when compared to other Antarctic species), the study of the fatty acid profile for this species is still to explore (Stübing 2004). In table 19, it is possible to compare the obtained results with those obtained by Stübing & Hagen (2003) and Phleger et al. (1998). For the samples collected for this study, the most abundant group of fatty acids was SFA, while for the reported literature was PUFAs/HUFAs. The most abundant saturated fatty acid for the collected samples for this study was stearic

acid (C18:0), while for the reported literature was palmitic acid (C16:0). The most abundant MUFA was the same for samples collected for this study and for samples collected for other studies (oleic acid, C18:1Δ9). PUFAs/HUFAs was mainly composed by EPA and DHA. of the Southern Ocean trophic chain and what will be at cause if that cange happens.

Table 19 - Comparison between collected samples and samples collected through previous years of *Euphausia triacantha*.

FA	Summer 1996, Antarctica Peninsula[1]	Summer 1996, East Antarctica[1]	Autumns 1999 and 2001[2]	Summer 2017[3]
C11:0	0.0%	0.0%	0.0%	0.4%
C12:0	0.0%	0.1%	0.0%	0.1%
C13:0	0.0%	0.0%	0.0%	0.2%
C14:0	1.0%	0.7%	6.6%	1.7%
C15:0	0.7%	0.4%	0.0%	1.1%
C16:0	13.9%	15.1%	18.6%	3.6%
C17:0	1.5%	2.2%	0.0%	2.6%
C18:0	6.6%	2.7%	1.1%	41.9%
C19:0	0.0%	0.2%	0.0%	0.0%
C20:0	0.4%	0.0%	0.0%	2.9%
C21:0	0.0%	0.0%	0.0%	0.1%
C22:0	0.2%	0.2%	0.0%	1.0%
C23:0	0.0%	0.0%	0.0%	0.0%
C24:0	0.0%	0.2%	0.0%	0.0%
C14:1Δ9	0.0%	0.0%	0.0%	1.0%
C15:1Δ10	0.0%	0.0%	0.0%	1.8%
C16:1Δ9	0.0%	2.0%	7.9%	0.0%
C16:1Δ11	2.1%	0.1%	0.0%	0.0%
C17:1Δ10	0.0%	0.0%	0.0%	1.4%
C18:1Δ9	17.2%	8.1%	16.9%	13.3%
C18:1Δ11	12.0%	11.7%	8.7%	0.0%
C18:1Δ13	0.9%	0.9%	0.0%	0.0%
C20:1Δ11	3.5%	0.4%	2.4%	0.3%
C20:1Δ13	0.9%	4.0%	0.0%	0.0%
C22:1Δ11	0.3%	0.1%	0.0%	0.0%
C22:1Δ13	3.6%	2.8%	0.0%	0.1%
C22:1Δ15	0.0%	0.6%	0.0%	0.0%
C24:1Δ15	0.9%	0.7%	0.0%	0.0%
C16:2ω-4	0.0%	0.0%	1.5%	0.0%
C18:2Δ9,12	1.8%	2.0%	1.8%	0.8%
C20:2Δ11,14	0.9%	1.3%	0.0%	0.2%
C22:2Δ13,16	0.0%	0.0%	0.0%	0.6%
C18:3Δ6,9,12	2.7%	0.0%	0.0%	0.0%
C18:3Δ9,12,15	0.0%	0.0%	0.0%	0.1%
C20:3Δ7,10,13	0.0%	0.0%	0.0%	5.4%
C20:3Δ7,10,14	0.3%	0.2%	0.0%	0.0%
C16:4(ω-1)	0.0%	0.0%	0.3%	0.0%
C18:4(ω-3)	0.1%	0.1%	1.3%	0.0%
C20:4Δ5,8,11,14	0.0%	0.7%	0.0%	0.1%
C20:4(ω-3)	0.4%	0.3%	0.0%	0.0%
EPA	12.2%	22.4%	17.1%	2.8%
C22:5(ω-3)	1.2%	1.0%	0.0%	0.0%
DHA	14.2%	18.4%	15.8%	16.5%
Others	0.2%	0.3%	0.0%	0.0%
∑SFA	24.4%	21.9%	26.3%	55.6%
∑MUFA	41.5%	31.3%	35.9%	17.9%
∑ PUFA/HUFA	33.9%	46.5%	37.8%	26.6%

1 - (Phleger et al. 1998), 2 - (Stübing & Hagen 2003), 3 – samples collected for this thesis

However, the contribution of these fatty acids differs from study to study. Further analysis for this species should be developed to assess the fatty acid composition and infer about body condition. However, since it reveals a better body condition and was

collected alongside *E. superba* (in colder waters), further investigation is necessary to understand what role *E. triacantha* may develop in the near future for the Southern Ocean trophic chain and if it will substitute *E. superba* as the base of this marine ecosystem.

4.2. Antarctic zooplankton biochemical composition at the different zones of the Southern Ocean with different environmental conditions

Euphausia superba can be found aroud South Georgia and in intermediate zones of the Southern Ocean (Cuzin-Roudy et al. 2014), which explains why most of the samples collected for this study were found closer to the island of South Georgia and only one sample from one swamp was found in warmer waters (Knox 2006). *E. superba* collected in cold waters revealed a profile with no presence of HUFAs, some PUFAs and MUFAs in low quantities and SFAs in higher amounts (see results). On the other hand, at warmer temperatures, there could also not be possible to find the presence of HUFAs for this species. Saturated fatty acids were the main contributors to the fatty acid profile at higher temperatures and further from South Georgia. The body condition for *E. superba* at higher temperatures appears to be lower than the one presented in the collected samples at lower temperatures, and so compared to previous years (Ericson et al. 2018). *E. superba* body condition and growth are dependent on sea-surface temperature and chlorophyll-*a* concentration (Virtue et al. 2010). Cripps et al. (1999) described the fatty acid profile of *E. superba* around South Georgia, also collected in three different regions. The fatty acids obtained for group C obtained by Cripps et al. (1999) and his peers are similar to those obtained in this thesis, where fatty acid concentration was lower than the one described by other authors (Clarke 1980, Fricke et al. 1984). However, these groups still revealed the presence of HUFAs, which was not present in this study. It is also possible to state that, at different locations, with different environmental conditions, such as phytoplankton blooms, the fatty acid profile of the species is altered (Cripps et al. 1999).

T. gaudichaudii is an important species of the Sub-Antarctic waters, playing a role that can be compared to that of *E. superba* in Antarctic waters (Padovani et al. 2012). In this study, *T. gaudichaudii* was collected in intermediate and warmer waters. *T. gaudichaudii* in warmer waters reveals a similar pattern of fatty acids between each other, revealing a prominence of HUFAs and SFAs in its composition, with an emphasis in DHA, present in very high quantities in *T. gaudichaudii* from warmer waters. EPA was also present in this species, although it appeared to be at a lower rate when compared to DHA. *Themisto gaudichaudii* collected in intermediate waters reveals a fatty acid profile rich in SFAs, followed by HUFAs, then MUFAs and finally PUFAs. The most abundant SFAs were C18:0, C16:0 and C14:0. DHA revealed a large abundance within

the fatty acid profile for this species, collected in intermediate waters. Between MUFAs and PUFAs, the most abundant fatty acid was oleic acid (C18:1Δ9). Samples collected in waters at warmer temperatures exhibit a better body condition than in waters at transitional temperatures. This species revealed a medium better body condition in warmer waters than in transitional waters. Observing at the fatty acid profile of *Themisto* gaudichaudii collected in intermediate waters, it reveals a similar fatty acid profile to the other collected species in transitional waters. Phleger et al. (1998) reveals a profile of fatty acids collected in Elephant Island and East Antarctica for *T. gaudichaudii* rich in PUFA (corresponding to 54.1% of the fatty acid composition), with emphasis in EPA and DHA (24.0% and 20.4%, correspondingly). Although the results obtained for EPA are distant to those obtained in this study, the results obtained for DHA are similar, even if they appear to be at slightly higher levels than what is described in literature (Fricke & Oehlenschläger 1988). Nonetheless, the results obtained describe that, at the temperatures at which these samples were collected, *T. gaudichaudii* is able to adapt, even at higher temperatures, as it is possible to observe as the better body condition for this species can be found in warmer waters. It is not possible to compare the obtained results with other studies in this region of the Southern Ocean, for they have not been done yet. Further investigation for this species in this region should be considered. Nonetheless, a comparison with fatty acid composition of specimens collected in different areas of the Southern Ocean for this species can be found above.

Thysanoessa spp. was found and collected in transitional and warmer waters. At warmer temperatures, this species revealed a fatty acid profile with DHA present at significant proportion. On the other hand, *Thysanoessa* spp. collected in transitional waters reveals a fatty acid profile characterized by the absence of HUFAs and dominated by SFAs, which is coincident to all the species collected at transitional temperatures waters. Since *Thysanoessa* spp. is more common in the northern zone of the Southern Ocean (Knox 2006), where it survives at higher temperatures and dwells in a low primary production region, it is expected that it exhibits a better body condition in warmer conditions, as it is corroborated in the present work. *Thysanoessa* has been overlooked in terms of fatty acid and biochemical composition, for which is very difficult to find other studies to compare this genus. Further investigation in the biochemical composition and role of this genus around South Georgia is necessary. Nonetheless, a comparison with fatty acid composition of specimens collected in different areas of the Southern Ocean for this genus can be found above.

Euphausia triacantha, as previously mentioned, was the only species collected in the three sampled areas, with representativity in cold, intermediate and warmer waters. *E. triacantha* collected in cold waters reveals a fatty acid profile rich in saturated fatty acids,

followed by the contribution of HUFAs. The saturated fatty acids that most contribute to the abundance of this group of fatty acids are stearic acid (C18:0) and palmitic acid (C16:0). On the other hand, the most abundant HUFA was EPA, immediately followed by DHA. This is the only species that reveals a larger amount of EPA in its composition than in DHA. Oleic acid (C18:1Δ9) was also somewhat relevant to the composition of the fatty acid profile for this species under this condition. *E. triacantha* at transitional waters reveals a profile dominated by SFAs, also followed by HUFAs. The most abundant SFAs present for this species were stearic acid (C18:0) and eicosanoic acid (C20:0) and the most abundant and only HUFA present was DHA. MUFAs were the third group to contribute to the fatty acid profile, with special emphasis to oleic acid (C18:1Δ9). In waters where temperature was higher, SFAs also contributed the most to the fatty acid profile, especially C16:0 and C18:0. SFAs were also followed by HUFAs at these temperatures, where DHA was the sole contributor to the HUFAs value to the final fatty acid profile. MUFAs representation was mainly based of oleic acid (C18:1Δ9). PUFAs contribution was not as significant as the other groups of fatty acids for all temperatures. In sum, it is possible to distinguish between species collected in colder waters, due to differences in fatty acid composition and abundance in the collected samples. Although it is also possible to observe some changes between species within the samples collected in warmer waters. The samples collected in intermediate waters revealed a profile alike, which was translated. Species collected in cold waters reveal a fatty acid profile and a body condition that can be distinguished through species: *E. superba* and *Euphausia triacantha*. *E. triacantha* reveals a better body condition than *E. superba* under these conditions. *E. triacantha* is also not common around South Georgia, for which is difficult to find literature that reports the condition of this species in this region. Further investigation is necessary ito overcome the lapse of information that exists for this species. Nonetheless, a comparison with fatty acid composition of specimens collected in different areas of the Southern Ocean for this species can be found above.

In conclusion, it is possible to observe that *T. gaudichaudii, E. triacantha* and *Thysanoessa* spp. all reveal a better body condition in warmer waters than in intermediate and cold waters.

4.3. Fatty acid trophic markers determined to identify and characterise the food sources of Antarctic zooplankton

In terms of feeding, all collected species revealed an omnivorous behavior, with the consumption of flagellates and small organisms being the two most present in the fatty acid trophic markers. Some samples revealed small organisms consumption; however,

it is not relevant when compared to other fatty acids present in their composition. Bacterial consumption is present in all samples collected for this study, but represent a minimal percentage of food source.

Euphausia superba feeds mainly on phytoplankton (Hamner et al. 1983), but it is also noted that protozoans and small copepods are consumed year-round by this species (Schmidt & Atkinson 2016). Phytoplankton is rich in omega-3 HUFAs, such as EPA and DHA. In the results obtained for this work, none of the samples of *E. superba* revealed a presence of these fatty acids. Nonetheless, species collected in the same regions, like *E. triacantha* (both cold and warmer waters) and *T. gaudichaudii* and *Thysanoessa* spp. (both in warmer waters) showed evidence of HUFA in their composition, with a main focus in EPA and DHA. This information reveals that, even though it was possible for the samples of *E. superba* to obtain these fatty acids in their feeding, they did not feed in preys rich in these. On the other hand, copepod biomarkers, ∑20:1+22:1 (Schmidt & Atkinson 2016), were present in these samples, in low quantities. According to Huntley et al. (1994), if *E. superba* stops feeding or feeds at very low rates during winter, the specimens would reduce their metabolism, utilize lipid stores and might shrink in size. The lipids would be “used up” instead of stored, which would lead to low quantities of fatty acids in their composition. This decrease in fatty acids is more common during winter, when algae availability is low, but it may also happen in summer (Cripps et al. 1999). Although it is common for *E. superba* to decrease its percentage of EPA and DHA during summer, due to a greater consumption of reproduction and winter survival related fatty acids (Hellessey et al. 2020), there are no reports in literature of collected *E. superba* with no percentage of EPA and DHA, like it was found in this thesis (Cripps & Atkinson 2000, Schmidt et al. 2006, Ericson et al. 2018, Hellessey et al. 2020). The differences between regions may be explained by phytoplankton. Samples collected in places that are closer geographically present similar fatty acid profiles.

Samples of *T. gaudichaudii* collected in warmer waters reveal a greater amount of HUFAs and SFAs in their composition, with greater prominence for HUFAs, specially DHA, present in very high quantities, revealing a feeding proeminent in flagellates. In fact, both diatom/flagellates ratios used to describe the FATMs indicate a diet richer in flagellates instead of diatoms. The abundance of DHA in this species indicates a flagellate-based diet, which is also coincident to the diet practiced by the samples collected in warmer waters. These results differ from those in literature (Dodge & Priddle 1987), being expected for the samples collected in the South Georgia region a higher dietary intake of diatoms, revealed by the high amounts of EPA (Dalsgaard et al. 2003). Nonetheless, this species also revealed an omnivorous behaviour, which also differs from literature, since to the species *T. gaudichaudiii* is a reported carnivory, being

presented in a higher trophic level (Froneman et al. 2000, Kruse et al. 2015). Nonetheless, this result is in line with the other three species collected for this study and is concordant to those results. Bacteria consumption and terrestrial detritus or green algae consumption is also present for the samples collected for this species, even though they account for smaller amounts of ingestion.

Thysanoessa spp., as all the collected species for this study, reveals the presence of DHA in the collected samples, which indicates dietary intake of flagellates. In fact, for this species, it was not possible to observe the intake of EPA from its fatty acid profile, even though other species collected in the same locations (intermediate and warmer waters) reveal the presence of this fatty acid. The fatty acid profile for *Thysanoessa* spp. also reveals the consumption of bacteria and terrestrial detritus or green algae. This species also exhibits an omnivory tendency in its feeding. *Thysanoessa macrura* is usually described as presenting an omnivorous behavior; however, it is more omnivorous in the Indian sector of the Southern Ocean than in other sectors (Falk-Petersen et al. 2000). Nonetheless, our results were coherent with those reported in literature for *T. macrura*. For *T. vicina*, it was not possible to find literature about this species feeding, but the obtained results are concordant to those obtained for other species collected for this study.

Euphausia triacantha reveals a fatty acid profile in cold waters that reveal an intake of both flagellates (DHA) and diatoms (EPA), with a larger consumption of the latter. This result is concordant with the results obtained in literature for Antarctic zooplankton, where *E. triacantha* is described as a carnivory species (Falk-Petersen et al. 2000). The diatom intake can be corroborated by the known richness of nutrients of the waters around South Georgia, which promote the development of diatoms, instead of flagellates, which tend to grow in nutrient-low regions (Constable et al. 2014). Fatty acid profile for samples collected in intermediate waters reveal a similar pattern to the other samples collected for other species (*Thysanoessa* spp. and *Themisto gaudichaudii*) in this environment. For this species, DHA was present in the fatty acid composition, indicating flagellate consumption. *Euphausia triacantha* also revealed consumption on small organisms like copepods, although this consumption is not significative, translating to very low percentages of present fatty acids correspondant to small organisms.

5. Conclusion

The present study highlights that, in general, sub-Antarctic species survive at higher temperatures with more ease than Antarctic species, revealing a better nutritive value and body condition. While samples of *Thysanoessa* spp., *Themisto gaudichaudii* and *Euphausia triacantha* reveal, in general, an expected fatty acid profile, characterized by the consumption of flagellates, samples collected for *Euphausia superba* reveal no consumption of diatoms nor flagellates, raising questions about this behaviour from a species that has been documented to be once prosperous in this region of South Georgia, with abundant feeding. More questions were raised when no sign of carnivory was found in the fatty acid profiles for this species. The sub-Antarctic species *Thysanoessa* spp., *Euphausia triacantha* and *Themisto gaudichaudii* revealed a better body condition and, consequently, a better adaptability to the increase of temperature than *Euphausia superba*, as it would have been expected for samples under higher temperatures. This results also suggests potencial phytoplankton alterations.

It is known that the South Georgia region is rich in diatom blooms, and so this was the result we were expecting, but not the one we obtained. It was clear that most samples collected for this study reveal a higher intake of flagellates than diatoms, which can be explained by temperature increase, which lead to chlorophyll-*a* blooms which, in turn, lead to flagellate blooms. Since 2017, this was one of the hottest years, as it is shown in records. It is expected that chlorophyll-*a* concentration had risen in the Southern Ocean during the time of the collection of these samples, explaining the flagellate food source. Thus, our study also suggest changes in phytoplankton community composition similarly to what is happening in Antartic *E. superba* and zooplankton community.

It was hypothesized that *E. superba* may not be feeding due to higher temperatures and phytoplankton community alterations, both of which have been observed in the Southern Ocean environment. *E. superba*, as an Antarctic species, does not tolerate higher temperatures, unlike the other species collected for this study. However, it seems that it does not tolerate changes in the phytoplankton community as it was thought it would. This urges politicians to take action, so that the structure of South Georgia's trophic chain is not compromised.

The results obtained in this study will serve as a starter for new research in phytoplankton and zooplankton communities and climate change interference in the South Georgia region (in particular) and Southern Ocean (in a more general analysis). These results are of great interest to the scientific community, since it raises many questions about the lowest trophic levels of the Antarctic trophic chain composition and adaptability. This results may also be of interest to local fisheries, that have been

collecting *E. superba* for the production of several products, such as dietary supplements, with a claim about its fatty acid composition that may not be occurring at the moment. An interesting approach would be the use of samples collected by local fisheries for longer studies of the zooplankton community, as it is suggested by Ericson et al. (2018).

Although these results are interesting in terms of ecology, they may not be representative enough of the total phytoplankton and zooplankton communities. Further studies should be developed in order to understand how phytoplankton and zooplankton communities evolve year-round, through the course of several years. For this study, it was only possible to assess the fatty acid composition of several zooplankton species, collected during just one season. Seasonal collections are recommended for a better understanding of climate change and its impacts in the Southern Ocean zooplankton communities. The quantification of chorophyll-*a* concentration during these collections would also help to understand the phytoplankton community and its behaviour better.

Actions must be taken immediately to avoid a complete change in zooplankton composition and, consequently, alterations to the trophic chains of this ecosystem.

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7. Supplementary Information

Table 20 - Obtained peak areas for each fatty acid for the replicas collected for samples CEs1, CEt3 and CEs4.

FA	1.1	1.2	1.3	3.1	3.2	3.3	4.1	4.2	4.3
C10:0	-	-	-	-	-	-	2494158	-	-
C11:0	5608068	10533275	4900350	4601320	5605200	5748481	-	8307688	260757
C12:0	-	2473600	3344011	-	-	-	427021	1304724	621566
C13:0	-	51846277	16410926	1867073	1964664	2751836	10255735	-	1176374
C14:0	-	-	-	6238094	64452703	6231640	1943792	2367089	1719999
C14:1Δ9	6081441	10556215	6040635	6375200	4605233	4851473	6428234	2850033	1512737
C15:0	-	10074267	-	41494884	7434781	32928137	525228	-	1542129
C15:1Δ10	7594033	12387815	8303865	11687920	19181831	9591237	260069	-	548394
C16:0	68868787	1.65E+08	-	-	1.18E+08	-	-	-	-
C16:1Δ9	-	34723518	12114503	-	-	-	-	2464804	2164259
C17:0	31992341	34181928	13896944	30737141	3764739	14545875	29775025	2949949	2559528
C17:1Δ10	45198673	66190582	53981798	69663975	7702934	55071913	34839229	40338376	-
C18:0	5.82E+08	8.35E+08	6.48E+08	3.97E+08	1.3E+08	3.94E+08	5.09E+08	8.18E+08	6.24E+08
C18:1Δ9	39203913	1.56E+08	-	-	1.46E+08	-	-	-	-
C18:2Δ9,12	-	-	-	9640887	11446639	9810712	16851031	-	17013046
C19	3.16E+08	6.25E+08	2.8E+08	1.74E+08	52764564	1.42E+08	2.88E+08	3.07E+08	2.45E+08
C18:3Δ6,9,12	-	-	-	-	-	-	1106326	-	-
C18:3Δ9,12,15	1.91E+08	-	-	5635688	-	-	-	-	-
C20:0	-	17534891	-	73526713	-	99821654	-	-	-
C20:1Δ11	-	22758803	-	-	17078728	-	1268263	8631542	4312639
C20:2Δ11,13	-	-	-	-	3375123	13022826	6162297	5076537	3983658
C20:3Δ7,10,13	41468824	42355708	32314751	35173940	-	45333727	-	-	-
C20:4Δ5,8,11,14	-	-	-	-	10430400	-	-	-	-
C21:0	23152771	21450962	25303893	4801946	-	-	16585197	-	-
EPA	-	-	-	-	1.73E+08	-	-	-	-
C22:0	-	-	-	7056573	-	22285531	77480104	-	-
C22:1Δ13	-	-	-	-	-	-	-	-	-
C22:2Δ13,16	-	-	-	5839110	-	7977102	-	3356570	3842253
C23:0	-	19384358	-	-	-	-	-	-	-
DHA	-	-	-	-	1.1E+08	-	-	-	-
C24:0	-	-	-	-	-	-	-	-	-
C24:1Δ15	-	-	-	-	-	-	-	-	-

Table 21 - Obtained peak areas for each fatty acid for the replicas collected for samples CEs5, ITg6 and ITspp7

FA	5.1	5.2	5.3	6.1	6.2	6.3	7.1	7.2	7.3
C10:0	-	-	-	-	-	-	-	-	-
C11:0	270342	1547804	420796	-	10090778	6034831	58065104	20494095	16179982
C12:0	1063338	2100049	1252052	-	5321972	2960875	-	-	-
C13:0	10511272	14779003	723704	362709	433713	1089852	-	-	-
C14:0	2498663	993400	2202466	291832	591747	-	29147748	29926391	39978124
C14:1Δ9	-	2564361	4824479	449727	288354	638679	12144531	11433846	14375797
C15:0	1078341	601759	491466	738320	1274129	-	-	-	-
C15:1Δ10	848855	2886997	4706766	5072240	6175748	9157920	10657836	7998677	9411359
C16:0	-	-	-	-	-	1.5E+08	-	-	61703983
C16:1Δ9	-	2025137	-	-	-	-	-	-	-
C17:0	-	29205310	6713904	6311900	7001322	-	16652874	13043150	55558617
C17:1Δ10	65214439	11086354	5715009	22275446	3575444	1358081	-	66918620	35090204
C18:0	5.17E+08	2.7E+08	5.29E+08	4.32E+08	3.81E+08	2.83E+08	6.24E+08	3.87E+08	-
C18:1Δ9	-	-	-	-	-	-	-	-	41646067
C18:2Δ9,12	-	7449168	-	-	46383460	35633990	-	-	-
C19	2.82E+08	3.18E+08	2.91E+08	1.87E+08	1.3E+08	1.17E+08	1.99E+08	1.26E+08	1.13E+08
C18:3Δ6,9,12	-	668373	-	716529	-	11360692	-	-	-
C18:3Δ9,12,15	-	-	-	348044	-	-	-	-	-
C20:0	-	2501785	2210719	24153862	-	57461734	20751773	41405900	-
C20:1Δ11	-	2078735	5745045	-	-	-	-	-	-
C20:2Δ11,13	-	1544659	3539301	-	-	-	-	-	-
C20:3Δ7,10,13	36483816	23208269	-	-	-	2234447	68619317	48096606	35661023
C20:4Δ5,8,11,14	-	-	-	-	-	-	-	-	-
C21:0	-	1551936	16116602	-	-	-	20301452	14680268	-
EPA	-	-	-	-	-	-	-	-	-
C22:0	-	4432466	-	5837447	-	-	-	24576571	-
C22:1Δ13	-	-	-	-	-	-	-	-	-
C22:2Δ13,16	-	3888409	3716529	2482428	-	1631372	-	-	-
C23:0	-	-	-	-	-	-	-	-	-
DHA	-	-	-	-	2.64E+08	1631372	-	-	-
C24:0	-	-	-	-	-	-	-	-	-
C24:1Δ15	-	-	-	-	-	-	-	-	-

Table 22 - Obtained peak areas for each fatty acid for the replicas collected for samples IEt8, IEt10 and IEt11

FA	8.1	8.2	8.3	10.1	10.2	10.3	11.1	11.2	11.3
C10:0	-	-	-	-	-	-	-	-	-
C11:0	-	5890771	-	-	4083612	-	6910170	-	-
C12:0	-	3158258	-	-	5883760	-	-	-	-
C13:0	-	-	-	-	12028903	-	-	-	-
C14:0	-	5757111	4936152	43360551	9949914	7931913	2028351	2933545	-
C14:1Δ9	7567223	6023792	9480576	-	11552084	10420437	9792418	6379604	8196115
C15:0	-	-	-	7137943	-	-	-	-	-
C15:1Δ10	12137898	10528342	11859226	13220367	18347571	14873710	14036316	7101240	13878366
C16:0	-	-	-	102839419	-	-	-	-	-
C16:1Δ9	-	-	-	-	-	-	-	-	-
C17:0	21376753	27940014	11233653	3113011	37884260	45320292	36526247	20755437	19740616
C17:1Δ10	-	-	-	12892251	-	-	-	-	-
C18:0	498756361	318006707	318200356	-	413634821	452445891	331009762	240816579	380711034
C18:1Δ9	-	-	329438454	149829411	-	-	-	261640801	-
C18:2Δ9,12	-	-	-	11131012	-	-	-	14581810	-
C19	175256034	142452862	146043471	80756844	149302422	192947811	178245020	116175671	146307647
C18:3Δ6,9,12	-	-	-	-	-	-	-	-	-
C18:3Δ9,12,15	-	-	-	-	-	-	-	-	-
C20:0	37309986	20868635	-	29745426	29691105	-	16551404	44686015	22265995
C20:1Δ11	-	-	-	6343369	-	-	-	-	-
C20:2Δ11,13	-	-	-	6334483	-	-	-	-	-
C20:3Δ7,10,13	70223253	35833000	-	-	35502368	39469794	40189671	-	52572703
C20:4Δ5,8,11,14	-	-	-	-	-	-	-	-	-
C21:0	18283249	8536432	-	-	10347416	-	-	-	-
EPA	-	-	-	-	-	-	-	-	-
C22:0	-	26603839	16503005	-	29455910	29646399	-	-	-
C22:1Δ13	-	-	-	-	-	-	-	-	-
C22:2Δ13,16	10361394	6765660	-	-	8090726	-	-	-	-
C23:0	-	-	-	-	-	-	-	-	-
DHA	-	-	-	296864730	-	-	-	-	-
C24:0	-	-	-	-	-	-	-	-	-
C24:1Δ15	-	-	-	-	-	-	-	-	-

Table 23 - Obtained peak areas for each fatty acid for the replicas collected for samples ITg12, ITg13 and WTg14

FA	12.1	12.2	12.3	13.1	13.2	13.3	14.1	14.2	14.3
C10:0	-	-	-	-	-	-	-	-	-
C11:0	9813975	7256692	33660749	14663237	18362378	-	-	-	-
C12:0	3657578	5438841	35506509	6448777	2764731	-	-	7506990	5670911
C13:0	-	-	47071638	-	2312197	-	-	-	-
C14:0	-	-	1.44E+08	-	-	1.18E+08	4293025	-	86233209
C14:1Δ9	-	2941220	-	-	2809869	3074997	-	-	-
C15:0	-	-	38127306	11308125	48060739	-	-	-	-
C15:1Δ10	6218340	4046970	14879828	7995073	9199562	6047279	10548117	4077620	9410192
C16:0	-	-	-	-	-	2.36E+08	-	-	1.26E+08
C16:1Δ9	-	-	-	-	-	-	59990517	-	21160893
C17:0	5780118	7712360	-	17208705	18901627	12622914	18209931	7749051	6228805
C17:1Δ10	-	-	-	-	1.1E+08	-	-	-	3658731
C18:0	2.47E+08	-	2.7E+08	1.84E+08	1.03E+08	3.29E+08	2.61E+08	5.05E+08	3.35E+08
C18:1Δ9	63568180	-	-	-	-	1.35E+08	-	-	-
C18:2Δ9,12	-	-	26440362	-	-	62138458	23136469	-	31025510
C19	2.65E+08	2.43E+08	1.72E+08	3.6E+08	2.23E+08	1.76E+08	1.29E+08	2.27E+08	1.6E+08
C18:3Δ6,9,12	-	-	-	-	-	-	9979271	10557848	-
C18:3Δ9,12,15	-	-	-	-	-	-	-	-	-
C20:0	59860757	30738350	28443188	53128787	-	-	25631391	22877308	34052883
C20:1Δ11	-	-	-	-	-	-	22772270	-	-
C20:2Δ11,13	-	-	-	-	10815991	-	-	-	-
C20:3Δ7,10,13	-	28670130	16794320	37947836	30841409	25273786	-	26028198	-
C20:4Δ5,8,11,14	-	-	-	-	-	-	-	-	-
C21:0	-	-	8957168	8630069	28316070	-	-	11081825	-
EPA	-	-	-	-	-	-	-	-	-
C22:0	8707353	13389233	46269440	29560425	51480065	12186674	-	6901556	-
C22:1Δ13	-	-	-	-	-	-	-	-	-
C22:2Δ13,16	7982824	-	-	-	-	-	-	-	-
C23:0	-	-	-	-	-	-	-	-	-
DHA	-	-	-	-	-	-	2.1E+08	-	2.35E+08
C24:0	-	-	-	-	-	-	-	-	-
C24:1Δ15	-	-	-	-	-	-	-	-	-

Table 24 - Obtained peak areas for each fatty acid for the replicas collected for samples WTspp15, WEt16 and WEs18

FA	15.1	15.2	15.3	16.1	16.2	16.3	18.1	18.2	18.3
C10:0	-	-	-	-	-	-	378172	664052	-
C11:0	-	-	-	3087921	2958556	-	-	1204344	220482
C12:0	-	4494270	-	2271263	533293	-	354814	1165060	355918
C13:0	-	4145689	-	-	1158125	771183	268234	1219958	1150328
C14:0	53997345	114090004	-	6862876	435149	5465613	931640	571142	710070
C14:1Δ9	3327814	6084073	20237011	5256699	7175092	9752366	3330679	2800819	7169836
C15:0	2622589	6144448	15197385	-	2724482	42932782	1134697	1310598	2887413
C15:1Δ10	2481024	-	11747352	11820869	12680646	-	-	925196	617561
C16:0	68679674	129503945	-	-	-	-	-	-	-
C16:1Δ9	-	-	-	-	-	-	-	-	-
C17:0	-	1848580	96460658	16746544	2178765	2941619	14236080	2277171	3004778
C17:1Δ10	-	8093663	46540008	-	10219435	77873362	9608632	12601402	-
C18:0	113156603	121693010	862206160	394094402	600702315	453401699	279173737	-	415672322
C18:1Δ9	-	108193848	-	-	-	-	-	-	-
C18:2Δ9,12	23344200	33812937	50952167	-	-	-	-	-	-
C19	149753883	116446993	206005395	121111526	204198283	185338619	157724597	198333104	200504206
C18:3Δ6,9,12	-	-	-	938076	-	-	-	-	-
C18:3Δ9,12,15	-	-	-	2337992	-	-	-	-	-
C20:0	30213121	75878076	67605647	12658287	-	-	-	-	-
C20:1Δ11	4493301	10678510	-	-	-	-	-	9976210	-
C20:2Δ11,13	-	-	-	-	4764434	-	13112488	17269415	18199781
C20:3Δ7,10,13	-	-	75408407	37395708	87810433	56526712	-	-	-
C20:4Δ5,8,11,14	11418096	11117905	-	-	-	-	-	-	-
C21:0	-	-	26619651	-	18750219	15166244	-	16114040	14644957
EPA	-	-	-	-	-	-	-	-	-
C22:0	-	-	-	2184587	-	-	31746872	-	-
C22:1Δ13	-	-	-	-	-	-	-	-	-
C22:2Δ13,16	-	-	-	3316634	12211452	8735226	2773938	1560053	2944050
C23:0	-	-	-	-	-	-	-	-	-
DHA	155000455	151499062	-	-	-	-	-	-	-
C24:0	-	-	-	-	-	-	-	-	-
C24:1Δ15	-	-	-	-	-	-	-	-	-

Table 25 - Obtained peak areas for each fatty acid for the replicas collected for samples WEt19, WTg20 and WTspp21

FA	19.1	19.2	19.3	20.1	20.2	20.3	21.1	21.2	21.3
C10:0	-	-	-	-	-	-	-	-	-
C11:0	-	3854842	-	-	-	-	-	-	-
C12:0	1605872	1946732	-	-	7583890	4964626	2702357	-	-
C13:0	993557	738783	2419423	-	1640778	-	-	-	-
C14:0	-	1636972	-	55304655	90561401	-	3094293	-	3775725
C14:1Δ9	386518	255012	12367306	-	-	-	-	7344270	3725246
C15:0	-	826842	48538563	7698130	-	-	3388125	-	-
C15:1Δ10	6652503	7430807	22355520	3437056	5991582	5521745	-	6475988	-
C16:0	143798418	-	-	158653068	-	-	428331865	-	-
C16:1Δ9	-	-	-	-	-	-	-	-	-
C17:0	-	-	35632906	6412826	5937572	6823329	8318808	11130052	14241352
C17:1Δ10	-	-	-	2399737	-	-	-	-	-
C18:0	167541947	169493096	355947528	-	434862655	-	529145776	-	-
C18:1Δ9	168835779	206146468	-	168984433	-	-	-	-	-
C18:2Δ9,12	12717925	14900707	-	-	41952436	-	11534417	11637596	16662255
C19	79397068	87958213	115785183	5677260	155486697	230376937	130444146	185600351	160576296
C18:3Δ6,9,12	-	3767731	-	-	13805840	-	-	-	-
C18:3Δ9,12,15	-	-	-	-	-	-	-	-	-
C20:0	33646686	32516171	7705536	-	40709051	7827044	21977302	-	37518578
C20:1Δ11	-	-	-	17122569	-	-	-	-	-
C20:2Δ11,13	3765047	-	-	-	-	-	-	101131248	-
C20:3Δ7,10,13	-	2913000	26114774	-	-	27217510	-	38851068	40149115
C20:4Δ5,8,11,14	-	-	-	7151455	-	-	-	-	-
C21:0	-	-	-	-	-	-	-	-	-
EPA	-	-	-	193546463	-	-	-	-	-
C22:0	-	-	-	-	8106431	9137733	59009648	112593639	16060657
C22:1Δ13	2675385	4623214	6169657	-	-	-	-	-	-
C22:2Δ13,16	-	-	-	-	2912338	5986512	-	-	-
C23:0	1100030	-	-	-	-	-	-	-	-
DHA	152653997	196041591	275804446	152015290	-	-	149895427	-	-
C24:0	-	-	-	-	-	-	-	-	-
C24:1Δ15	-	-	-	-	-	-	-	-	-

Table 26 - Obtained peak areas for each fatty acid for the replicas collected for sample CEs22

FA	22.1	22.2	22.3
C10:0	-	-	-
C11:0	-	615574	-
C12:0	901908	-	-
C13:0	489965	12806439	-
C14:0	1976690	1103425	-
C14:1Δ9	-	962835	10951416
C15:0	542040	544591	
C15:1Δ10	624754	3554398	10225154
C16:0	-	-	-
C16:1Δ9	-	-	31676659
C17:0	10736869	11004681	27414784
C17:1Δ10	15869021	-	31124866
C18:0	452740338	242125671	-
C18:1Δ9	-	-	-
C18:2Δ9,12	-	-	-
C19	246269559	148123437	258481753
C18:3Δ6,9,12	-	-	-
C18:3Δ9,12,15	-	-	309869242
C20:0	-	1987451	-
C20:1Δ11	10881076	2014831	-
C20:2Δ11,13	-	-	-
C20:3Δ7,10,13	-	17832367	30217543
C20:4Δ5,8,11,14	-	-	-
C21:0	20012594	-	-
EPA	-	-	-
C22:0		44169557	50834852
C22:1Δ13	-	-	-
C22:2Δ13,16	-	-	-
C23:0	-	-	-
DHA	-	-	-
C24:0	-	-	-
C24:1Δ15	-	-	-