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Habitat, trophic ecology and mercury levels of two Antarctic octopod species

Dissertation of MSc in Ecology, supervised by Prof. Dr. José Carlos Caetano Xavier and Prof. Dr. Jaime Albino Ramos presented to the Department of Life Sciences of the Faculty of Sciences and Technology of the University of Coimbra

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Cover photo of Grytviken, South Georgia (Antarctica): Courtesy of José C. Xavier

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In memory of Francisco Manuel da Silva (30/09/29 – 10/03/08),

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Main Contents

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Abstract

Cephalopods (e.g. octopus, squid) play important roles in Antarctic marine ecosystems and are a major portion of the diet of Antarctic top predators. However, there is still a lack of crucial ecological information regarding Southern Ocean cephalopod species. This knowledge becomes even poorer when referring to Antarctic benthic octopod species, since most past studies had mainly focused on squid, particularly those with potential interest for future fishery exploitation.

The *Adelieledone polymorpha* (Robson, 1930) and *Pareledone turqueti* (Joubin, 1905) are two sympatric octopod species occurring abundantly in overlapping benthic ecosystems on the South Georgia shelf. Albeit *A. polymorpha* and *P. turqueti* are similar externally, the remarkable morphological difference between their beaks has raised the hypothesis that both octopod species exploit distinct trophic niches within the same habitat. Through a wide analysis combining different methodologies applied onto the beaks, the goal of this study was to understand the ecology of both species by relating their beak morphology (through physicochemical properties analysis), their habitat and trophic levels (through stable isotopic signatures (carbon and nitrogen)) and bioaccumulation/biomagnification food web processes through trace metal concentrations (total mercury, T-Hg). Moreover, assessing mercury levels in benthic octopods allowed the evaluation of pollution levels around South Georgia.

My results show that *A. polymorpha* and *P. turqueti* inhabit the same habitat while exploring different trophic niches, at least partially. Both species exhibited an expected dietary ontogenic shift, translated by an increase of $\delta^{15}N$ from the juvenile to the adult life stages, presumably for preying on larger and different preys. Moreover, the beaks of both species seem to present differences in density between species. Therefore, the different beak physicochemical properties and other dissimilarities in the digestive apparatus (e.g. posterior salivary gland) between both species seem to be adaptations to different trophic niches.

Regarding total mercury (T-Hg), few conclusions were drawn about the trophic ecology of both species since it is still unknown how differently tissues from octopods uptake mercury. However, the highest T-Hg concentrations were found on *P. Turqueti* flesh. Relative to other benthic octopod species in the northern hemisphere, similar values of T-Hg were found on *A. polymorpha* and *P. turqueti*. These results show that ecosystem’s health of Antarctica may be threatened due to higher pollution levels of some toxicants, however future studies are needed.

Keywords: Sympatry; Cephalopods; Beak morphology; Stable isotopic analysis; Heavy metals.

Resumo

Os cefalópodes (p.e. polvos, lulas) desempenham papéis importantes nos ecossistemas marinhos no oceano Antártico e constituem uma grande porção na dieta de predadores topo. No entanto, existe ainda uma falta de informação sobre estes organismos. Este conhecimento torna-se ainda mais pobre relativamente às espécies antárticas bentónicas, uma vez que estudos anteriores se focaram maioritariamente em espécies de lula, particularmente aquelas com potencial comercial.

O *Adelieledone polymorpha* (Robson, 1930) e o *Pareledone turqueti* (Joubin, 1905) são duas espécies simpátricas de polvos que ocorrem nos ecossistemas bentónicos ao largo da Geórgia do Sul. Apesar de *A. polymorpha* e *P. turqueti* serem similares externamente, a diferença notável entre a morfologia dos bicos de ambas originou a hipótese de que os dois polvos exploram nichos tróficos distintos no mesmo habitat. Através de um estudo multidisciplinado, o objetivo deste estudo consistiu em perceber a ecologia de ambas as espécies de polvo, relacionando as assinaturas de isótopos estáveis (carbono e azoto) e concentrações de metais pesados (mercúrio) com a morfologia dos bicos. Além disso, consistiu em avaliar os níveis de mercúrio nas duas espécies de polvos bentónicos, de forma a compreender os níveis de poluição por mercúrio nos ecossistemas bentónicos em redor da Geórgia do Sul.

Este estudo mostra que *A. polymorpha* e *P. turqueti* habitam nos mesmos habitats enquanto exploram nichos tróficos parcialmente diferentes. Ambas espécies apresentaram mudanças ontogénicas de dieta, traduzidas por um aumento de $\delta^{15}N$ entre as fases da vida juvenil e adulta, provavelmente resultante de uma predação de presas maiores e diferentes. Adicionalmente, os nossos resultados indicam diferenças de densidade entre os bicos de ambas as espécies. Concluímos que, as diferentes propriedades físicas dos bicos e outras diferenças que ocorrem no aparelho digestivo de ambas as espécies aparentam ser adaptações a diferentes nichos tróficos.

Relativamente ao mercúrio total (T-Hg), poucas conclusões puderam ser retiradas sobre a ecologia trófica de ambas espécies uma vez que ainda não se sabe como os diferentes tecidos absorvem mercúrio. No entanto, as maiores concentrações de T-Hg foram encontradas no músculo de *P. turqueti*. Adicionalmente, concentrações de T-Hg similares a outras espécies de polvo do hemisfério norte foram encontradas em *A. polymorpha* e *P. turqueti*. Estes resultados mostram que a saúde de ecossistemas antárticos pode estar ameaçada por um aumento do nível de poluição causado por certos tóxicos, contudo há ainda a necessidade realizar mais estudos para uma melhor compreensão da sua ecologia.

Palavras-chave: Simpatria; Cefalópodes; Morfologia dos bicos; Análise de isótopos estáveis; Metais pesados.

CHAPTER I – Introduction

1. Why study ecology in Antarctica and Southern Ocean?

Polar regions are some of the most important ecosystems due to their contribute to climate regulation worldwide (Sarmiento et al., 2004). Man has become the main responsible for interfering with Earth's physicochemical processes, including these ice-covered regions (IPCC, 2013; Oreskes, 2004) where its effects are visible (Kennicutt II et al., 2014; Meltofte, 2013; Xavier, Brandt, et al., 2016). For example, both Antarctic and Arctic ice-shelves are decreasing due to warmer temperatures and contributing to rising sea temperatures, sea-level rise, and decreased ocean salinity, affecting other ecosystems globally (Hoegh-Guldberg & Bruno, 2010; Miles et al., 2013). Still, more studies are required to fully understand climate change impact on polar regions (Gutt et al., 2015; Hanna et al., 2013; Murphy et al., 2007; SCAR, 2011).

Associated to climate change effects, decreasing ocean productivity is affecting polar marine food webs and the ecosystem itself (Constable et al., 2014; Montes-Hugo et al., 2009; Schofield et al., 2010). Due to structural shifts, different distributions or behaviours from some organisms are expected as an adaptation to climate disturbances and changing distributions of preys (Xavier, Peck, et al., 2016). Species, from benthic to pelagic ecosystems, are unable to disperse in rapidly changing environments are likely to face extinction (Griffiths, et al., 2017; Griffiths et al., 2013; Hays et al., 2005; Perry et al.Reynolds, 2005). Recent numerous efforts on polar ecology research has helped to understand how ecosystems are affected and, afterwards, develop adequate conservation measures that hopefully will help to mitigate climate change effects globally (Chown et al., 2017; Kennicutt II et al., 2014; Xavier, Brandt, et al., 2016).

Marine polar ecosystems are also pressured by fishery activities targeting commercially valuable species (Haug et al., 2014). In Antarctic waters, large populations of Antarctic krill (*Euphausia superba*), an important element in Antarctic marine ecosystems (Xavier & Peck, 2015), are used for human consumption and animal industries (Everson, 2000). Moreover, as Antarctic krill properties are discovered new krill-based products developed, it is expected that the demand for Antarctic krill will increase the pressure on nature stocks and food webs dependent (directly or indirectly) on it (Nicol et al., 2000; Nicol & Foster, 2003). Modelling projections show that different species distribution will force commercial fisheries to encompass larger areas to supply the demand and also search for alternative target species, such as cephalopods (e.g. octopods, squids) (Hill et al., 2013; Perry et al., 2005). Therefore, present and future fishery management policies need ecological information about these Antarctic marine organisms to guarantee a sustainable marine environment (Allison et al., 2009; Constable, 2011; Xavier, Brandt, et al., 2016).

In Antarctica, where this thesis will focus, there are only two marine protected areas (MPA's) under Antarctic treaty regulations today: one created in 2009 located in the South Orkney Islands' southern shelf (CCAMLR, 2009a, 2012) and a more recent one, created in 2016, containing Ross Sea region. Since 2012, still in the Southern Ocean but not under Antarctic Treaty regulations, there is a British MPA for South Georgia and South Sandwich Islands (De Santo, 2013), whose region is still requiring further ecological information to provide evidence on conservation measures to be implemented. Hence, ecology research of benthic Antarctic organisms, such as octopods, can provide information relevant to marine food web interspecific interactions locally (Xavier, Brandt, et al., 2016; Xavier & Peck, 2015).

1.1. South Georgia marine ecosystem

Approximately 300km south the Antarctic Polar Front, where cold Antarctic and warm sub-Antarctic waters meet, and benefiting from upwelling nutrient-rich currents, South Georgia is one of the most productive areas in the Southern Ocean (Fig. 1), supporting numerous populations of Antarctic marine organisms, from small phytoplankton/zooplankton (Atkinson et al., 2001; Hogg et al., 2011) to large top predators (Croxall et al., 1984). Antarctic krill is one of the most important species in the entire Antarctic food web breeding around South Georgia, thus being a dominant species (Fig. 2) (Croxall & Wood, 2002). Also in the Antarctic food web, cephalopods constitute an important mid-trophic food source for large predators (Xavier & Cherel, 2009). Consequently, large breeding colonies of penguins and other seabirds, seals, fish and whales, inhabit the South Georgia region, making it one of the richest in biodiversity in the Southern Ocean (Fig. 3) (Hogg et al., 2011; Murphy et al., 2007).

Despite its geography and high productivity, South Georgia region is rapidly changing due to climate change effects due to rising temperatures and decreased ocean salinity, a shift in phytoplankton size has leading to structural shifts in the food web (Whitehouse et al., 2008; Xavier et al., 2013). Antarctic krill densities are decreasing while salps, a gelatinous zooplankton organism, are becoming more abundant due to higher tolerance to environmental stress and grazing efficiency (Atkinson et al., 2004; Flores et al., 2012). Since predators do not prefer salps to Antarctic krill (and are energy deficient), negative feedbacks in species distributions, feeding habitats and population size are expected (Fraser & Hofmann, 2003; Moline et al., 2004). Alternative food-web ways emerging recently, with other organisms such as myctophid fish, can play a relevant role in energy transfer up the food chain (Murphy et al., 2007). The role of cephalopods, mostly octopods, still needs to be fully understood (Collins & Rodhouse, 2006).

Figure 1. Location of the South Georgia region.

Current fishery activities in the Southern Ocean target fish and Antarctic krill, therefore, ecological research has been ongoing for several decades and providing crucial information for future policies related to fishery and ecosystem's conservation management (Xavier et al., 2015; Xavier, Brandt, et al., 2016). As a result, South Georgia region is now protected by a restricted zone for bottom-fishing activities and Antarctic krill fisheries are now highly regulated by the Commission for the Conservation of Antarctic Marine Living Resource (CCAMLR, 2009b). However further needs for research on a wide range of marine organisms, such as cephalopods (including octopod fauna) have been identified (Collins et al., 2004; Collins & Rodhouse, 2006; Hill et al., 2007).

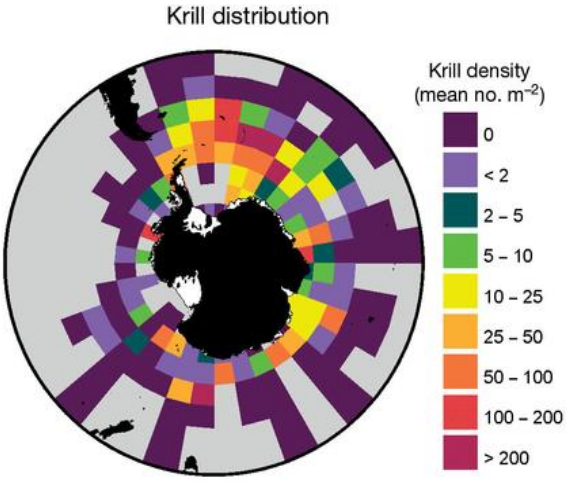

Figure 2. Circumpolar distribution of the Antarctic krill (Euphausia superba) with the arithmetic values of krill density (ind. m^{-2}) derived from KRILLBASE (adopted from Flores et al., 2012).

1.2. Antarctic Cephalopod Ecology

The Southern Ocean cephalopod fauna is diverse and distinctive, supporting over 50 known species of 16 different families of octopods (Octopoda) and squid (Decapoda) which, some of them, are endemic to Antarctic waters (Collins & Rodhouse, 2006; Xavier et al., 2018). The number of species and their taxonomy is still changing as new techniques and innovative approaches are applied to this field of study (Allcock, 2005; Allcock et al., 2003).

Cephalopods are very important to the Antarctic food web, particularly in South Georgia region, playing both roles of predator and prey (Clarke, 1996; Collins & Rodhouse, 2006) and constituting a big percentage of the diet of numerous Antarctic top predators (Fig. 3), including albatrosses (Alvito et al., 2014; Xavier et al., 2007), penguins and other seabirds (Casaux & Ramón, 2002; Rodhouse et al., 1998), seals (Negri et al., 2016; Rodhouse, 2012), sharks (Cherel & Duhamel, 2004), Patagonian toothfish (Cherel et al., 2004; Xavier et al., 2002) and sperm whales (Cherel, Ridoux, et al., 2009; Clarke, 1980).

Figure 3. Vertebrate predators of South Georgia: Black-browed Albatross (top-left), Elephant Seal (top-right), Weddell Seal (bottom-left) and Gentoo Penguins (bottom-right). (Photos: José C. Xavier, MARE-UC).

The complexity of ecological systems and the multiplicity of stressors make future environmental impacts in Antarctic cephalopods very difficult to predict (Gutt et al., 2015; Xavier et al., 2015; Xavier, Brandt, et al., 2016). Within Antarctic cephalopods, the ecology of octopods, are not known particularly on the most northern islands, such as South Georgia.

1.3. Antarctic Octopods of South Georgia

Since previous cephalopod researchers mainly focused on squid species with commercial value (e.g. *Martialia hyadesi*) or relevant to predators' diets, there is still considerable work to be carried out on Antarctic octopod species (Rodhouse, 1998). Cephalopods distribution is continuously improving as new specimens are caught on fishing/scientific nets or its remains identified on the diet of Antarctic top predators (Xavier, Raymond et al., 2016). The Southern Ocean counts with 34 octopod species distributed through Antarctic and sub-Antarctic waters (Collins & Rodhouse, 2006; Xavier et al., 2018). Endemism can be found in Antarctic/sub-Antarctic waters and, it is thought that, they may have evolved after the formation of the Antarctic circumpolar current (Allcock & Piertney, 2002). With 13 recognised species, *Pareledone* is the most abundant genus that can be found in Southern ocean marine ecosystems (Allcock, 2005).

In South Georgia region, seven octopod species and one yet to be confirmed have been caught. Previous bottom trawl surveys suggest suggest that species like *Thaumeledone gunteri* (Robson, 1930), *Staurotheutis gilchristi* (Robson 1924), *Cirroctopus glacialis* (Robson, 1930), *Graneledone* sp. and *Opisthoteuthis hardyi* (Villanueva et al., 2002), occur in the South Georgia region at higher depths while *Pareledone turqueti* (Joubin, 1905) and *Adelieledone polymorpha* (Robson, 1930) occur mainly at shallower depths. Same studies also indicate that, octopod fauna from South Georgia is dominated by *P. turqueti* and *A. polymorpha* at lower depths, being replaced at higher depths by *Thaumeledone gunteri* (Collins et al., 2004; Yau et al., 2002).

1.4. Case Study: Two Benthic Sympatric Octopod Species of South Georgia

In ecology, two species are considered sympatric when both are found sharing the same habitat in the same time. This niche overlap might translate itself in increased competition for natural resources among those species or in the adoption of different adaptations to reduce competition. In Namibian waters (South Atlantic) for example, two deep-sea octopod species, *Opisthoteuthis agassizii* and *Opisthoteuthis vossi*, have similar distributions but, higher abundances of *O. vossi* were registered at higher depths during the night, not confirmed to be related to a different feeding ecology (Villanueva & Guerra, 1991). In Galician waters (North Atlantic), two squid species, *Todaropsis eblanae* and *Illex coindetti*, present similar distributions and a similar diet. However, in regions with higher abundances of crustaceans, *T. eblanae* seems to have a broader diet possibly due to a benthic predatory activity (Rasero et al., 1996).

Within Antarctic octopods, *Pareledone turqueti* (Joubin, 1905) and *Adelieledone polymorpha* (Robson, 1930) are two sympatric in the Southern Ocean (Fig. 4). These two benthic octopod species inhabit the Scotia Sea coastal waters from the Antarctic Peninsula to South Georgia, where higher abundances are registered (Collins et al., 2004; Negri et al., 2016). Since both species are prey to higher trophic predators, past dietary studies of Antarctic top predators have contributed to both species' distribution (Alvito et al., 2014; Casaux & Ramón, 2002; Rodhouse et al. , 1992) but their allometric equations (Xavier & Cherel, 2009) are still poor. During a survey around South Georgia, both species could be found in a depth range of 100-400 meters, however the *P. turqueti* tends to be distributed at shallower depths (Yau et al., 2002). Ocean topography seems to affect the genetic flow between populations which, over time, may lead to speciation (Allcock et al., 1997).

Figure 4. *Pareledone turqueti* (left) and *Adelieledone polymorpha* (right) specimens (Photos: Michael Oellermann, Alfred Wegener Institute).

In addition to both species distribution and main predators, some studies have added crucial information to fully understand how these two species can coexist in the same habitats (Collins et al., 2004; Negri et al., 2016). Despite their external similarities, *P. turqueti* has body less prone to damage while *A. polymorpha* is more delicate and easily damageable, what might indicate that the latter is more adapted to an off-bottom lifestyle. On the other hand, *P. turqueti* seems to be more muscular and robust what might be an adaptation to a rocky benthic environment (Daly & Rodhouse, 1994). Moreover, both species have toxic saliva composed by cephalotoxin and paralyzing enzymes for external digestion of the prey (Allcock et al., 2003; Daly, 1996). The toxic saliva is produced by the posterior salivary gland (PSG), which is significantly larger in *A. polymorpha* indicating that this species might rely heavily on its saliva (Fig. 5) (Allcock et al., 2003; Daly, 1996; Gibbs & Greenaway, 1978). In contrast, *P. turqueti* has a smaller PSG, a bigger buccal mass and a weaker venom, evidence that this species might rely more on bite force to prey on its toxic saliva (Undheim et al., 2010).

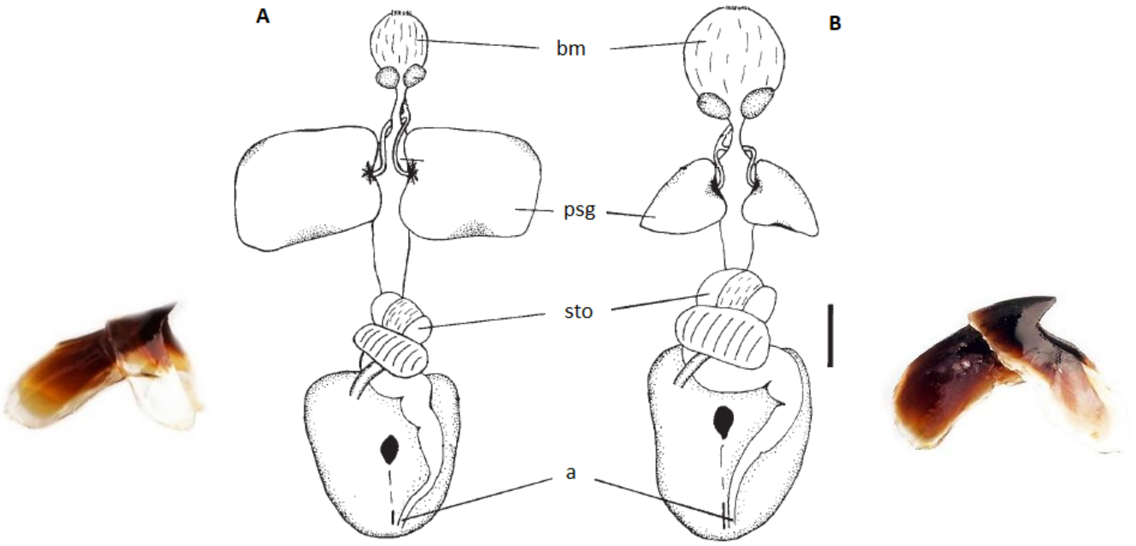

Figure 5. Drawings of the digestive systems of *Adelieledone polymorpha* (A) and *Pareledone turqueti* (B). Abbreviations: a – anus; bm – buccal mass; psg – posterior salivary gland; sto – stomach (adapted from Allcock et al., 2003). Photos of lower beaks of *A. polymorpha* (lower hood length (LHL): 2.3mm; left) and *P. turqueti* (LHL: 3.8mm; right) are also presented. (Xavier & Cherel, 2009).

Both species' beaks are important identifying features of these octopod species. The beaks of *A. polymorpha* present a morphology unseen in other octopod species due to its small size and upturned sharp rostrum (Fig. 5) (Allcock et al., 2003; Daly & Rodhouse, 1994). Possibly, *A. polymorpha* unique beak might be an adaptation to a different trophic niche as past studies have identified few differences on their diets but no conclusions were drawn due to limited data (Daly & Rodhouse, 1994). Through macroscopic identification, both species diet are mainly constituted by amphipods, polychaetas, and other benthic organisms (Piatkowski et al., 2003). The presence of thick-shelled bivalves was only verified on the diet of *P. turqueti* (Daly, 1996; Piatkowski et al., 2003) but more information on their feeding ecology is required.

1.5. Beaks and Dietary Studies applied to Antarctic Octopods

One approach developed to study trophic interactions involving cephalopods as prey, is the use of their beaks (Fig. 6)(Clarke, 1962a; Xavier, Ferreira, et al., 2016). Important for feeding, cephalopod beaks are chitinous structures that have been intriguing researchers for a long time. The beaks are constituted by two mandibles, an upper and a lower beaks, and present different morphologies among species, what makes them good structures for species identification (Clarke, 1986; Ogden et al., 1998; Xavier & Cherel, 2009). Since cephalopod beaks are very resistant, they can go through the digestive track of predators without being degraded. Therefore, the use of top predators as biological samplers to identify and study the biology and ecology of cephalopods has been a major contributor for advances in cephalopod researches (Clarke, 1980, 1996; Rodhouse et al., 1992; Xavier et al., 2014). Other than identifying cephalopod species, different beak measurements allow estimations of total weight and size of the specimen body in life, through allometric equations (Clarke, 1962b; Jackson, 1995), which can provide important information about population structure, growth, reproduction, distribution, trophic ecology, trace metals, and others (Xavier, Ferreira, et al., 2016).

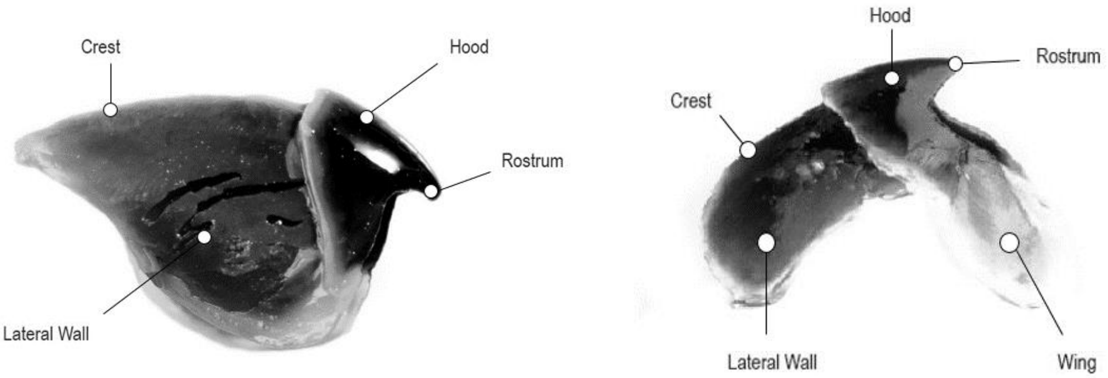

Figure 6. Legend of the octopods' upper (left) and lower (right) beaks.

1.6. Trophic Ecology Studies: Indirect Methodologies

Trophic ecology of Antarctic cephalopods was, traditionally, studied using direct methodologies such as the identification of squid or octopod remains (mostly beaks) in the diet of predators. However, new approaches to study trophic ecology of cephalopods, using their beaks, have been developed, such as physicochemical properties analyses, stable isotope analyses and trace metal analyses.

1.6.1. Physicochemical Properties of Beaks

Through techniques as Scanning Electron Microscopy (SEM), X-Ray based methods and other techniques, cephalopod beaks ontogeny and mechanical properties are starting to be discovered. The cephalopod beaks are mainly constituted by chitin, water and proteins that define a gradient of stiffness when in different proportions (Miserez et al., 2007). A gradient of stiffness and colour can be found within beaks from the rostrum to the crest, being the rostrum the stiffest and darkest part of the beak and, in opposition, the crest being the most flexible and light coloured (Miserez et al., 2008). The gradient of stiffness is largely caused by an increment towards the rostrum of proteins rich in amino acids that mechanically stabilize and strengthen the beak (Miserez et al., 2010; Miserez et al., 2008; Tan et al., 2015).

Regarding the beak ontogeny, the rostrum, also known by rostral tip, is formed at early stages of life and, as the individual ages, the beak continuously develops towards the crest, where the newly formed chitin is, without renovating tissue (Fig. 4)(Cherel & Hobson, 2005). Such physicochemical properties of beaks may allow cephalopods to feed on different prey and explore different habitats.

1.6.2. Stable Isotope Analysis (SIA)

This technique has been applied to cephalopod's beaks in order to obtain isotopic signatures, provided by the different stable isotope ratios. Since stable isotope signatures can be found in natural gradients, important information can be inferred based on its ratio values (Cherel & Hobson, 2005; Hobson & Welch, 1992). Since the rostrum and the outer part of the crest are, respectively, the oldest and the newest parts of the beak, life-history traits can be identified when applying SIA to different parts of the same beak (Cherel, Fontaine, et al., 2009; Cherel & Hobson, 2005; Guerra et al., 2010; Queirós & Xavier pers. comm.). The stable isotope ratios of carbon ($^{13}C/^{12}C$, $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, $\delta^{15}N$) are used to study the habitat and trophic ecology of species, respectively: since ^{15}N bioaccumulates from lower to higher trophic levels (mean value of increase per trophic level: 2.5-3.4‰; Cherel & Hobson, 2005), nitrogen stable isotope ratio ($\delta^{15}N$) can be used as indicator of a consumer's trophic position. On the other hand, carbon stable isotope ratios ($\delta^{13}C$) vary little along trophic levels and are used to determine the primary sources in a trophic network (Cherel & Hobson, 2005; Hobson & Welch, 1992; McCutchan et al., 2003). In a marine context, a positive gradient of $\delta^{13}C$ can be found from off-shore/neritic to inshore/benthic species and from lower to higher latitudes. Thus, $\delta^{13}C$ values able us to characterise organisms preferential habitat, since it varies according to latitude, proximity to coast and depth (Guerreiro et al., 2015; Jaegar et al., 2010; Seco et al., 2016).

1.6.3. Trace Metals: Mercury Analysis

Complementary to habitat and trophic levels analyses, heavy metal analyses are important, not only to assess bioaccumulation/biomagnification food web processes and the human risk of exposure to marine organisms, but also to quantify ecosystem's pollution levels for future mitigation Antarctic policies (Xavier, Ferreira, et al., 2016). As anthropogenic activities grow, so does the amount of toxic waste that ends up in the environment (Halpern et al., 2008). Mercury (Hg), one of the most concerning trace metals for the ecosystem, enters in the aquatic ecosystems and is methylated by bacteria, and, from then on, is bioaccumulated and biomagnified along the trophic levels (Atwell et al., 1998; Wiener et al., 2007). In Antarctic marine ecosystems, past studies have confirmed that total Hg concentrations increase from phytoplankton to top predators (Bargagli et al., 1998; Tavares et al., 2013). In addition, aquatic organisms, including cephalopods, absorb mercury through their diet since Hg levels on muscle tissue tend to be higher than in other tissues (Mieiro et al., 2012; Penicaud et al., 2017). Among cephalopods, benthic octopods seem to accumulate higher concentrations of mercury on the flesh (Storelli et al., 2010).

Additionally, since mercury is bioaccumulated along the trophic chain, previous studies have shown that both Hg and $\delta^{15}N$ values correlate positively and that, conjugated, can be used to study trophic relationships (Atwell et al., 1998; Bearhop et al., 2000). Therefore, after a careful interpretation of mercury concentrations on Antarctic organisms, it is possible to have some insights about trophic ecology of the species in study by comparing mercury concentrations between Antarctic organisms of the same region, occupying very similar habitats (Bargagli et al., 1998; Dehn et al., 2006).

Recent studies show that cephalopod beaks have mercury, the use of trace metals, mercury for example, enables a new approach for cephalopod ecology studies (Xavier, Ferreira, et al., 2016). However, more studies are needed since it is still unclear how much do Hg concentrations in cephalopod beaks differ from other softer tissues and how does it interferes with the individual's biology and physiology (Xavier, Ferreira, et al., 2016).

1.7. Objectives

The main goal of this thesis is to understand the ecology of two octopod species in study, *P. turqueti* and *A. polymorpha*, using a novel approach combining different methodologies of physicochemical beak properties, stable isotopic and trace metal analyses. In addition, allometric equations (relating beak size to mantle length (ML) and total mass (M)) from complete individuals caught in bottom trawling fishing nets were produced. Specifically, this study addressed three main points:

(1) Evaluation of both species beak's physicochemical properties, structure and stiffness, using the techniques of SEM, XRD, micro CT and AFM. Since beaks from *A. polymorpha* are constituted by a lighter brown chitin and more delicate at touch, it is believed that beaks from *A. polymorpha* are less stiffer that beaks from *P. turqueti* (Miserez et al., 2008). Due to the close phylogenetic relation of these two octopod species, no differences in chemical composition and microstructural organization are expected to be found (Cárdenas et al., 2004). If verified both hypotheses, a trophic niche constituted by hard-tissue prey may be unavailable to *A. polymorpha* due to its beak's limitations. On the other hand, the unique shape of the beak of *A. polymorpha* may be an adaptation to a specific trophic niche not explored by benthic octopods like *P. turqueti*;

(2) Comparison of the habitat and trophic ecology of both octopod species through stable isotopic analyses on their upper and lower beaks. Using different sections within beaks, it is also intended to study possible habitat and trophic shifts on the life cycle of both species, and the effects of untanned chitin in the results. Since tanned and untanned chitin might be biochemically different, lower δ^{15}N are expected on the latter (Cherel, Fontaine, et al., 2009). It is expected not to find great differences in δ^{13}C values between species since the primary production source responsible for the energy flow in the South Georgia coastal ecosystem is the same. Shifts in habitat throughout specimens' life cycle are also not expected. On the other hand, both species might increase in trophic level from juvenile to adult and a broader range of δ^{15}N values are expected to be found on *P. turqueti* due to its apparent generalist behaviour (Cherel, Fontaine, et al., 2009; Cherel & Hobson, 2005; Guerra et al., 2010);

(3) Comparison of mercury concentrations in the flesh and beaks of *P. turqueti* and *A. polymorpha*, in relation to their habitat and trophic level. It is still unknown how beaks accumulate mercury comparatively to soft tissues – however, lower concentrations are expected to be found on cephalopod beaks (Xavier, Ferreira, et al., 2016). Positive relationships are expected between Hg concentrations on flesh and beaks. Moreover, since mercury is bioaccumulated/biomagnified and *P. turqueti* may occupy a wider trophic niche constituted by

prey of higher trophic level, higher mercury levels are expected to be found on *P. turqueti* tissues.

In summary, this thesis aims to provide a comparative analysis of the properties of beaks, the habitat, trophic level, trace metal levels of *P. turqueti* and *A. polymorpha* by applying a range of scientific techniques. This thesis also aims to contribute data to support future decisions in ecosystem's management of South Georgia MPA.

CHAPTER II – Materials & Methods

2. Sampling and First Proceedings

In this thesis, beaks of *Pareledone turqueti* and *Adelieledone polymorpha* were collected from different sources. Some beaks were collected from boluses (i.e. indigestible material regurgitated voluntarily) (Xavier, Croxall et al., 2005) by Black-Browed Albatrosses (*Thalassarche melanophris*) and Blue-eyed Shags (*Phalacrocorax atriceps*) during 2015 and 2016, respectively, and from individuals caught on scientific trawlers in the years 2004 and 2013. These materials were collected by members of Marine and Environmental Sciences Centre of the University of Coimbra (MARE-UC) and/or collaborators. The British Antarctic Survey (BAS) provided all the support related to the permits for the fieldwork (as fieldwork was carried out from a BAS research base): The animal procedures used in this study were reviewed and approved by the joint BAS Cambridge University Animal Welfare and Ethical Review Committee. Permits to operate were issued by the Government of South Georgia and the South Sandwich Islands. For each objective of the thesis, it will be specified which beaks were used. After being collected, all samples were kept in plastic bags and frozen at -20°C. Cephalopod species were after identified at BAS facilities (Cambridge, UK).

All sub-adult/adult octopod's upper (n=83) and lower (n=91) beaks were after analysed in MARE facilities in the University of Coimbra, Portugal. The beaks were clean and measured, with a digital Vernier calliper (Fig. 5) and a measuring magnifier, to the nearest 0.01mm for the upper beak' hood length (UHL) and crest length (UCL), and lower beak hood length (LHL)(Rodhouse et al., 1998; Xavier et al., 2002). All samples were kept in ethanol 80% filled Eppendorf's with respective labelling and allometric equations built with the available data. For some beak samples, due to the unavailability of mantle length and mass values, allometric equations were given by Xavier & Cherel, 2009.

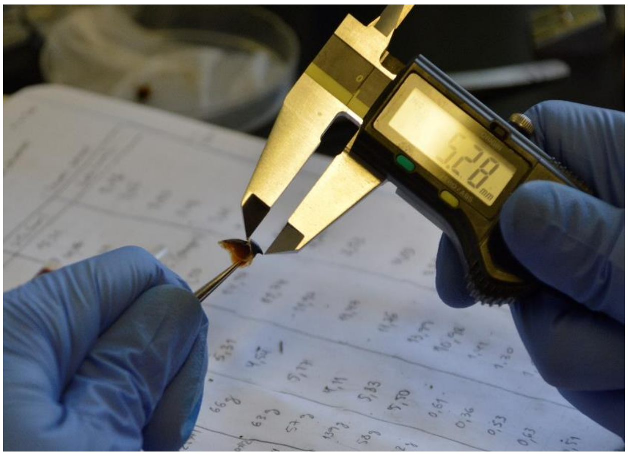

Figure 7. Measuring the hood length (UHL) from an upper beak of *Pareledone turqueti* in MARE-UC.

2.1. Physicochemical Properties Analysis

To study the structure and stiffness of both octopod species beaks, several specimens of *P. turqueti* and *A. polymorpha* were collected during a trawl survey carried out by Marine Resources Assessment Group (MRAG) and BAS researchers on behalf of the Government of South Georgia and South Sandwich Islands, between January and February of 2004. The specimens were caught along South Georgia coast at depths ranging from 100 to 400 meters during 30-minutes sessions and thereafter frozen and taken for identification at BAS (Cambridge, UK). After the standard proceedings (see 2.), the physicochemical properties of beaks were analysed in 3B's Research Group in Biomaterials, Biodegradables and Biomimetics of the University of Minho (Braga, Portugal), using multiple techniques (see below).

Scanning Electron Microscopy (SEM)

The microstructure of the beaks (per species, $n_{Upper}=2$ and $n_{Lower}=2$) was evaluated using JSM-6010 LV (JEOL, Japan). For that, beaks were fractured under different conditions: dried sample (fractured after drying beaks in vacuum oven at 37°C for 24h); frozen sample (fractured after immersing beaks in liquid N_2); hydrated sample (immediately fractured after taking them from ethanol-water solution). Since different fracturing conditions might affect the fracture propagation and consequently the observed microstructure, fracturing the beaks under different conditions was an attempt to observe what conditions would preserve better the natural morphology of the microstructure using longitudinal and transversal cuts (Fig. 8).

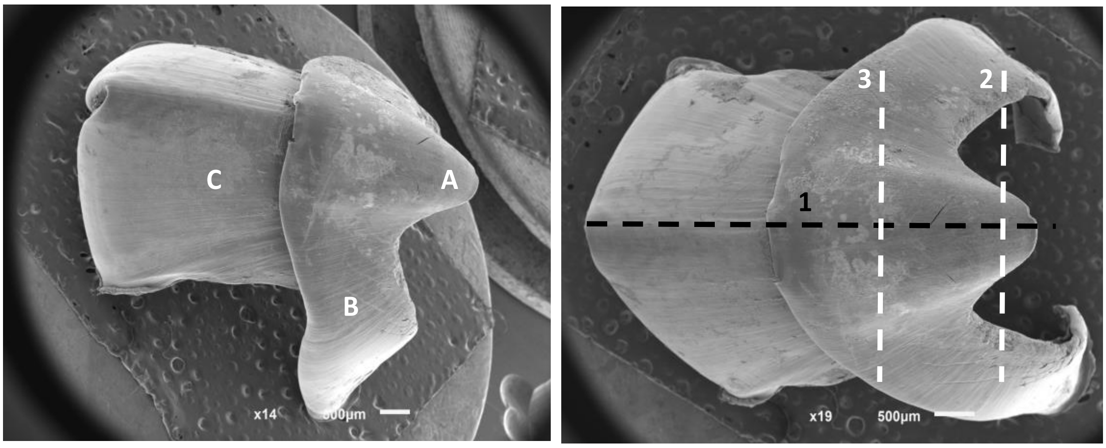

Figure 8. Scanning electron micrographs of *Pareledone turqueti* lower beak with indications of the main structures and cut directions used in this study. A – rostrum; B – wing; C – lateral wall. 1 – longitudinal fracture; 2 – transversal fracture of the rostrum; 3 – transversal fracture of the hood.

X-ray Powder Diffraction (XRD)

For characterizing the mineral structure, the beaks (per species, $n_{Upper}=1$ and $n_{Lower}=1$) were ground into fine powder and analysed by XRD since different materials interact differently with the radiation. The qualitative analysis of crystalline phases presented on the samples were obtained using a conventional Bragg-Brentano diffractometer (Bruker D8 Advance DaVinci, Germany) operated with a Cu-Kα anode (λ = 1.5406 Å), scanning from a dispersion range 2θ between 5° to 50° at a speed of 2°/min (Cárdenas et al., 2004; Miserez et al., 2007)

Micro Computed Tomography (micro CT)

To study the structural features of the beaks (e.g. geometry and density) of the two species in study, *A. polymorpha* and *P. turqueti*, a high-resolution microcomputed tomography (uCT) SkyScan 1272 (Bruker, Kontich, Belgium) was used. Specimens at adult stages were selected for better comparison of the density and geometry (per species, $n_{Upper}=1$ and $n_{Lower}=1$). For scanning, a voltage range of 50kV and current source of 200µA was applied and a pixel size between 8µm and 16µm was selected for the octopodid species, *A. polymorpha* and *P. turqueti* respectively. The acquisitions were obtained without filter application and the rotation step used was 0.3° up to a total of 360°.

The images obtained by x-ray were then 3D reconstructed using the CTvox software according to a threshold directly proportional to material hardness/density.

Atomic Force Microscopy (AFM)

To study the stiffness of the beaks, a Dimension Icon atomic-force microscope (Bruker) system with a RTESPA probe was employed. In this technique, a probe tip at the end of a flexible cantilever scans across the sample and registers its topography based on the cantilever's deflection caused by the interaction forces between the probe tip and sample surface, building an image with the resultant data. AFM is also used to study physicochemical properties of biomaterials through indentation tests that provide information about stiffness, maximum indentation, the Young's modulus and others (Variola, 2015).

Several tests were carried out in two regions of the both species beaks (per species, $n_{Upper}=1$ and $n_{Lower}=1$): the rostrum (region 1) and the middle of the hood (region 2). The stiffness of the beaks is given by calculated values of Young's modulus and presented in gigapascals (1 GPa = 1000 MPa)

2.2. Stable Isotope Analysis (SIA)

For analysing stable isotope ratios, beaks were randomly collected from specimens of *P. turqueti* and *A. polymorpha* captured (1) along South Georgia coast at a depth range of 100-300 meters on 30-minutes trawl surveys realised by BAS researchers and the Government of South Georgia and the South Sandwich Islands, during January 2013 and, (2) from boluses regurgitated by specimens of Black-browed Albatross and Blue-eyed Shag inhabit in Bird Island, South Georgia (54°00'N 38°03'W), during January 2015 and 2016, respectively.

After the standard proceedings (see 2.), beaks (per species, n_{Upper}=10 and n_{Lower}=10) with approximated sizes were selected to compare individuals at similar life-stages. To confirm if the chitin composition has impact on stable isotopic ratios, the upper beaks' crest of *A. polymorpha* was separated in two subsamples of tanned and untanned chitin. For the same purpose, lower beaks' lateral wings of *A. polymorpha* were selected. To assess if there were significant differences in $\delta^{13}C$ and $\delta^{15}N$ values along each species life-cycle, the upper beaks were sectioned in subsamples of the rostrum and crest (Cherel, Fontaine, et al., 2009; Guerra et al., 2010). The lower beaks of both species were also used for comparison.

All beak subsamples were then dried at 50°C for 24h and ground into powder in a mill, using small metal spheres of 1 mm diameter, to homogenize the subsamples. Afterwards, around 0.3 to 0.5 mg of each subsample were encapsulated in tin cups to analyse $\delta^{13}C$ and $\delta^{15}N$ values. At MAREFOZ lab (Coimbra, Portugal), all subsamples were analysed with a continuous-flow Isotope Ratio Mass Spectrometer (IRMS) and the results presented in the δ notation in parts per-mille (‰). The δ values are calculated by the formula $\delta X = [(R_{sample} / R_{standard}) -1] \times 1000$, where X represents ^{13}C and ^{15}N, and R_{sample} represents the ratios $^{13}C/^{12}C$ ($\delta^{13}C$) and $^{15}N/^{14}N$ ($\delta^{15}N$). $R_{standard}$ represents the V-PDB (Vienna Pee Dee Belemnite) and atmospheric N_2 (AIR) for $\delta^{13}C$ and $\delta^{15}N$, respectively (Cherel & Hobson, 2005; Guerreiro et al., 2015; Hobson & Welch, 1992).

2.3. Trace Metal Analysis

To analyse total mercury (T-Hg) concentrations, individuals from both species were collected during a trawling survey from January to February 2004 carried out by MRAG and BAS research on behalf of the Government of South Georgia and South Sandwich Islands. All specimens were caught on several 30-minutes events along South Georgia coast at depths ranging from 100 to 400 meters. Prior to the trace metal analysis, beak and flesh were sampled from random individuals to assess mercury concentrations. This trace metal analysis was performed in University of Aveiro, Portugal. Only the lower beaks were used.

Thereafter, beak and flesh samples (per species, n_{Lower}=10 and n_{Flesh}=10), were dehydrated during 24h through lyophilization and then cut down into smaller pieces. All samples were weighted and analysed in an Advanced Mercury Analyzer (AMA) LECO 254, using mussel tissue (SRM 2976 by NIST) as reference, which determines total mercury (T-Hg) concentrations by atomic absorption spectrometry with thermal decomposition and gold amalgamation (Xavier et al., 2016b). For all flesh samples, two replicates per sample were performed in order to obtain a more accurate analysis. On the other hand, only one replicate per beak sample was performed due to their small weight.

2.4. Statistical Analysis

The statistical analyses followed a planned methodology. All individuals used were randomly selected and caught along South Georgia.

Since the results of the conducted analyses of physicochemical properties are mainly qualitative, a descriptive interpretation of the output is provided taking into account species and beak type (upper or lower) as grouping variable. Regarding stiffness, differences in the Young's modulus were assessed between species and beak's regions (rostrum and middle section of the hood) using Mann-Whitney test due to the non-normal distribution of the data. For the SIA, significant differences were tested by a series of one-way ANOVA's and, for comparison of stable isotopic signatures between juvenile and adult stages, a multivariate ANOVA was performed using species and beak region (hood or crest) as grouping variable. For the isotopic niche overlap, the metrics SEA, SEAc and TA were calculated using Stable Isotope Bayesian Ellipses in R (SIBER). Regarding the mercury analysis, one-way ANOVA's were performed in order to check if there were significant differences between species and soft/hard tissues (flesh/beak, respectively). No correlations between mercury and nitrogen isotopes were performed since the beak samples used were not from the same individuals.

Prior to all analyses, differences in mass and LHL between species were checked and allometric equations for mass (M) and mantle length (ML) built using Pearson's Correlation. The normality of all data was checked using Shapiro-Wilks test. All statistical analyses were performed using the software R 3.4.1 and RStudio 1.0.153 (R Core Team, 2017).

CHAPTER III – Results

3. General Results (Allometric Equations)

Based on the beak data resultant of the 2004 bottom trawling survey around South Georgia (including beaks not used in this study) (Table 1), allometric equations relevant for predator studies for both octopod species were created. The beaks were collected from randomly selected sub-adult/adult individuals. The allometric equations produced are (M = mass (g); LHL = lower hood length (mm); ML = mantle length (mm)):

***Pareledone turqueti*:**

$LnM = 0.7756 + 2.4659LnLHL$ ($r^2 = 0.7380$, $n = 24$, $min_M = 6g$ and $max_M = 139g$)

***Adelieledone polymorpha*:**

$M = - 98.327 + 72.602LHL$ ($r^2 = 0.8373$, $n = 37$, $min_M = 3g$ / $max_M = 267g$)

$LnML = 2.9628 + 1.511LnLHL$ ($r^2 = 0.9689$, $n = 5$, $min_{ML} = 35mm$ / $max_{ML} = 73mm$)

Through the relationship between the LHL and body mass, beaks of *A. polymorpha* are generally smaller comparing to *P. turqueti* ($F_{2,58} = 103.03$, $p < 0.001$) (Fig. 9). In Table 1, all beak measurements recorded, of beaks used and not used in this study, are presented.

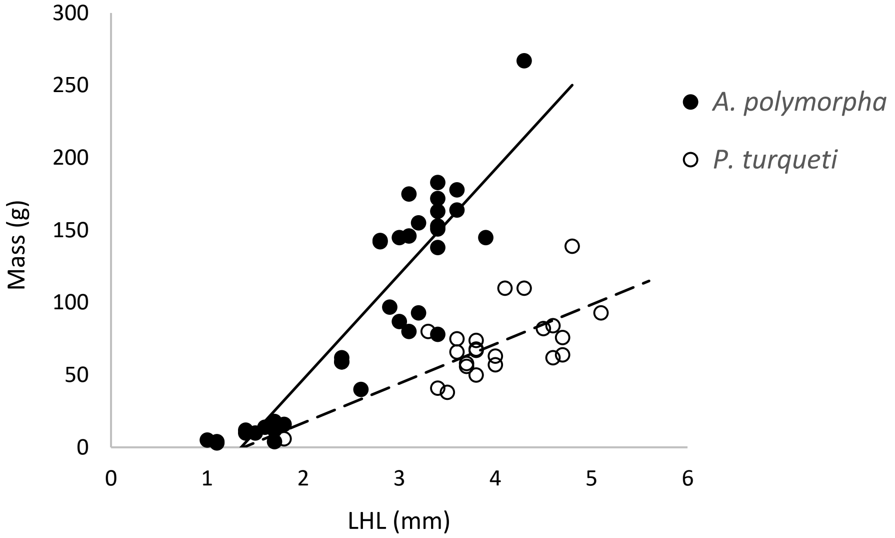

Figure 9. Plot of the relationship between the mass and the lower beak hood length (LHL) for *Adelieledone polymorpha* (n = 37) and *Pareledone turqueti* (n = 24). Solid and dashed tendency lines of *A. polymorpha* ($r^2 = 0.8373$, $p < 0.01$) and *P. turqueti* ($r^2 = 0.4839$, $p < 0.01$) respectively.

Table 1. Values of the upper hood length (UHL), upper crest length (UCL) and lower hood length (LHL) of both octopod species, *Adelieledone polymorpha* and *Pareledone turqueti*, upper and lower beaks. The estimated values for the mass and mantle length were calculated using both known values and estimates given by the allometric equations given by Xavier and Cherel in 2009. The mean values are presented ± SD.

Taxa	Upper Beak							Lower Beak				Estimated Mass (g)	Estimated Mantle Length (mm)
	UHL (mm)				UCL (mm)			LHL (mm)					
	n	Mean	Min	Max	Mean	Min	Max	n	Mean	Min	Max	Mean	Mean
A. polymorpha	40	2.90 ± 0.60	1.47	3.93	8.73 ± 1.58	4.27	10.51	45	2.81 ± 0.65	1.15	3.73	106.81 ± 58.94	63.39 ± 16.42
P. turqueti	43	5.00 ± 0.90	2.55	6.63	11.94 ± 2.07	5.57	15.22	46	3.73 ± 0.92	1.25	5.91	63.40 ± 32.70	67.38 ± 12.32

3.1. Physicochemical Properties Analysis

3.1.1. Scanning Electron Microscopy

After observing fractures at different conditions (hydrated, dried and frozen), we concluded that dried samples were the best at preserving and thus providing better representations of the natural microstructure of the beak. Through the micrographs of the different fractures (see Fig. 8), we can observe that both *A. polymorpha* and *P. turqueti* lower beaks are composed by a very compact structure (Fig. 10 and 11). In the lower beaks of both species, it is possible to distinguish two layers with different structural arrangements (Fig. 10A and B). In the transversal fracture of the lower beak's hood of *P. turqueti*, fibrillary and spherical structures were found in the inner region of the beak (Fig. 12).

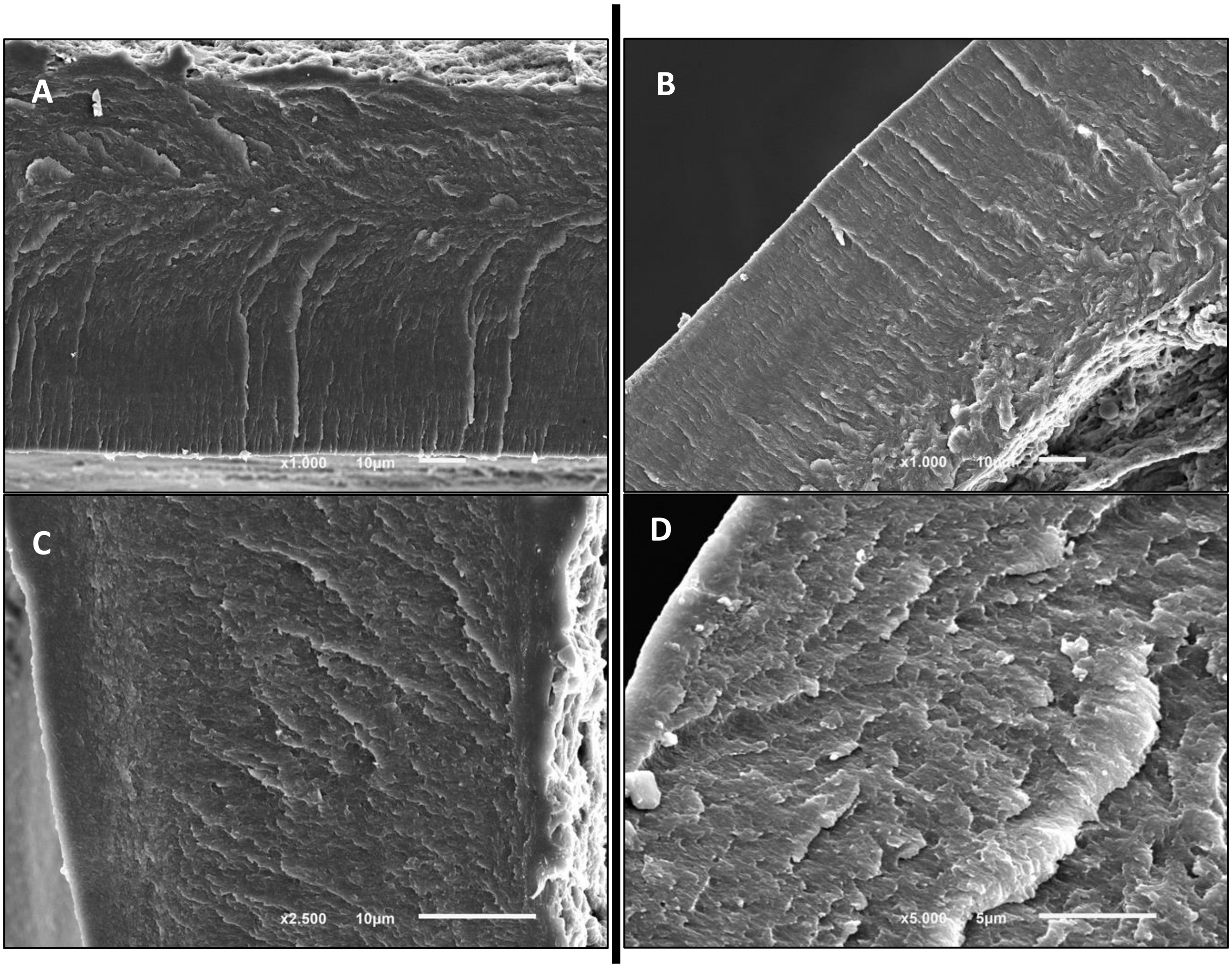

Figure 10. Scanning electron micrographs of *Adelieledone polymorpha* (left side: A and C) and *Pareledone turqueti* (right side: B and D) lower beak longitudinal fractures.

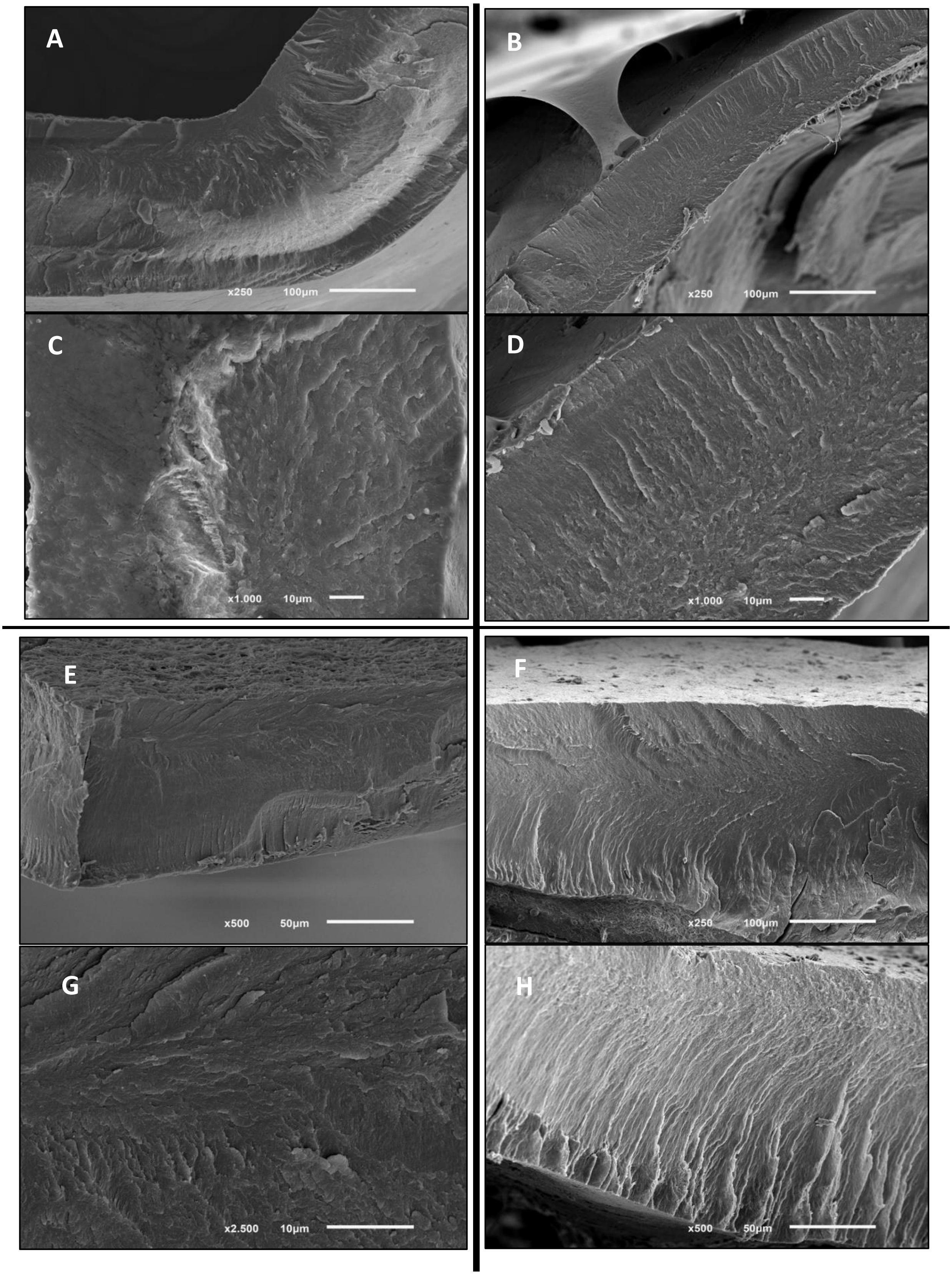

Figure 11. Scanning electron micrographs of *Adelieledone polymorpha* (left side) and *Pareledone turqueti* (right side) lower beak transversal fractures of the rostrum (from A to D) and transversal factures of the hood (from E to H).

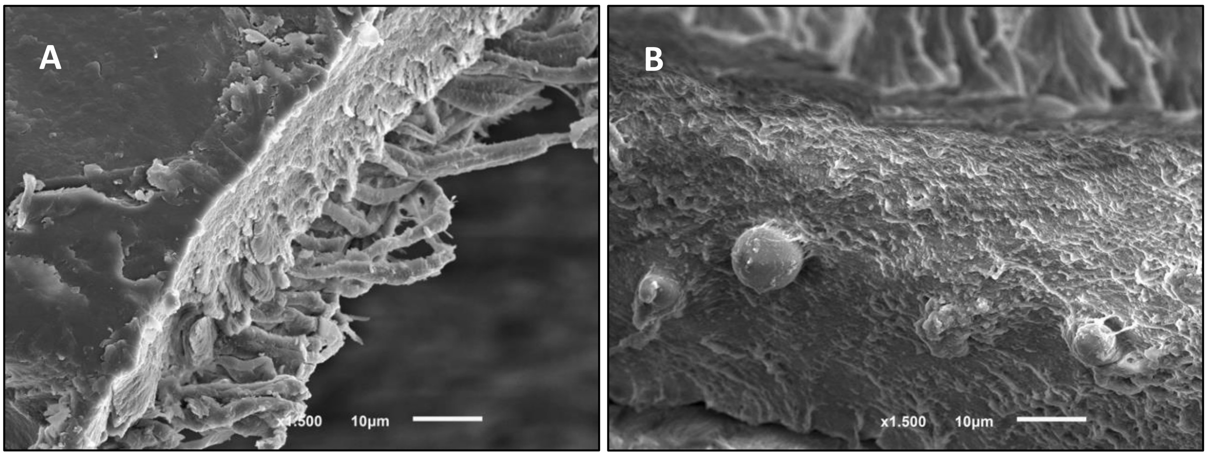

Figure 12. Scanning electron micrographs of fibrillary (A) and spherical (B) structures found on the transversal cut of the lower beak's hood of one *Pareledone turqueti* specimen.

The upper beak of both species seems to present the same compact structure also observed in the lower beaks (Fig. 14). In the upper beaks, it is also possible to distinguish two layers with two different arrangements of the microstructure (e.g. Fig. 14G and H). The picture taken with backscattered electrons (Fig. 14B) demonstrated that the chemical composition is similar along the fracture, since heavy elements appear lighter and light elements appear darker. In the upper beaks of *A. polymorpha* and *P. turqueti,* fibrillary structures were also observed in the inner surface of the transversal fractures of the rostrum (Fig. 13).

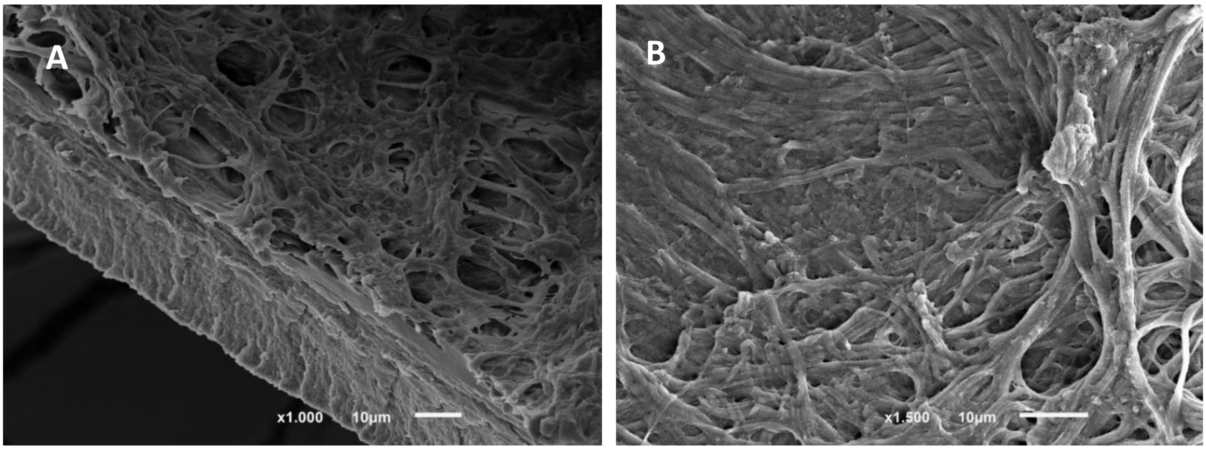

Figure 13. Scanning electron micrographs of *Adelieledone polymorpha* (A) and *Pareledone turqueti* (B) upper beak transversal fractures of the rostrum

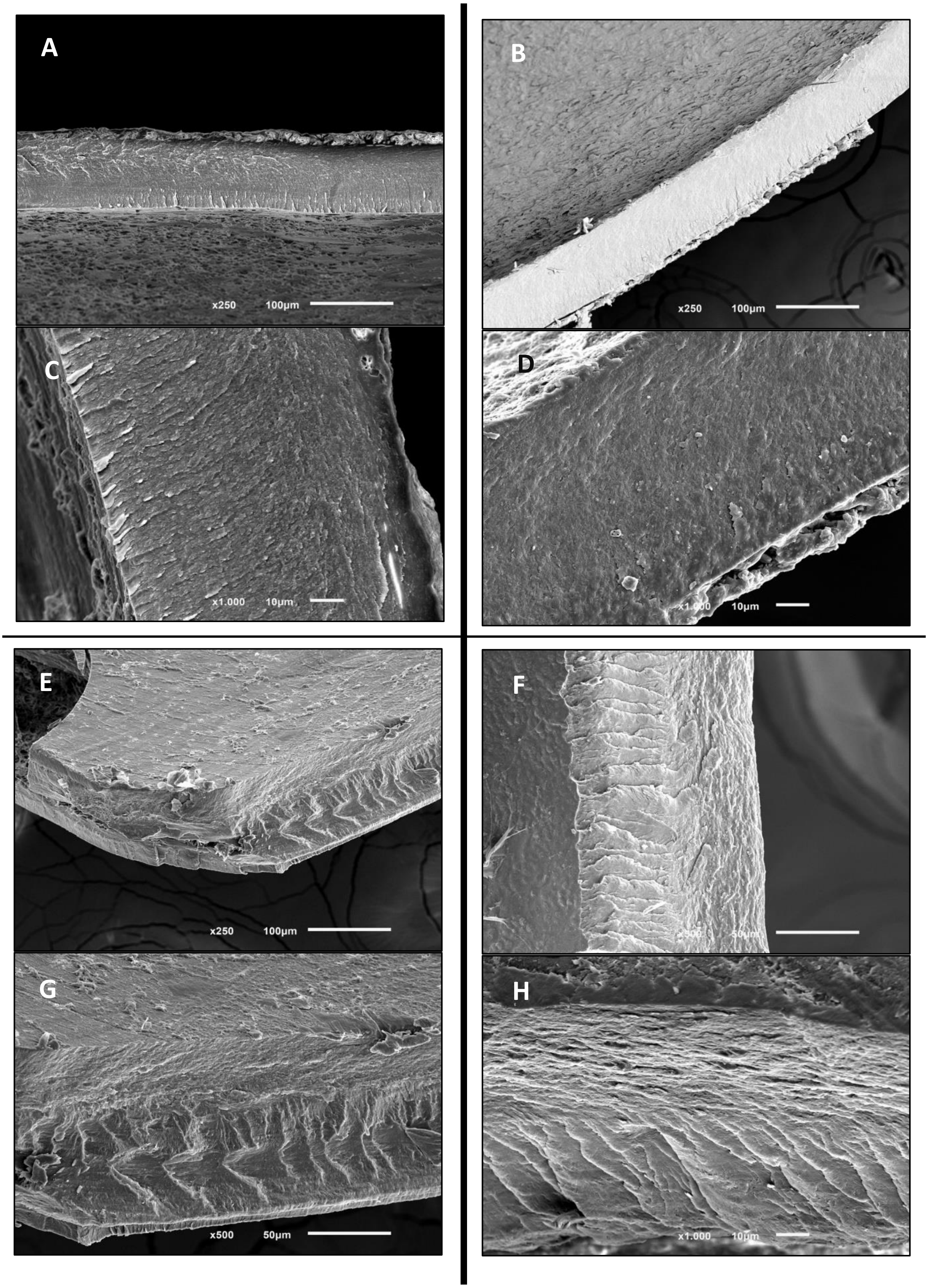

Figure 14. Scanning electron micrographs of *Adelieledone polymorpha* (left side) and *Pareledone turqueti* (right side) upper beak longitudinal fractures (A to D) and transversal factures of the rostrum (E to H).

3.1.2. X-Ray Powder Diffraction (XRD)

From the XRD spectra (dispersion range 2θ of 5-50°), both species present a beak structure mainly amorphous. The only crystalline phase identified was *α*-chitin, demonstrated by intense peaks at 2θ = 9 and 19° and weak peaks 12, 23 and 27° (Fig. 15).

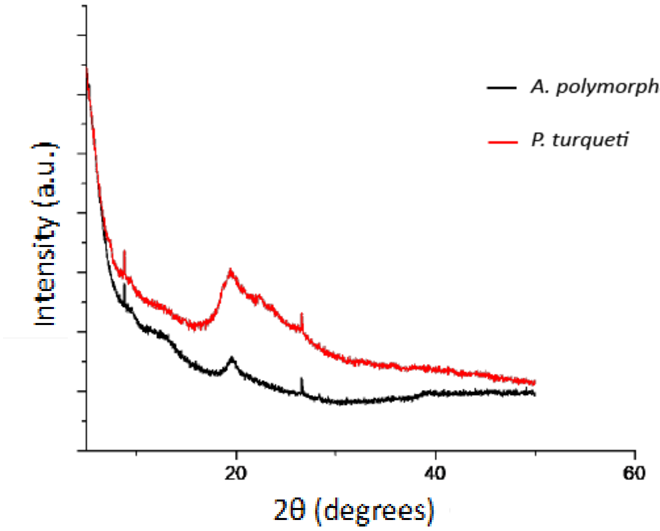

Figure 15. The XRD spectra (dispersion range 2θ of 5-50°) comparing the beak structure of *Adelieledone polymorpha* (black) and *Pareledone turqueti* (red).

3.1.3. Micro Computed Tomography (micro CT)

Through the micro CT scans, it was concluded that the upper beaks and lower beaks of *A. polymorpha* and *P. turqueti* present a very compact structure without porosities (Fig 16). Although not quantifiable, the beak density seems to decrease from the centre to the peripheral regions of the beak. Moreover, the beaks of *P. turqueti* seem to be denser than *A. polymorpha* beaks (Fig. 16). With this 3D imagery technique, it was also possible to notice that both upper and lower beaks of *A. polymorpha* are less curve and present sharper edges. On the other hand, the beaks of *P. turqueti* present a bigger curvature in its geometry and bulky edges (Fig. 17).

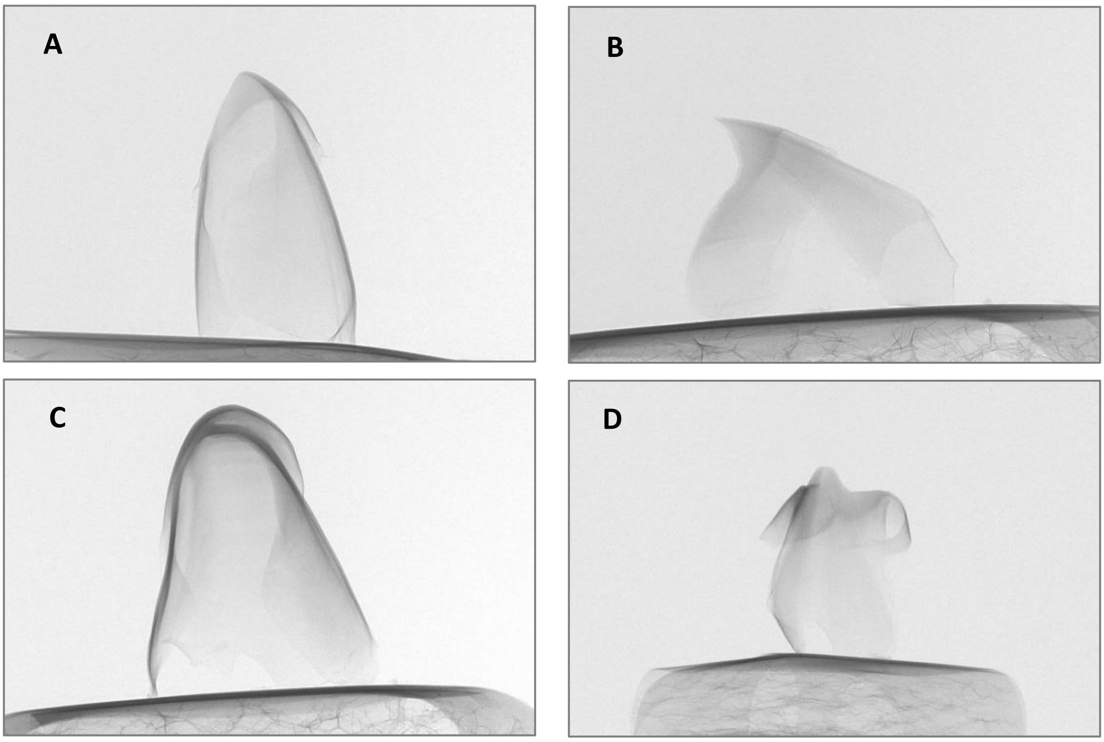

Figure 16. Micro CT scans of the beak structure of *Adelieledone polymorpha* (A – Upper; B – Lower) and *Pareledone turqueti* (C – Upper; D – Lower). Beak measures (in mm): *A. polymorpha* – UHL = 5.83, UCL = 13.99 and LHL = 4.52; *P. turqueti* – UHL = 2.59, UCL = 8.65 and LHL = 3.23.

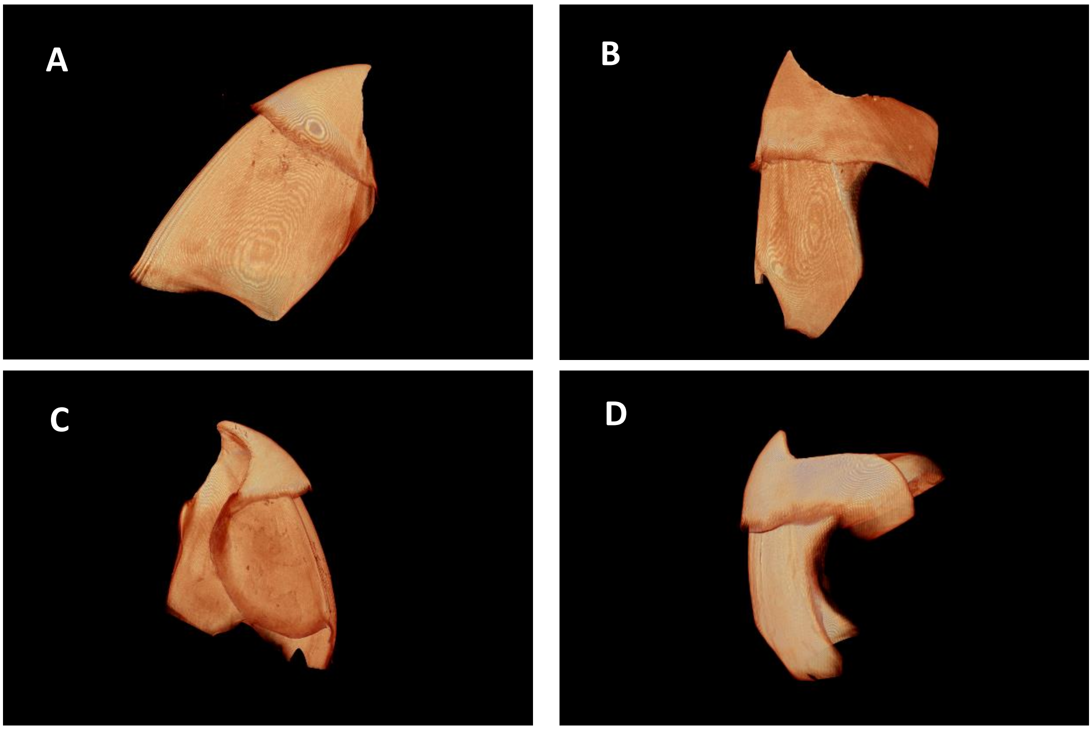

Figure 17. 3D images of the beak structure of *Adelieledone polymorpha* (Beaks: A – Upper; B – Lower) and *Pareledone turqueti* (Beaks: C – Upper; D – Lower). Beak measures (in mm): *A. polymorpha* – UHL = 5.83, UCL = 13.99 and LHL = 4.52; *P. turqueti* – UHL = 2.59, UCL = 8.65 and LHL = 3.23.

3.1.4. Atomic Force Microscopy (AFM)

Regarding beaks, significant differences were found between species in region 1 and 2 of the upper beak ($U < 0.001$, $p = 0.004$; $U < 0.001$, $p = 0.004$, respectively) and region 2 of the lower beak ($U < 0.001$, $p = 0.001$). Within beaks, region 1 and 2, there were also significant differences recorded in the upper beak of *A. polymorpha* ($U < 0.001$, $p = 0.004$) and lower beak of *P. turqueti* ($U < 0.001$, $p = 0.003$)(Table 2).

Table 2. Values of the Modulus recorded on both octopod species beak samples. The values are expressed in GPa (1GPa = 1000 MPa). The values highlighted in grey were not considered to obtain the modulus values.

Adelieledone polymorpha				*Pareledone turqueti*			
Upper Beak		Lower Beak		Upper Beak		Lower Beak	
Region 1	**Region 2**	**Region 1**	**Region 2**	**Region 1**	**Region 2**	**Region 1**	**Region 2**
113.355	36.694	5.701	8.276	43.011	11.639	7.236	211.574
110.429	2.302	5.722	9.019	29.026	22.170	7.358	247.964
70.320	4.458	5.903	2.978	42.353	14.860	5.525	186.174
109.970	2.584	7.121	0.675	9.521	13.729	5.301	68.931
75.914	4.086	6.447	1.060	6.762	34.307	4.503	44.746
51.469	1.380	5.819	3.341	7.679	19.986	5.101	44.211
---	3.481	---	2.650	93.511	---	5.487	---
---	0.961	---	3.798	69.384	---	---	---
---	0.0693	---	3.513	---	---	---	---
---	---	---	3.915	---	---	---	---

3.2. Stable Isotopic Analysis (SIA)

3.2.1. Differences between tanned and untanned chitin

As a preliminary study in order to avoid bias results in the analysis, differences between tanned and untanned chitin from the crest were assessed (Table 3). Such differences were only found for the upper beak δ^{15}N ($F_{1,17} = 7.73$, $p = 0.01$) but not for δ^{13}C ($F_{1,17} = 4.23$ $p = 0.06$)(Fig. 18). No significant differences were found in C/N ratios for the upper ($F_{1,17} = 2.70$, $p = 0.12$) and the lower beaks ($F_{1,14} = 1.66$, $p = 0.23$)(Fig. 19). Therefore, in subsequent analyses, only beak sections of tanned chitin were used.

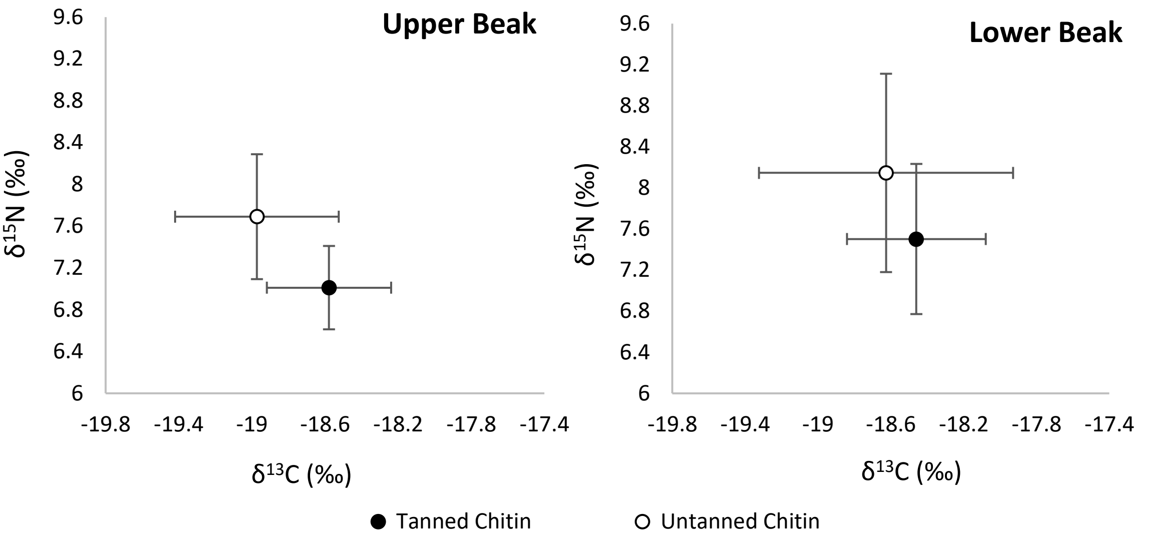

Figure 18. Isotopic values of tanned and untanned chitin registered in the upper (left) and lower beaks (right) of *Adelieledone polymorpha*. Upper beak: Tanned chitin (n = 10) and untanned chitin (n = 9). Lower beak: Tanned chitin (n = 10) and untanned chitin (n = 6). Means and SD are shown.

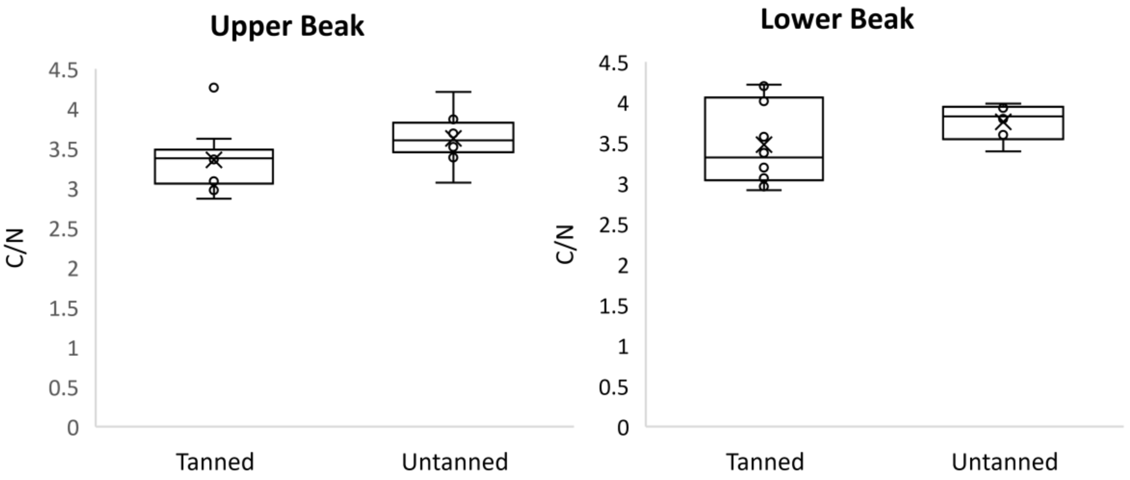

Figure 19. Values of carbon/nitrogen ratio registered in the upper and lower beaks of *Adelieledone polymorpha*. Upper beak: Tanned chitin (n = 10) and untanned chitin (n = 9). Lower beak: Tanned chitin (n = 10) and untanned chitin (n = 6). The distribution of data is displayed in a boxplot with the mean (cross), median (line), 1st/3rd quartile (box), minimum/maximum (whiskers) and C/N values (circles) registered.

3.2.2. Habitat and trophic levels of *P. turqueti* and *A. polymorpha*

Differences were found between the upper beaks' rostrum and crest (representing juvenile and adult stages, respectively) isotopic signatures (Table 3) of both octopod species ($F_{1,32}$ = 11.42, $p < 0.001$), a significant enrichment of $\delta^{15}N$ on the upper crest was found on *A. polymorpha* and on *P. turqueti* ($F_{1,32}$ = 16.85, $p < 0.001$). No significant differences were found in the δ^{13}C. An isotopic niche overlap of 95.6% was calculated for the upper beak's rostrum of the two species. Focusing only on upper beaks' crest (adult stage), an isotopic niche overlap of 19.2% between species ($F_{1.16}$ = 4.08, p = 0.03) was found, only δ^{15}N values showed to be significantly higher on *P. turqueti* ($F_{1,16}$ = 9.05, p = 0.01) (Fig. 20 and 21; Table 4).

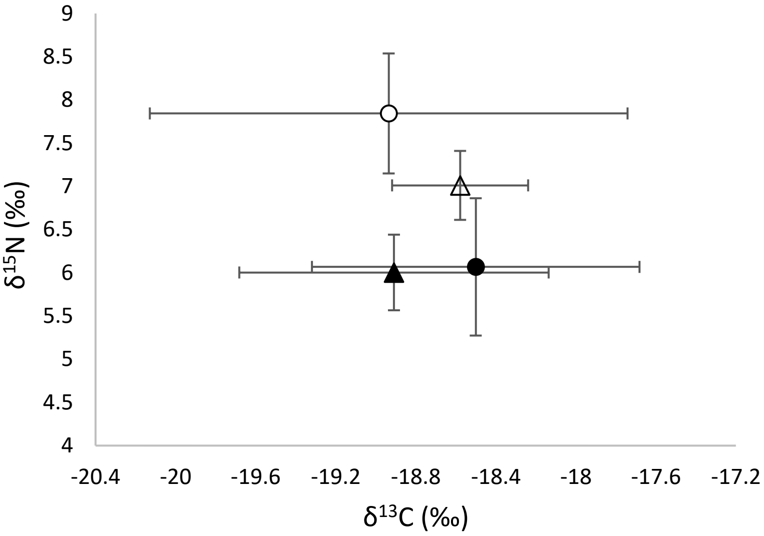

Figure 20. Carbon and nitrogen stable isotope values of the upper beak's rostrum (filled) and crest (empty) of *Adelieledone polymorpha* (triangles) and *Pareledone turqueti* (circles). Means and SD are shown.

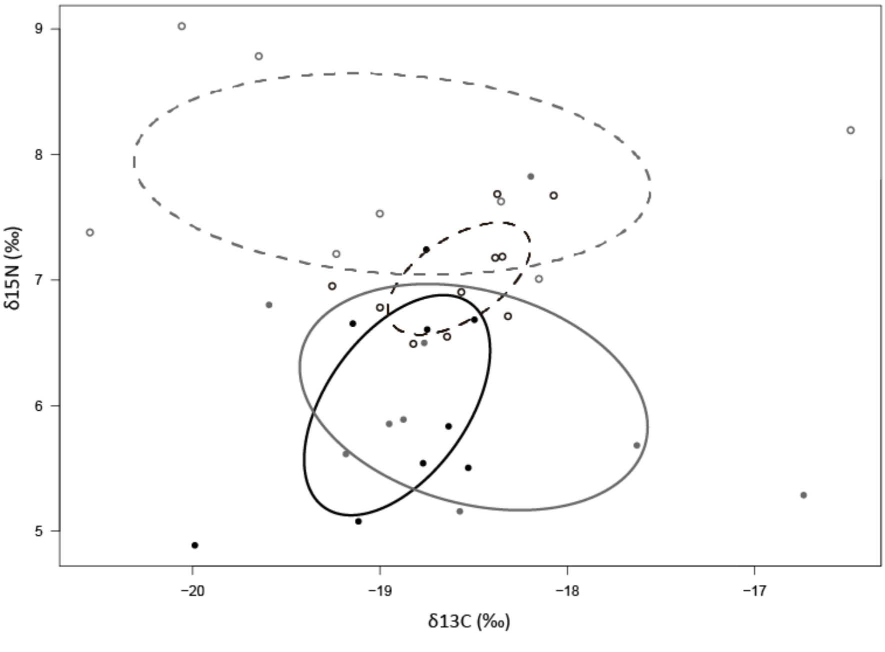

Figure 21. Bivariate δ^{13}C and δ^{15}N plot for upper beaks' rostrum (solid lines and filled circles) and crest (dashed lines and empty circles) of *Adelieledone polymorpha* (black) and *Pareledone turqueti* (grey), estimated by the ellipse corrected for the SIBER analysis (Stable Isotope Bayesian Ellipses in R).

Table 3. Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) and C/N mass ratios registered values in *Adelieledone polymorpha* and *Pareledone turqueti.* Mean ± SD, minimum and maximum values are shown.

		$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)			C/N mass ratio		
Species	n	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max
Adelieledone polymorpha										
Lower Beak (Whole)	10	-18.05 ± 0.61	-19.16	-16.73	7.17 ± 0.57	6.15	8.08	3.15 ± 0.06	3.07	3.30
Lower Beak										
Wing (Tanned chitin)	10	-18.46 ± 0.38	-19.26	-17.68	7.50 ± 0.73	5.99	8.56	3.48 ± 0.47	2.92	4.22
Wing (Untanned chitin)	6	-18.63 ± 0.70	-19.23	-17.42	8.15 ± 0.97	6.38	9.30	3.76 ± 0.20	3.40	3.98
Upper Beak										
Rostrum	9	-18.91 ± 0.44	-19.99	-18.50	6.00 ± 0.44	5.08	7.24	3.17 ± 0.13	2.94	3.35
Crest (Tanned chitin)	9	-18.58 ± 0.34	-19.26	-18.07	7.01 ± 0.40	6.50	7.70	3.35 ± 0.38	2.87	4.26
Crest (Untanned chitin)	9	-18.97 ± 0.45	-19.55	-17.87	7.69 ± 0.30	6.55	8.37	3.63 ± 0.30	3.07	4.21
Pareledone turqueti										
Lower Beak (Whole)	10	-18.37 ± 0.73	-19.62	-17.36	6.80 ± 0.52	6.04	7.59	3.17 ± 0.26	2.92	3.75
Upper Beak										
Rostrum	10	-18.75 ± 0.81	-19.59	-16.74	6.17 ± 0.81	5.16	7.83	3.56 ± 0.81	3.07	4.63
Crest	9	-18.95 ± 1.13	-20.55	-16.49	7.84 ± 0.65	7.01	9.02	3.76 ± 0.56	3.10	4.93

Using the lower beaks, there was an isotopic niche overlap of 79.5% with no significant differences found between *A. polymorpha* and *P. turqueti* (Fig. 22 and 23; Table 4).

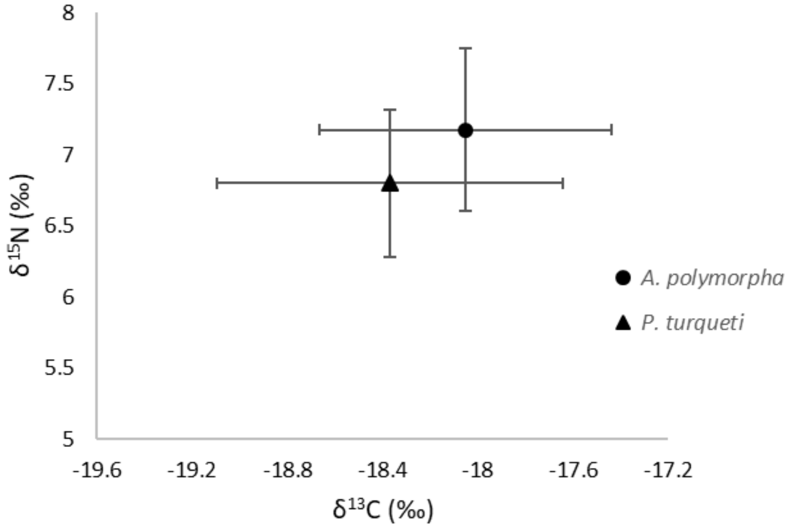

Figure 22. Total isotopic values of the lower beaks for comparing the habitat and the trophic ecology of *Pareledone turqueti* (n = 10) and *Adelieledone polymorpha* (n = 10). The figure shows the mean and the SD of the total quantities of both stable isotope ratios. Means and SD are shown.

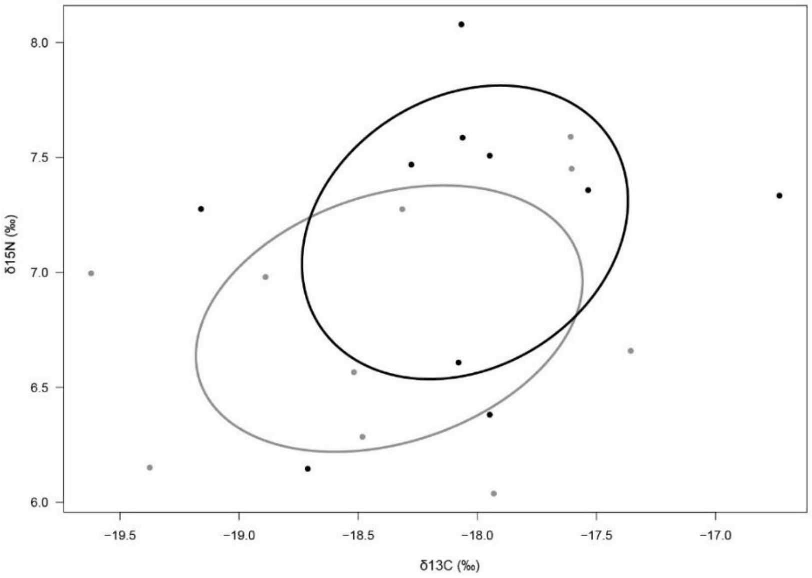

Figure 23. Bivariate $\delta^{13}C$ and $\delta^{15}N$ plot for lower beaks of *Adelieledone polymorpha* (black) and *Pareledone turqueti* (grey), estimated by the ellipse corrected for the SIBER analysis (Stable Isotope Bayesian Ellipses in R).

Table 4. Values of SEA, SEAc and TA calculated by the Standard Ellipses analysis using the SIBER package (Stable Isotope Bayesian Ellipses in R).

	Species	Beak	SEA	SEAc	TA
Fig. 21	*A. polymorpha*	Upper beak (rostrum)	1.037	1.185	1.726
		Upper beak (crest)	0.392	0.441	0.765
	P. turqueti	Upper beak (rostrum)	2.217	2.533	4.359
		Upper beak (crest)	2.945	3.435	4.862
Fig. 23	*A. polymorpha*	Lower Beak	1.192	1.341	2.549
	P. turqueti	Lower Beak	1.261	1.418	2.408

3.3. Trace Metals: Mercury Analysis

At first, the total mercury concentration (T-Hg) was analysed for the two tissues and compared between species. The T-Hg concentrations found on the flesh were 6x ($F_{1,12} = 50.96$, $p < 0.001$) and 10x higher ($F_{1,14} = 55.43$, $p < 0.001$) than the concentrations found on the lower beaks of *A. polymorpha* and *P. turqueti*, respectively (Fig 24).

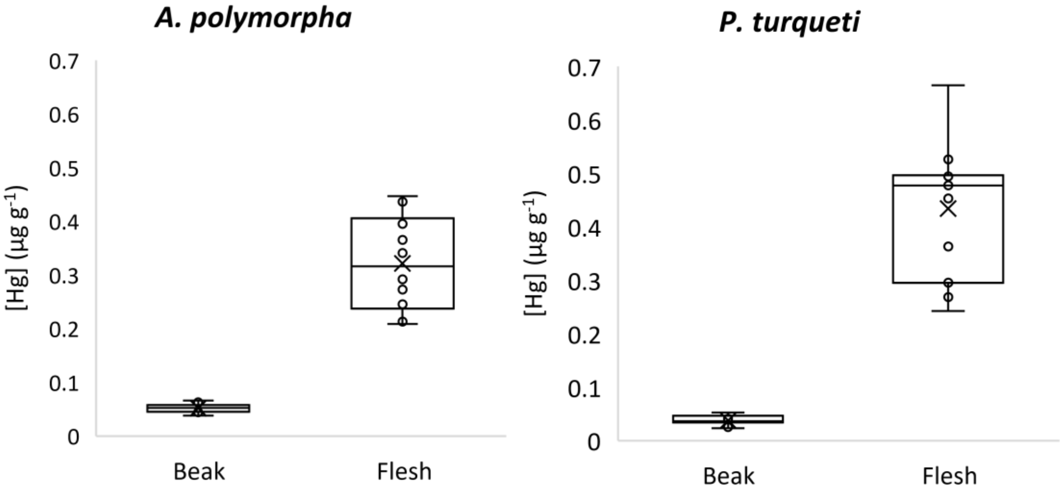

Figure 24. Total mercury total concentrations registered in the two tissues in study, lower beaks and flesh, of *Adelieledone polymorpha* (n = 10) and *Pareledone turqueti* (n = 11). The distribution of data is displayed in a boxplot with the mean (cross), median (line), 1st/3rd quartile (box), minimum/maximum (whiskers) and concentration values (circles) registered.

Afterwards, *P. turqueti* flesh had higher T-Hg concentrations (0.434 ± 0.128 µg g^{-1}) when comparing with *A. polymorpha* (0.322 ± 0.088 µg g^{-1})($F_{1,19}$ = 5.38, p = 0.03). In the lower beaks, the higher T-Hg concentrations were in *A. polymorpha* (0.052 ± 0.008 µg g^{-1}) and showed to be significant ($F_{1,19}$ = 13.09, p = 0.00) when comparing with the concentrations found on *P. turqueti* (0.038 ± 0.008 µg g^{-1})(Fig 25; Table 5). No correlations on T-Hg concentrations were found between both tissues and LHL within each species.

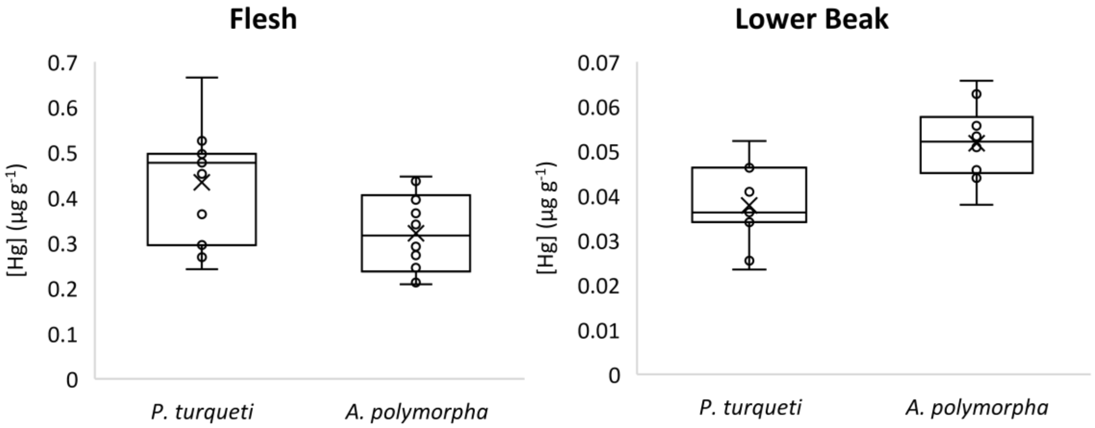

Figure 25. Total mercury total concentrations registered in the flesh and lower beak of *Adelieledone polymorpha* (n = 10) and *Pareledone turqueti* (n = 11). The distribution of data is displayed in a boxplot with the mean (cross), median (line), 1st/3rd quartile (box), minimum/maximum (whiskers) and concentration values (circles) registered.

Table 5. Total mercury (T-Hg) concentrations on the lower beak and flesh of *Adelieledone polymorpha* and *Pareledone turqueti*. The T-Hg values are expressed in µg g^{-1} (ppm).

Species	N	Lower Beak				Flesh			
		Mean	SD	min	max	Mean	SD	min	max
A. polymorpha	10	0.052	0.008	0.038	0.066	0.322	0.084	0.209	0.448
P. turqueti	11	0.038	0.008	0.024	0.052	0.434	0.122	0.242	0.526

CHAPTER IV – Discussion

4. Main Findings

This study provides results indicating that both octopod species, *A. polymorpha* and *P. turqueti*, occupy the same benthic ecosystems around South Georgia throughout their life cycle. Other than that, results indicate that both species occupy different trophic niches, at least partially. *P. turqueti* may occupy a wider trophic niche whereas *A. polymorpha* may occupy a more specialised one niche, as a consequence of the different beak morphology and other dissimilarities in the digestive apparatus (e.g. buccal mass, posterior salivary gland). In addition, we postulate that beak morphology differences can be explained not only with different diets but also with different feeding strategies, as *A. polymorpha* seems to rely more on its toxic saliva and *P. turqueti* on its bite force to hunt prey.

To my knowledge, this study may well be the first to tackle the octopod beak physicochemical properties and relating it to habitat and trophic ecology inferred by stable isotopes and trace metals analyses, since similar past studies mainly focused on macroscopic prey identification and analysing beaks morphometrically. Bellow, all topics will be discussed in more detail.

4.1. Allometric equations for *Adelieledone polymorpha* and *Pareledone turqueti*

The allometric equations of both species provided in this study were built using a wide range of mass (M) and mantle length (ML) values of randomly selected individuals caught along South Georgia coast, which can be valuable for trophic interactions studies. The sample size used is very similar to the used by Xavier and Cherel in 2009 to create *A. polymorpha* and *P. turqueti* allometric equations, however the data used in this study is more recent thus providing an updated version for the equations. Moreover, this study's allometric equations are specific for *A. polymorpha* and *P. turqueti* individuals inhabiting South Georgia coastal habitats, which will be of possible use for ongoing predator diet projects (including monitoring programs, such as ecosystem monitoring program of the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) (Constable, 2011; Xavier et al., 2002). In fact, there is a lack of significant allometric equations for Antarctic octopods (Xavier et al., 2018; Xavier & Cherel, 2009). Although the r^2 for this study's allometric equations is very significant, both studies equations are very similar and therefore should be taken into account in order to assess which one fits the best.

4.2. Evaluation of the physicochemical properties of the beaks: structure and stiffness of *A. polymorpha* and *P. turqueti*

The initial assumption of my study was that the beaks from *A. polymorpha* were less stiff than the beaks of *P. turqueti* since the former present lighter brown chitin colour and a more fragile appearance (Miserez et al., 2008). Although the AFM technique was not performed under the best conditions due to the irregular and curvy shape of the beaks, preliminary conclusions can be taken from the results of this preliminary study. A positive gradient of stiffness along the beak's hood, starting at the rostrum, was found in the majority of the samples. Moreover, the upper beak of *P. turqueti* seems to be stiffer comparing with the upper beak of *A. polymorpha*. Since some results do not make sense regarding cephalopod beak's ontogeny – the lower beak's rostrum of *P. turqueti* was more compliant (lower modulus values) than the middle section of the hood (higher modulus values) – we are not confident in the AFM results due to bad testing conditions resultant from the irregular beak geometry. Further testing combining AFM and other techniques focused on biomaterial's mechanical properties, such as nanoindentation tests, are required for taking stronger conclusions.

The second assumption of our study was that *A. polymorpha* and *P. turqueti* beaks would present similar chemical compositions and microstructure since both species are closely related (Family: Megaleledonidae). The upper and lower beaks of *A. polymorpha* and *P. turqueti* present a very similar compact structure composed by two different structural arrangements observed in the SEM micrographs, nothing like the lamellar structure observed on jumbo squid (*Dosidicus gigas*) beaks by Miserez and his colleagues (2007). The fibrillary and spherical structures observed on the inner surfaces of both species beaks seem to have a protein origin and to not affect adjacent beak's microstructure. Both octopod species beaks also present a similar amorphous structure constituted by α-chitin, identified on XRD (XRD). Results from prior XRD studies show that α-chitin and β-chitin can be found on squid beaks (Miserez et al., 2007) and, the former, found in crustacean shells (Cárdenas et al., 2004).

The most noticeable difference between species lies on their different beak geometry. Whilst the beaks of *P. turqueti* are bulky with rounded edges, very similar to the beaks of other benthic octopod species known for feeding on hard-shelled preys, the beaks of *A. polymorpha* present a unique shape with sharper edges and fragile appearance. Both species beaks are very compact and free of porous structures and, although not quantifiable, the beaks of *A. polymorpha* are structurally less dense comparing with the beaks of *P. turqueti*. However, even though I'm confident with this study results, future analyses with a wider range of beak sizes may provide stronger conclusions.

In addition to the different beak morphology, *A. polymorpha* and *P. turqueti* present great dissimilarities on their digestive apparatus, such as the buccal mass and the posterior salivary gland (PSG) responsible for producing toxic saliva. Comparing with *P. turqueti*, the PSG of *A. polymorpha* is significantly larger which is an indicator of the venom's importance for the species' feeding behaviour (Gibbs & Greenaway, 1978). Contrarily, the large-sized buccal mass of *P. turqueti* indicates that it relies heavily on bite force to hunt prey. Although further testing is needed, *P. turqueti* seems to have a venom less adapted to sub-zero water temperatures (Undheim et al., 2010). All these dissimilarities between species, including beak morphology, indicates that both species coexist occupying, at least partially, different trophic niches probably determined by an adaptative toxin evolution associated to a specialisation of the morphology of the beak.

4.3. Habitat and trophic ecology of *A. polymorpha* and *P. turqueti* through stable isotopic analyses on their upper and lower beaks

Regarding isotopic signatures on tanned and untanned chitin, our assumption was that, since both type of chitin are biochemically different (Miserez et al., 2008), different isotopic signatures were expected. Moreover, since chitin has a higher C/N ratio than protein and is impoverished in ^{15}N relative to diet, different C/N ratios, $\delta^{13}C$ and $\delta^{15}N$ among the two types of chitin were expected (Cherel, Fontaine et al., 2009). Despite not significant, this study results showed a difference between the C/N ratios of tanned and untanned chitin, with slightly higher values registered in the latter. The biochemical composition of the chitin seems to not affect $\delta^{13}C$ values. On the other hand, higher $\delta^{15}N$ were registered in the untanned chitin relative to the tanned part. Nevertheless, the beaks used in this study were generally small (sub-adult individuals) and, for that reason, the interpretation of the $\delta^{15}N$ is not only affected by the chitin content but also by the ecological trophic context of the sampled individuals. In order to get more trustworthy results, it is advised to remove untanned chitin of the beaks for future isotopic studies, especially the ones focusing on $\delta^{15}N$, due to the differences found among tissues.

For the Southern Ocean plankton, a gradient shows an enrichment of $\delta^{13}C$ from higher to lower-latitude waters and, using that gradient, it is possible to estimate the latitude of the habitats occupied (Brault et al., 2018; Cherel & Hobson, 2007). However, the $\delta^{13}C$ values in South Georgia region can range greatly, from -19 to -23‰ at the base of the food web, since the southwestern sector of the South Atlantic Ocean, where the South Georgia is located, includes two distinct oceanic masses, the Polar and the Sub Antarctic fronts (Brault et al., 2018; Ceia et al., 2015; Stowasser et al., 2012). Due to the influence of the two oceanic fronts, establishing a gradient of $\delta^{13}C$ values for South Georgia marine habitats becomes a daunting task. Since *A. polymorpha* and *P. turqueti* are two benthic species, no significant differences of $\delta^{13}C$ values the juvenile and adult stages were expected, meaning that throughout their lifecycle both species inhabit benthic ecosystems. Furthermore, both species seem to occupy benthic habitats of South Georgia as no differences in $\delta^{13}C$ were found.

The results of this study indicate an increase in trophic position from juvenile to adult life stages for both species as expected. An enrichment in $\delta^{15}N$ suggests a typical and continuous increase in trophic position of consumers during their life cycle since bigger preys from higher trophic levels become available as the individuals grow (Cherel, Fontaine, et al., 2009; Cherel & Hobson, 2005; Guerra et al., 2010). Even though $\delta^{15}N$ values can vary geographically from the bottom trophic levels to the top, *A. polymorpha* and *P. turqueti* inhabit the same ecosystems thus having identical background isotopic signatures.

Between species, significant differences in the $\delta^{15}N$ values were only found on the crest of the upper beak. No isotopic differences were found in the lower beaks between species. However, since different isotopic signatures among cephalopod's upper and lower beaks have already been described (Cherel & Hobson, 2005) and a recent study shows that isotopic signatures of the upper beaks present more precise results (Queirós et al., *in press*), we are confident in the results of this study. Therefore, through the stable isotopic signatures this study adds to the known feeding ecology that, both species explore partially different trophic niches, exploring different prey items available in function of the adaptations that both species possess on their digestive apparatus, such as beak, buccal mass and posterior salivary gland size.

4.4. Total mercury (T-Hg) in *A. polymorpha* and *P. turqueti* in relation to their habitat and trophic levels

Although expected, no positive relationships were found between T-Hg concentrations on flesh and beaks. Comparing to beaks, higher concentrations of T-Hg are expected to be found on the muscle. Moreover, since mercury is bioaccumulated and *P. turqueti* may occupy a wider trophic niche constituted by preys of higher trophic level, higher total mercury levels were also expected to be found on *P. turqueti* tissues.

As postulated, the *P. turqueti* presented significantly higher concentrations of T-Hg on the flesh compared to *A. polymorpha*. Since both species inhabit the same ecosystems, it is hypothesized that the differences in T-Hg may be rooted in the different trophic niche that species seem to explore and in different mercury intake and elimination processes that both species' tissues may have (Penicaud et al., 2017; Seixas et al., 2005). On the other hand, the higher concentrations of T-Hg on beaks were found on *A. polymorpha*. Regarding mercury levels on cephalopod hard-tissues, such as beaks, there is until today very few data however it is known that although chitin has a low Hg uptake capacity, the link chitin-mercury is very strong and almost irreversible (Barriada et al., 2008). In 2016, Xavier and colleagues begun to tackle T-Hg concentrations on Antarctic squid beaks and comparing it with available data from mercury concentrations of muscle (Anderson et al., 2009).

Regarding all existent literature concerning mercury levels, there is a notorious lack of knowledge for Antarctic cephalopods. To my knowledge, this study is the first tackling T-Hg concentrations on Antarctic octopods and to compare simultaneously T-Hg concentrations on beaks and flesh. The T-Hg values registered in the flesh of *A. polymorpha* and *P. turqueti* (0.322 ± 0.088 and 0.434 ± 0.128, respectively) are very similar to the values documented in other benthic species of the northern hemisphere (Table 7) and higher than the ones documented in Antarctic squid species (Table 8). In addition, the T-Hg values observed in the lower beaks of *A. polymorpha* and *P. turqueti* (0.052 ± 0.009 and 0.038 ± 0.009, respectively) are also higher than the T-Hg values observed in Antarctic squid species (Table 8).

Since high Hg concentrations have been reported on Antarctic benthic organisms, such as demersal fish and benthic molluscs (Bargagli et al., 1998), and benthic organisms are more exposed to Hg (Bustamante et al., 2006), higher Hg values were expected when comparing with other Antarctic pelagic cephalopods. Moreover, when comparing with octopods from other regions around the globe, low Hg values were expected since Antarctica has been considered as a pristine environment with the lowest Hg concentrations ever registered (Bargagli et al., 1993; Sheppard et al., 1991). This study results highlight the importance of assessing concentrations of heavy metals, such as mercury and cadmium, since there still is a lack of knowledge to what extent heavy metals are impacting Antarctic marine organisms and ecosystems.

4.5. Relevance to support future decisions in ecosystem's management of South Georgia Marine Protected Area (MPA)

The Southern Ocean is home to ancestral, highly endemic and diverse cephalopod fauna worthy of being protected and contemplated on future ecological studies. Since cephalopods constitute an important food source for Antarctic organisms, scientific research on Antarctic cephalopod ecology has become increasingly important on the development of future policy strategies. In the case of the biodiversity-rich region of South Georgia, benthic octopod species

Table 7. Mercury concentrations, expressed in µg g^{-1} (ppm), registered in the flesh/mantle and digestive gland of octopod species in several locations around the world. The mean values ± SD and, within brackets, the minimum and maximum values are presented. ML stands for mantle length values (mm).

Species	Local	N	ML	Flesh/Mantle	Digestive Gland	References
Octopodoidea						
Bathypolypodidae						
Bathypolypus baierii	Bay of Biscay	2	76 ± 0	0.337 ± 0.071 (0.287-0.387)	1.650 ± 0.453 (1.330-1.970)	Bustamante et al. (2006)
	West Scotland	2	44 ± 21	0.415 ± 0.092 (0.350-0.480)	0.791 ± 0.239 (0.622-0.961)	Bustamante et al. (2006)
Bathypolypus sponsalis	Bay of Biscay	5	-	0.216 ± 0.032 (0.185-0.269)	1.003 ± 0.233 (0.796-1.370)	Bustamante et al. (2006)
Benthoctopus sp.	Celtic Sea	1	153	0.921	1.090	Bustamante et al. (2006)
Eledonidae			-			
Eledone cirrhosa	Bay of Biscay	6	85 ± 16	0.298 ± 0.052 (0.265-0.402)	0.457 ± 0.036 (0.422-0.524)	Bustamante et al. (2006)
	Celtic Sea	2	51 ± 4	0.504 ± 0.418 (0.208-0.800)	0.243 ± 0.168 (0.124-0.362)	Bustamante et al. (2006)
	Faroe Island	4	100 ± 8	0.144 ± 0.071 (0.079-0.234)	0.184 ± 0.065 (0.091-0.241)	Bustamante et al. (2006)
	Tyrrhenian Sea	223	20-145	0.01-1.82	-	Barghigiani et al. (2000)
Eledone moschata	Kastela Bay (Adriatic)	-	-	0.505	-	Buzina et al. (1989)
	Kastela Bay (Adriatic)	-	-	0.370	-	Buzina et al. (1989)
	Mediterranean Sea	31	-	0.36 ± 0.07 (0.22-0.47)	-	Storelli & Marcotrigiano (2004)
Megaleledonidae						
Adelieledone polymorpha	**South Georgia**	**10**	**-**	**0.322 ± 0.088 (0.209-0.447)**	**-**	**Current study**
Pareledone turqueti	**South Georgia**	**11**	**-**	**0.434 ± 0.128 (0.242-0.665)**	**-**	**Current study**
Octopodidae						
Octopus cyanea	New Caledonia	7	113 ± 16.3 (82-131)	0.090 ± 0.023 (0.069-0.139)	-	Chouvelon et al. (2009)
Octopus dofleini	Aleutian Islands	5	-	0.038 ± 0.011 (max 0.064)	-	Burger et al. (2007)
Octopus vulgaris	Azores	96	80-260	0.064 ± 0.006	-	Monteiro et al. (1992)
	Bay of Biscay	13	115 ± 19	0.381 ± 0.099 (0.236-0.640)	1.141 ± 0.242 (0.844-1.690)	Bustamante et al. (2006)
	Italy (Modena)	-	-	0.04	-	Plessi et al. (2001)
	Mediterranean Sea	-	-	0.40 ± 0.28 (0.20-0.94)	-	Storelli (2009)
	Portugal (Cascais)	-	760 ± 90 (TL)	0.48 ± 0.16	-	Seixas et al. (2005)
	Portugal (Cascais)	10	-	0.60 ± 0.20	-	Raimundo et al. (2010)
	Portugal (Matosinhos)	11	-	0.28 ± 0.14	-	Raimundo et al. (2010)
	Portugal (Olhão)	12	-	0.35 ± 0.04	-	Raimundo et al. (2010)
	Portugal (Viana)	-	770 ± 48 (TL)	0.22 ± 0.08	-	Seixas et al. (2005)
	Tyrrhenian Sea	-	-	1.65 ± 0.64	-	Renzoni et al. (1973)
Octopus salutii	Bay of Biscay	6	58 ± 15	0.426 ± 0.161 (0.302-0.767)	1.950 ± 0.923 (1.001-3.560)	Bustamante et al. (2006)
	Mediterranean Sea	-	-	0.42 ± 0.20 (0.11-0.84)	-	Storelli (2009)
	South Adriatic Sea	-	-	0.27 ± 0.08	0.84 ± 0.46	Storelli & Marcotrigiano (1999)

Table 8. Mercury concentrations, expressed in µg g^{-1} (ppm), registered in the beak and flesh/mantle of Antarctic cephalopod species. The mean values are presented ± SD and, within brackets, the minimum and maximum values. ML stands for mantle length values (mm).

Species	N	ML	Beak	Flesh/Mantle	References
Octopus					
Megaleledonidae					
Adelieledone polymorpha	**10**	**-**	**0.052 ± 0.009 (0.038-0.066)**	**0.322 ± 0.088 (0.209-0.447)**	**Current study**
Pareledone turqueti	**11**	**-**	**0.038 ± 0.009 (0.024-0.052)**	**0.434 ± 0.128 (0.242-0.665)**	**Current study**
Squid					
Cranchiidae					
Galiteuthis glacialis	4	425 ± 21.5	0.008 ± 0.004 (0.04-0.11)	-	Xavier, Ferreira, et al. (2016)
	3	-	-	0.23 ± 0.08 (0.18-0.31)	Anderson et al. (2009
Gonatidae					
Gonatus antarcticus	4	241 ± 3.75	0.013 ± 0.003 (0.009-0.017)	-	Xavier, Ferreira, et al. (2016)
	2	-	-	0.60 ± 0.02 (0.58-0.61)	Anderson et al. (2009)
Ommastrephidae					
Martialia hyadesi	2	-	-	0.05 ± 0.01 (0.04-0.05)	Anderson et al. (2009
Onychoteuthidae					
Kondakovia longimana	6	554 ± 37.7	0.008 ± 0.003 (0.007-0.013)	-	Xavier, Ferreira, et al. (2016)
	2	-	-	0.10 ± 0.02 (0.08-0.11)	Anderson et al. (2009
Moroteuthis knipovitchi	5	274 ± 17.5	0.025 ± 0.015 (0.009-0.047)	-	Xavier, Ferreira, et al. (2016)
	4	-	-	0.16 ± 0.09 (0.07-0.29)	Anderson et al. (2009

such as *A. polymorpha* and *P. turqueti* support the trophic web as prey of numerous fish species and large breeding colonies of seabirds and pinnipeds (see introduction). Therefore, and since benthic habitats around South Georgia are very complex ecosystems yet to be fully understood (Rogers et al., 2015), cephalopod ecology can be an efficient tool contributing to the reinforcement of the status of protected area in the region (CCAMLR, 2009b; De Santo, 2013).

Due to the important role that cephalopods play on the ecosystem, filling the existent gaps of knowledge relative to the ecology of *A. polymorpha* and *P. turqueti* can provide highly relevant information for future management decisions taking place in South Georgia. Therefore, understanding the interspecific interactions (e.g. prey-predator), population dynamics and detailed distribution is imperative, since we believe that both octopod species can be used as bioindicators of the ecosystem's health due to their relevance for lower and higher trophic levels in the South Georgia food web. Moreover, we also believe that octopods can be used to assess pollution levels of heavy metals, such as mercury, and understand the dynamic of these pollutants throughout the trophic chain and in the ecosystem.

In addition, the Southern Ocean is a resourceful region with fishery activities highly valuable economically targeting krill, fish and, potentially in the future, squid species. For that reason, discovering if *A. polymorpha* and *P. turqueti* are potentially valuable octopod species for being targeted on future fisheries is important in order to assess what resources South Georgia has to offer and what management measures should be implemented.

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