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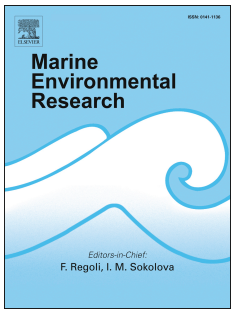

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Show your beaks and we tell you what you eat: Different ecology in sympatric Antarctic benthic octopods under a climate change context

Ricardo S. Matias[a]*, Susan Gregory[b,c], Filipe R. Ceia[a], Alexandra Baeta[a], José Seco[d,e], Miguel S. Rocha[f,g], Emanuel M. Fernandes[f,g], Rui L. Reis[f,g,h], Tiago H. Silva[f,g], Eduarda Pereira[d], Uwe Piatkowski[i], Jaime A. Ramos[a] and José C. Xavier[a,b]

* Contact Number: (+351) 918 393 687 / E-mail: ricardomatias.bio@gmail.com

a. Marine and Environmental Sciences Centre (MARE), Department of Life Sciences, Faculty of Sciences and Technology of the University of Coimbra, 3000-456 Coimbra

b. British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge, CB3 0ET, United Kingdom

c. Government of South Georgia and the South Sandwich Islands, Stanley, Falkland Islands

d. Centre for Environmental and Marine Studies (CESAM), Department of Chemistry, University of Aveiro, Campus Universitário de Santiago 3810-193 Aveiro, Portugal

e. School of Biology, University of St Andrews KY16 9ST, Scotland UK

f. 3B's Research Group, I3Bs – Research Group on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal

g. ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

h. The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal

i. GEOMAR, Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany

Abstract

Sympatry can lead to higher competition under climate change and other environmental pressures, including in South Georgia, Antarctica, where the two most common octopod species, *Adelieledone polymorpha* and *Pareledone turqueti*, occur side by side. Since cephalopods are typically elusive animals, the ecology of both species is poorly known. As beaks of cephalopods are recurrently found in top predator's stomachs, we studied the feeding ecology of both octopods through the evaluation of niche overlapping and specific beak adaptations that both species present. A multidisciplinary approach combining carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope signatures, mercury (Hg) analysis and biomaterials' engineering techniques was applied to investigate the beaks. An isotopic niche overlap of 95.6% was recorded for the juvenile stages of both octopod species, dropping to 19.2% for the adult stages. Both *A. polymorpha* and *P. turqueti* inhabit benthic ecosystems around South Georgia throughout their lifecycles ($\delta^{13}C$: -19.21 ± 1.87‰, mean ± SD for both species) but explore trophic niches partially different during adult life stages ($\delta^{15}N$: 7.01 ± 0.40‰, in *A. polymorpha,* and 7.84 ± 0.65‰, in *P. turqueti*). The beaks of *A. polymorpha* are less dense and significantly less stiff than in *P. turqueti*. Beaks showed lower mercury concentration relative to muscle (*A. polymorpha* - beaks: 0.052 ± 0.009μg·g^{-1}, muscle: 0.322 ± 0.088μg·g^{-1}; *P. turqueti* - beaks: 0.038 ± 0.009μg·g^{-1}; muscle: 0.434 ± 0.128μg·g^{-1}). Overall, both octopods exhibit similar habitats but different trophic niches, related to morphology/function of beaks. The high Hg concentrations in both octopods will have negative consequences on their top predators and may increase under the present climate change context.

Keywords: Cephalopods, Sympatry, South Georgia, Stable Isotopes, Mercury, Biomaterials

1. Introduction

Sympatry in Antarctic cephalopods has started to be addressed, in the sense that close-related species with similar ontogenetic/phylogenetic life-history strategies may display different patterns of genetic differentiation (Strugnell *et al.*, 2017). Two species are considered sympatric when sharing the same geographical region thus frequently encountering each other while exploiting the available natural resources. To avoid competition and probable extinction of the inferior competitor, ecological niche theory predicts sympatric species exploit differently the habitat allowing their coexistence (Hardin, 1960). Niche differentiation, documented in various organisms, such as birds, reptiles (MacArthur, 1958; Pacala & Roughgarden, 1985) and cephalopods (Bennice *et al.*, 2019), can occur by using different habitats or consuming different prey. However, few studies have documented competition between sympatric cephalopods, particularly in polar regions, failing to describe interspecific relationships that might influence marine biota. A better understanding of cephalopods will allow to understand interspecific relationships in the Antarctic ecosystem, as cephalopods play an important roles by constituting strong links between trophic (Collins & Rodhouse, 2006; Xavier *et al.*, 2018). Climate change associated environmental factors are likely to influence the current structure and functioning of Antarctic ecosystems (Constable *et al.*, 2014; Gutt *et al.*, 2015; Rintoul *et al.*, 2018), thus raising concern about the future of keystone cephalopod species.

The octopods *Adelieledone polymorpha* (Robson, 1930) and *Pareledone turqueti* (Joubin, 1905) are the two most abundant sympatric species of benthic Antarctic cephalopods (family: Megaleledonidae) living on the South Georgia shelf (Collins *et al.*, 2004). They are the main prey for some top predators, such as pinnipeds and commercially valuable fish like the Patagonian Toothfish, *Dissostichus eleginoides* (Negri *et al.*, 2016; Rodhouse *et al.*, 1992; Xavier *et al.*, 2002). Around South Georgia, *A. polymorpha* and *P. turqueti* have been found down to 15-862 and 25-640 meters deep, respectively (Allcock, 1997). Even though both species can be found at similar depths, *P. turqueti* is more abundant at lower depths relative to *A. polymorpha* (Yau *et al.*, 2002). Since both species produce large-egg hatchlings and seem to show high parental investment (e.g. brooding), adult dispersal is limited (Barratt *et al.*, 2008; Schwarz *et al.*, 2018; Villanueva & Norman, 2008). Notwithstanding, the arms of *A. polymorpha* have higher number of suckers of smaller diameter and its body is more fragile (i.e. more prone to damage when caught on nets) than *P. turqueti*, which might indicate that both species exploit habitats differently (Daly & Rodhouse, 1994). Great dissimilarities can also be found in the digestive system of the two species: different sizes of posterior salivary gland (PSG) and buccal masses; different beak morphology (Xavier & Cherel, 2009). Whilst *P. turqueti* beaks present features common to other benthic octopods, the lower beak of *A. polymorpha* has an unique shape that clearly differs from all other octopods (Allcock *et al.*, 2003; Daly & Rodhouse, 1994), indicating that both species occupy different trophic niches. Previous dietary studies have recorded a broadly similar diet composed by amphipods, polychaetes and other invertebrates on both species, but differing in the identification of the presence of few remains of hard-shelled prey, octopods and fishes in the diet of *P. turqueti* (Daly,

1996; Piatkowski *et al.*, 2003). As these both species possess clearly different beaks, such characteristics may allow a better understanding of the trophic differences found in their diets.

Due to morphological and trophic specific traits in *A. polymorpha* and *P. turqueti*, our research aims to test for differences in habitat use and trophic ecology of these sympatric octopod species and assess their specific roles in the Antarctic marine ecosystem. It is hypothesized that both octopods occupy benthic habitats within the South Georgia coastal region while exploring trophic niches composed of different prey communities. The different methods of exploiting different sorts of prey are made possible by functional morphological adaptations that both species possess, such as the diverse characteristics of their beaks (Allcock *et al.*, 2003; Daly & Rodhouse, 1994). As cephalopod diversity of South Georgia marine ecosystems and the pivotal role that these organisms have on marine food webs are still not entirely understood, filling existing gaps of knowledge is crucial to fully understand the benthic functional diversity and which ecological drivers dictate the community (Alvito *et al.*, 2014; Collins *et al.*, 2004; Xavier *et al*., 2003). Furthermore, the gathered knowledge will inform future conservation measures implemented through the recently established South Georgia and South Sandwich Islands Marine Protected Area (Hogg *et al.*, 2016; Rogers *et al.*, 2015; Trathan *et al.*, 2014).

To accomplish this study, beaks of *A. polymorpha* and *P. turqueti* were analysed morphologically and their habitat and trophic levels investigated using a multidisciplinary approach:

(1) Applying novel biomaterial engineering techniques, including scanning electron microscopy (SEM), X-ray diffraction (XRD), high-resolution microcomputed tomography (μCT) and nanoindentation test (NNI), provide detailed information on microstructure, composition and density of hard tissues (Cárdenas *et al.*, 2004; Miserez *et al.*, 2007). Since both species are closely related, we expected to find no differences in the beak's microstructure between them, with beak morphology being the key factor in determining physical properties, such as stiffness.

(2) Stable isotope analyses have been successfully applied to study the trophic signals in cephalopod beaks (Cherel & Hobson, 2005; Zimmer *et al*., 2007). Through stable isotope ratios of carbon ($^{13}C/^{12}C$, $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, $\delta^{15}N$) applied in different regions of the beaks, we studied ontogenetic shifts in species' habitat and trophic ecology. Values of $\delta^{13}C$ were used to determine habitat (e.g. higher vs lower latitude, inshore vs offshore) and $\delta^{15}N$ values to trophic niche (Cherel & Hobson, 2007; Hobson & Welch, 1992). Since *A. polymorpha* and *P. turqueti* are benthic octopods, we believe that both inhabit coastal ecosystems on the South Georgia shelf throughout their lifecycle. Ontogenetic dietary shifts are expected in both species, although a broader range of $\delta^{15}N$ values is expected to be found in *P. turqueti* due to its more generalist feeding behavior (Daly, 1996; Piatkowski *et al.*, 2003).

(3) Mercury has been successfully applied to trophic studies before, as its concentration is biomagnified along trophic levels (Atwell *et al.*, 1998; Bargagli *et al.*, 1998). We expect that *P. turqueti* feeds on prey of higher trophic levels thus presenting higher total mercury (T-Hg) concentrations in its tissues relative to *A. polymorpha*. As little is known about cephalopods' beaks mercury uptake, T-Hg concentrations on beaks will also be assessed and compared to T-Hg concentrations in the same individual's muscle. T-Hg concentrations on beaks are expected to be lower relative to muscle.

2. Materials and Methods

Beaks of *A. polymorpha* (n_{Upper}= 40, n_{Lower}= 45) and *P. turqueti* (n_{Upper}= 43, n_{Lower}= 46) were randomly selected from: whole individuals ($n_{A.\ polymorpha}$= 30, $n_{P.\ turqueti}$= 31) captured at depths ranging from 100 to 400 meters, during 30-minutes bottom trawling stations carried out along South Georgia coast, in 2004 (Fig. 1), by the British Antarctic Survey (BAS) on behalf of the Government of the South Georgia & the South Sandwich Islands (GSGSSI); boluses regurgitated by Blue-eyed Shag (*Leucocarbo georgianus*) and Black-browed Albatross (*Thalassarche melanophris*; only 1 sample) breeding on Bird Island, South Georgia (54°00'N 38°03' W), collected by BAS researchers in 2013 (*A. polymorpha*: n_{Upper}= 10, n_{Lower}= 15; *P. turqueti*: n_{Upper}= 12, n_{Lower}= 15). 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 GSGSSI. Samples were preserved at -20°C. At the laboratory, all samples were cleaned and the upper hood length (UHL) and the upper crest length (UCL), from upper beaks, and the lower hood length (LHL) from lower beaks, were measured to the nearest of 0.01 mm using a digital calliper (bigger items) and a measuring lens in a stereomicroscope (smaller items). Estimated mantle length (ML in mm) and mass (M in g) were calculated from loose beaks using allometric equations given by Xavier & Cherel in 2009 (see Results: Table 1). Even though similar-sized individuals were selected and all beaks used presented sub-adult characteristics (e.g. light-coloured wings), beaks of *P. turqueti* were generally larger than *A. polymorpha* beaks (see in results: Table 1; Allcock *et al.*, 2003; Daly & Rodhouse, 1994). Thereafter, samples for stable isotope analysis (*A. polymorpha*: n_{Upper}= 10, n_{Lower}= 10; *P. turqueti*: n_{Upper}= 10, n_{Lower}= 10) were kept in 80% ethanol filled microtubes and the remain samples kept dry until further analyses.

2.1. Physical Properties of Beaks

To evaluate beak microstructure, the upper and lower beaks of both species were fractured, sputter-coated with gold and observed by scanning electron microscope JSM-6010 LV (JEOL, Japan) (Cárdenas *et al.*, 2004; Miserez *et al.*, 2008; Miserez *et al.*, 2007). Respecting the long axis of beaks, longitudinal and transversal observations were set by standard mounting. Backscattered electrons enabled the characterization of chemical composition. Since fracture propagation can be affected by different conditions (e.g. humidity), preliminary tests were carried out to observe which conditions would better preserve the natural morphology of the samples' microstructure (dried in vacuum oven at 37°C for 24h, hydrated from an ethanol-water solution and frozen by immersion on liquid N_2). Following SEM observations were performed on dried samples as they preserved better the natural microstructure of the beaks (per species, n_{Upper}= 2 and n_{Lower}= 2).

Prior to quantitative analysis of crystalline phases, all beak samples (per species, n_{Upper}= 1 and n_{Lower}= 1) were dried and ground into powder. Afterwards, the powder pattern was recorded by XRD using a conventional Bragg-Brentano diffractometer (Bruker D8 Advance DaVinci, Germany) operated

with a Cu-Kα anode (λ = 1.5406Å). The powder pattern of the bulk material was scanned from 2θ range between 5° and 50° at a speed of 2°/min (Cárdenas *et al.*, 2004; Miserez *et al.*, 2007).

Structural features (e.g. geometry and density) of *A. polymorpha* and *P. turqueti* beaks (n_{Upper}= 1, n_{Lower}= 1) were examined using µCT, SkyScan 1217 (Bruker, Kontich, Belgium; Ho & Hutmacher, 2006). For scanning, a voltage range of 50kV and current source of 200µA were applied. Pixel sizes between 8µm and 16µm were selected for *A. polymorpha* and *P. turqueti*, respectively. No filters were applied on the acquisitions and the rotation of the step used was 0.3° up to 360°. All 3D images obtained by x-ray diffraction were reconstructed using CTvox software according to a threshold directly proportional to the materials hardness/density.

For nanoindentation tests (NNI), upper beaks (n = 1) from both species were prepared by imbedding them in methacrylate resin and polishing by microtomy until the zone of interest (rostrum) was exposed. Nanoindentation tests were performed in a Micro Materials NanoTest equipment in ambient air using a Berkovich tip. The properties of the rostrum were determined by performing indentations along upper beak's exposed longitudinal section, from the rostrum to the end of the hood, determined by interest regions (5 in *A. polymorpha* and 9 in *P. turqueti*) separated by 100µm between each other (Fig. 2). All indentations were performed at a loading rate of 1 mN/s to a peak of 4 mM, held at load for 10 s and unloaded at 1 mN/s.

2.2. Stable Isotope and Mercury Analyses

Stable isotope analyses were performed using beaks of *A. polymorpha* (n_{Upper}= 10, n_{Lower}= 10; $ML_{Estimated}$= 94.37 ± 24.85mm; $M_{Estimated}$= 107.21 ± 36.33g wt) and *P. turqueti* (n_{Upper}= 10, n_{Lower}= 10; $ML_{Estimated}$= 63.74 ± 9.15mm; $M_{Estimated}$= 49.47 ± 23.93g wt) collected from boluses regurgitated by Blue-Eyed Shags and Black Browed Albatross specimens (Xavier & Cherel, 2009). All beaks of *A. polymorpha* (UHL = 2.43 ± 0.72mm, UCL = 6.62 ± 1.77mm, LHL = 2.83 ± 0.50mm) and *P. turqueti* (UHL = 4.55 ± 0.96mm, UCL = 10.82 ± 2.31mm, LHL = 3.45 ± 0.67mm) presented sub-adult characteristics such as not completely darkened crest and wings. Each upper beak was sectioned in two pieces (rostrum's tip and crest, representing juveniles and adult life-stages, respectively (Queirós *et al.*, 2018; Fig. 2). In the lower beaks, one wing (representing adult life-stage, Fig. 2) was sectioned and the remaining beak was analysed as a whole (entire beak without 1 wing). Upper beaks' crest and lower beaks' wing of *A. polymorpha* were divided in tanned and untanned chitin, to assess the influence of chitin composition on stable isotope ratios. Untanned chitin subsamples were not used for habitat and trophic ecology analysis, as differences in protein content between tissues (Miserez *et al.*, 2008) might have an impact on $\delta^{15}N$ not only due to their different biochemical composition but also because chitin has a higher C/N ratio than protein and is impoverished in ^{15}N relative to diet (Cherel *et al.*, 2009). As chitinous parts are also impoverished in ^{15}N relative to other tissues, direct comparisons between the isotope ratios of predator and prey should be prevented. Due to their small mass and size respectively, beak samples used in SIA were different from the ones for trace metal analysis.

Prior to analysis, all beak samples of both species (see *n* values in Results: Table 2) were dried at 60°C for 24h and then ground into fine powder. Approximately 0.35 mg of each sample was encapsulated. Stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) were analysed using a continuous-flow Isotope Mass Spectrometer (CFIRMS). Results were calculated by the formula, $\delta X = [(R_{Sample} / R_{standard}) - 1] \times 1000$, where the *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 Vienna-PeeDee belemnite standard (V-PDB) and the atmospheric N_2 (AIR) for $\delta^{13}C$ and $\delta^{15}N$, respectively (Cherel & Hobson, 2005; Hobson & Welch, 1992). Replicate measurements of internal laboratory standards (acetanilide) indicate measurement errors < 0.1 ‰ both for $\delta^{13}C$ and for $\delta^{15}N$.

For mercury analysis, muscle and lower beaks of *A. polymorpha* and *P. turqueti* were collected from whole individuals caught on bottom trawling surveys. All samples (*A. polymorpha:* n_{Lower}= 10, n_{Muscle}= 10; *P. turqueti*: n_{Lower}= 10, n_{Muscle}= 11) were lyophilised during 24h and homogenized. T-Hg concentrations were determined by atomic absorption spectrometry, using an Advanced Mercury Analyzer (AMA) LECO 254, with thermal decomposition and gold amalgamation (Seco *et al.*, 2019; Xavier *et al.*, 2016). The detection limit of the equipment is 0.01ng and the accuracy of the analysis was verified using mussel tissue (ERM – CE278K; 97 ± 16%) as certified reference material (CRM) for calculating recovery efficiency. Analyses were performed in duplicate, when possible, blanks were analysed at the beginning of each set of samples and the coefficient of variation between replicates never exceeded 10%.

2.3. Statistical analysis

Mass (M) and mantle length (ML) allometric equations were calculated (see in results: Table 1) using Pearson's Correlation from individuals with available M values (*A. polymorpha,* n = 37, *P. turqueti,* n = 24) and, only for those individuals, differences in M, UHL, UCL and LHL were checked between species using T-tests. For the rest of the beaks used in this study (i.e. not collected from whole individuals) the M and ML values were estimated using the allometric equations given by Xavier and Cherel (2009). The results of the physical properties' analysis are mainly qualitative thus a descriptive and comparative interpretation of the output is provided between beak type (upper or lower) and species. For the NNI results, one-way ANOVA's were performed to determine statistically significant differences ($p < 0.05$) between the samples after meeting the assumption of normality (Kolmogorov-Smirnov), using the GraphPad Prism 6 (GraphPad Software, La Jolla California USA). Regarding SIA, a series of one-way ANOVA's were used to assess differences in $\delta^{13}C$ and $\delta^{15}N$ between tanned and untanned chitin and a multivariate ANOVA was performed using species and beak region (rostrum and crest) as grouping variables. The carbon and nitrogen isotopic niche overlap were calculated using Stable Isotope Bayesian Ellipses in R (SIBER) encompassing 95% of the proportion of data. For the mercury analysis, one-way ANOVA's were performed to check significant differences between species and soft/hard tissues (muscle/beak, respectively). No correlations between mercury and stable isotopes

were performed since the beak samples used were not from the same individuals. Excluding NNI results, the normality of data was tested using Shapiro-Wilks test and statistical analyses performed using R software (R Core Team, 2017).

3. Results

Only for beaks collected from whole individuals with available mass data, significant differences between species were recorded on UHL ($t = 11.77$, $p < 0.001$), UCL ($t = 8.48$, $p < 0.001$) and LHL ($t = 6.70$, $p < 0.001$), showing that for individuals with similar mass, the upper and lower beaks of *A. polymorpha* (n = 37) are generally smaller relative to *P. turqueti* (n = 24). Some allometric equations were calculated, since strong correlations were found in both species between LHL and the mass (*P. turqueti*: LnM = 0.7756 + 2.4659LnLHL [$r^2 = 0.738$, n = 24]; *A. polymorpha*: M = -98.327 + 72.602LHL [$r^2 = 0.837$, n = 37]) and mantle length (*A. polymorpha*: LnML = 2.9628 + 1.511LnLHL [$r^2 = 0.969$, n = 5]).

3.1. Physical properties

A similar compact and chemically homogenous structure was observed in transversal and longitudinal fractures of both species' upper and lower beaks. Between the inner and outer surface of the beaks, it was also possible to distinguish two strongly-linked layers with different structural arrangements (Fig. 3) and only in the outer surface, spherical and fibrillary structures were recorded. In addition, through XRD spectra (Fig. 4) beaks of *A. polymorpha* and *P. turqueti* were mainly amorphous structures composed by α-chitin, the only crystalline phase found, manifested by the intense peaks at $2\theta = 9$ and 19° and weak peaks at 12, 23 and 27°. Also, μCT scans confirmed that both upper and lower beaks are very compact structures without porosities, increasing in density from peripheral to core regions of the beak. Not precisely quantifiable, beaks of *A. polymorpha* are sharper and less dense relative to *P. turqueti* beaks (Fig. 5). Regarding *A. polymorpha* and *P. turqueti*, both Hardness and Young's Modulus values are statistically different ($p < 0.05$) when comparing the tip of the rostrum (region 1) with the end of the hood (region 5 and 9, respectively). Moreover, the single upper beak of *P. turqueti* was significantly harder (Hardness = 0.269 GPa; Young's Modulus = 4.99 GPa) ($p < 0.05$) than the beak of *A. polymorpha* (Hardness = 0.253 GPa; Young's Modulus = 4.69) (Fig. 6).

3.2. Habitat and trophic niche

Differences between tanned ($n_{Upper} = 9$, $n_{Lower} = 10$) and untanned ($n_{Upper} = 6$, $n_{Lower} = 9$) chitin stable isotopes, $^{12}C/^{13}C$ and $^{14}N/^{15}N$, and C/N ratios were assessed in *A. polymorpha* upper and lower beaks (Table 2). Differences were only found in upper beaks' $\delta^{15}N$ values ($F_{1,17} = 7.73$, $p = 0.010$) and only beak sections of tanned chitin were used in subsequent analyses to avoid biased results. An isotopic niche overlap of 79.5% was found, showing no significant differences between the isotopic values of both species' lower beaks ($n_{Ind} = 10$) ($F_{1,18} = 0.79$, $p = 0.52$). A significant enrichment of $\delta^{15}N$ was found

from the rostrum to the crest of the upper beaks of *A. polymorpha* ($n_{rostrum}$= 9, n_{crest}= 9) and *P. turqueti* ($n_{rostrum}$= 10, n_{crest}= 9) ($F_{1,32}$= 16,85, p < 0.001). No differences were found between species and between rostrum and crest $\delta^{13}C$ values. Between *A. polymorpha* (n_{crest}= 9) and *P. turqueti* (n_{crest}= 9), an isotopic niche overlap of 19.2% of the upper beak's crest subsamples ($F_{1,16}$= 4.08, p = 0.03) was found, but only $\delta^{15}N$ values showed to be significantly different ($F_{1,16}$ = 9.05, p = 0.01), with the crest region of the beak presenting higher levels of ^{15}N relative to the rostrum (Fig. 7).

3.3. Trace metal analysis

Mercury concentrations found in muscle were 6 ($F_{1,12}$= 50.96, p < 0.001; *A. polymorpha*, n = 10) and 10 times ($F_{1,14}$= 55.43, p < 0.001; *P. turqueti*, n = 11) higher than concentrations in lower beaks, respectively. The muscle T-Hg concentrations of *A. polymorpha* (0.322 ± 0.088μg·g^{-1}) were significantly lower ($F_{1,19}$=5.38, p=0.03) than *P. turqueti* (0.434 ± 0.128μg·g^{-1}) whilst, T-Hg concentrations on *A. polymorpha* lower beaks (0.052 ± 0.008μg·g^{-1}) were significantly higher ($F_{1,19}$= 13.09, p = 0.002) than *P. turqueti* (0.038 ± 0.008μg·g^{-1}; Fig. 8). For both octopod species, no correlations were found between T-Hg concentrations of both tissues and between tissues and LHL.

4. Discussion

4.1. Physical properties of the beaks of *A. polymorpha* and *P. turqueti*

Regarding physical properties, similar chemical compositions and microstructure were expected to be found in both species' beaks. However, contrary to the lamellar structure observed in squid beaks (Miserez *et al.,* 2007), the upper and lower beaks of both octopod species present a similar and very compact structure composed by two different structural arrangements (Fig. 3), suggesting that both beaks possess similar function when taking prey. 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. From backscattered electrons, the chemical composition of both beaks of *A. polymorpha* and *P. turqueti* appears to be homogenous. XRD spectra confirmed that both octopod beaks present a similar amorphous structure constituted by only one crystalline phase found, α-chitin (Fig. 4). Prior XRD studies show that β and α-chitin can be found in squid beaks (Miserez *et al.*, 2007) and also in crustacean shells (Cárdenas *et al.*, 2004). In addition, μCT's indicate that both species' beaks are very compact and free of porous structures (Fig. 5).

The most noticeable differences between beaks of both species are their different morphology and structural density which seems to affect the hardness and stiffness of the beak. The unique beak of *A. polymorpha* seems to be structurally less dense and sharper relative to the beak of *P. turqueti,* which has a bulky shape very similar to those found in other benthic octopod species that feed on hard-shelled prey (Guerra & Nixon, 1987). Mechanical properties of the beaks may be related to the differences between the feeding ecology of the two-octopod species. In fact, reported differences are in agreement with previous studies that found hard-shelled organisms exclusively in the diet of *P. turqueti*, being prey that require stronger beaks for hunting and feeding (Daly, 1996). Considering the only single upper beak as representative of the species, the harder and stiffer beak of *P. turqueti* supports a generalist feeding behaviour. Beak values of hardness and stiffness of the jumbo squid *Dosidicus gigas*, a generalist predator which feeds on fish, crustaceans and cephalopods (Nigmatullin *et al.*, 2001), are roughly the double of the ones observed for *A. polymorpha* and *P. turqueti* when comparing with the values reported by Miserez and his colleagues, in 2007. These differences may be related to the different β-chitin based microstructure with a lamellar organization as well as with the larger size of the analysed beaks for that study and, for that reason, future studies may be needed to address ontogenetic variations in the physical properties of the beaks.

Besides the different beak morphology, previous studies reported dissimilarities between the digestive apparatus of *A. polymorpha* and *P. turqueti* (Allcock *et al.*, 2003) indicating that both species might be adapted to different trophic niches. For instance, the posterior salivary gland (PSG), responsible for producing toxic saliva, is significantly larger in *A. polymorpha*, indicating the venom's importance for the species' feeding behaviour (Gibbs & Greenaway, 1978; Undheim *et al.*, 2010). On the other hand, the large-sized buccal mass of *P. turqueti* enables stronger bites and, consequently, the exploration of a wider range of prey unavailable to *A. polymorpha* (Allcock *et al.*, 2003; Daly &

Rodhouse, 1994; Piatkowski *et al.*, 2003). Moreover, taking into account that *A. polymorpha* presents a less muscular body relative to *P. turqueti*, the dissimilarities between species suggest that *A. polymorpha* is adapted to exploit a specific trophic niche in the water column (Daly & Rodhouse, 1994).

4.2. Habitat and trophic niche of *A. polymorpha* and *P. turqueti*

Establishing gradients of $\delta^{13}C$ for the South Georgia region can be a difficult task since $\delta^{13}C$ values can vary spatially (i.e. not linearly related with the latitude and/or inshore/offshore gradients), from -19 to -23‰, both at the base of the trophic web and at the consumers level, due to the encounter of various water masses and fronts (i.e. Southern Antarctic Circumpolar Current Front, South Georgia shelf water, Antarctic Zone water) around South Georgia (Brault *et al.*, 2018; Ceia *et al.*, 2015; Stowasser *et al.*, 2012). However, since *A. polymorpha* and *P. turqueti* are sympatric, holobenthic (Barratt *et al.*, 2008) and no significant differences were found between $\delta^{13}C$ values registered in the juvenile and adult stages (represented by the beak's rostrum and crest, respectively), of both species, it is hypothesized that both species occupy the benthic ecosystems around South Georgia throughout their lifecycles. Regarding trophic niches, an enrichment in $\delta^{15}N$ from juvenile to adult life stages was expected for both species, since preys of bigger size and of higher trophic levels become available as individuals grow (Cherel & Hobson, 2005; Á. Guerra et al., 2010). Even though $\delta^{15}N$ values can vary geographically from bottom to top trophic levels (Alvito *et al.*, 2014; Guerreiro *et al.*, 2015; Seco *et al.*, 2016), *A. polymorpha* and *P. turqueti* inhabit the same ecosystems thus having identical background carbon and nitrogen isotope signatures.

Significant differences found on upper beak's crest $\delta^{15}N$ values suggest that *A. polymorpha* and *P. turqueti* explore different trophic niches during their adult life stage. Even though no differences were found on $\delta^{15}N$ values for lower beaks, different isotope signatures have been recorded between individuals' upper and lower beak (Cherel & Hobson, 2005) and a recent study suggests that upper beaks present isotopic signatures more reliable to be used in trophic models, as lower beaks present a slower growing rate which makes harder to study isotopic signatures of specific periods (Queirós *et al.*, 2018). For that reason, we are confident that both species might occupy partially different trophic niches, differing in some prey items available in function of the adaptations that both species present, such as the different physical properties of the beak, buccal mass and posterior salivary gland size. Interpretation of mercury results can also give as insight about trophic differences (see next topic).

4.3. Mercury in *A. polymorpha* and *P. turqueti* in relation to other octopods

The Southern Ocean has some of the highest concentrations of organic Hg (the most toxic form of mercury) reported for open waters (Cossa *et al.*, 2011) and information regarding Hg levels on Antarctic cephalopods is scarce. To our knowledge, this study is the first to tackle Hg concentrations on Antarctic octopods and to compare it on muscle and beaks. T-Hg concentrations found in the muscle of *P. turqueti* are higher relative to those found in *A. polymorpha*. Since mercury is biomagnified

throughout the trophic web, with top predators presenting higher concentrations, the mercury analysis suggests that *P. turqueti* feeds on preys of higher trophic levels than *A. polymorpha*, corroborating with the $\delta^{15}N$ results. On the other hand, *A. polymorpha* presented higher T-Hg concentrations regarding the lower beaks. Apparently, lower beaks also presented 6 to 10 times less T-Hg levels relative to muscle, similar values also found on several Antarctic squid species (Xavier *et al.*, 2016). As mercury intake rates and elimination processes can vary between tissues and species (Penicaud, *et al.*, 2017; Seixas *et al.*, 2005), any comparative ecological interpretations regarding habitat and trophic niches should be considered with caution. Future studies should consider correlating mercury with nitrogen stable isotope ratios.

Since benthic organisms tend to accumulate Hg due to living close to the sediment, were this element is more bioavailable (Bargagli *et al.*, 1998; Bustamante *et al.*, 2006), the T-Hg values registered in lower beaks and muscle of *A. polymorpha* and *P. turqueti* were higher (3 to 5 times higher) than the ones registered in lower beaks and muscle of some Antarctic squid species with more pelagic behaviour (see Supporting Information, Table A1). When compared with other octopods, concentrations of T-Hg found in the muscle of *A. polymorpha* and *P. turqueti* were similar to those registered in muscle of other northern hemisphere octopod species (see Supporting Information, Table A2). Moreover, cephalopods are among the most contaminated prey (Cipro *et al.*, 2018) of marine top predators, such as Antarctic seabirds, which seem to rely heavily on these prey containing high Hg levels (Anderson *et al.*, 2009). Establish a base line of mercury concentration for cephalopods is important to understand this contaminant in the Southern Ocean trophic webs. Therefore, evaluating how mercury accumulates on cephalopod tissues (e.g. muscle and beaks) may be a good tool to estimate Hg body burden on cephalopods, and how much Hg concentration magnifies along the trophic chain.

5. Conclusion

As a warming trend has been recorded in South Georgia region (Whitehouse *et al.*, 2008), understanding how ecosystems are going to respond due to environmental change has become increasingly important in the development of future policy strategies. As South Georgia region is within the northern boundary of the distribution of *A. polymorpha* and *P. turqueti*, the complexity of ecosystems and multiplicity of stressors make environmental impacts very hard to predict. Through our study, we confirmed that both species inhabit the benthic ecosystems of South Georgia during their lifecycle. According to literature, the low dispersal of the species may be rooted in their reproductive strategies (Barratt *et al.*, 2008; Villanueva & Norman, 2008). Moreover, both *A. polymorpha* and *P. turqueti* deal with interspecific competition by occupying overlapping trophic niches, but with some differences. While *P. turqueti* is able to feed on hard-shelled mollusks, such as bivalves, and crustaceans due to its harder beak, *A. polymorpha* has an unique beak morphology more suitable for predating organisms of softer tissues (Allcock *et al.*, 2003; Daly, 1996; Daly & Rodhouse, 1994; Piatkowski *et al.*, 2003). Different feeding strategies may also determine which one will be more successful in the

changing future, as *A. polymorpha* seems to rely heavily on its highly cold-adapted venom (i.e. bigger PSG; Undheim *et al.,* 2010) while *P. turqueti* predates by using its stronger muscles of the buccal mass and bulkier beak. Since both octopods are highly abundant and a major prey for some top predators breeding in South Georgia region, understanding their ecology, and using them as bioindicators (i.e. high abundance, opportunistic behavior and short lifecycle), can be a powerful tool contributing to fill existing gaps of knowledge and reinforcing the status of protected areas in this region.

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Zimmer, I., Piatkowski, U., & Brey, T. (2007). The trophic link between squid and the emperor penguin *Aptenodytes forsteri* at Pointe Géologie, Antarctica. *Marine Biology*, *152*(5), 1187–1195. https://doi.org/10.1007/s00227-007-0766-1

1 **Figures**

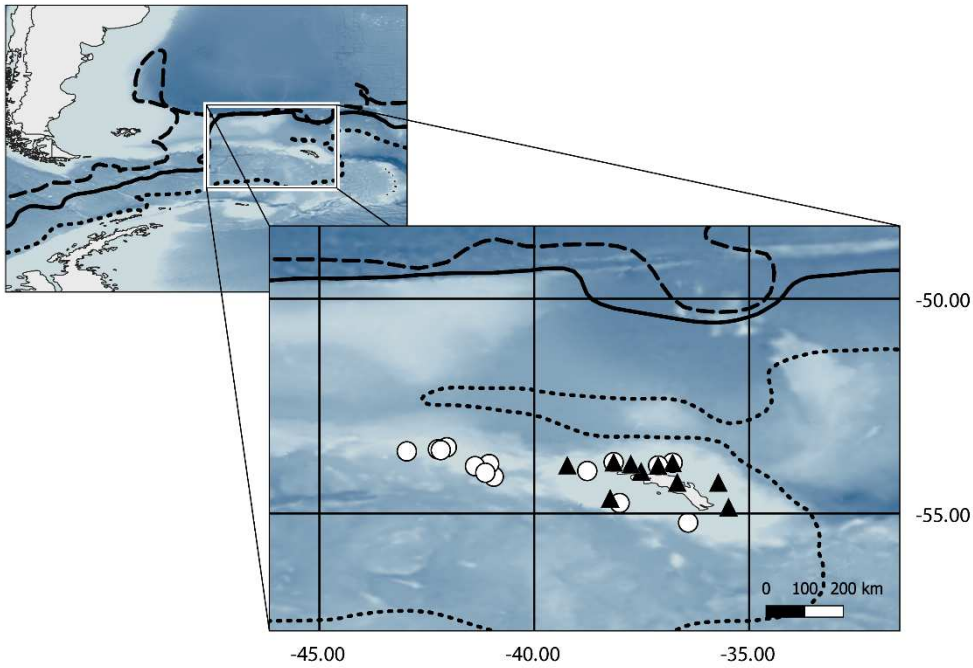

Figure 1. South Georgia region, where specimens of *Adelieledone polymorpha* (black triangles) and *Pareledone turqueti* (white circles) used in this study were caught. In the figure, bathymetry is represented by the background gradient (darker and lighter colours mean deeper and shallower waters respectively) and the oceanic currents - Sub Antarctic Front (dashed line), Antarctic Polar Front (solid line) and the Southern Antarctic Circumpolar Current (pointed line).

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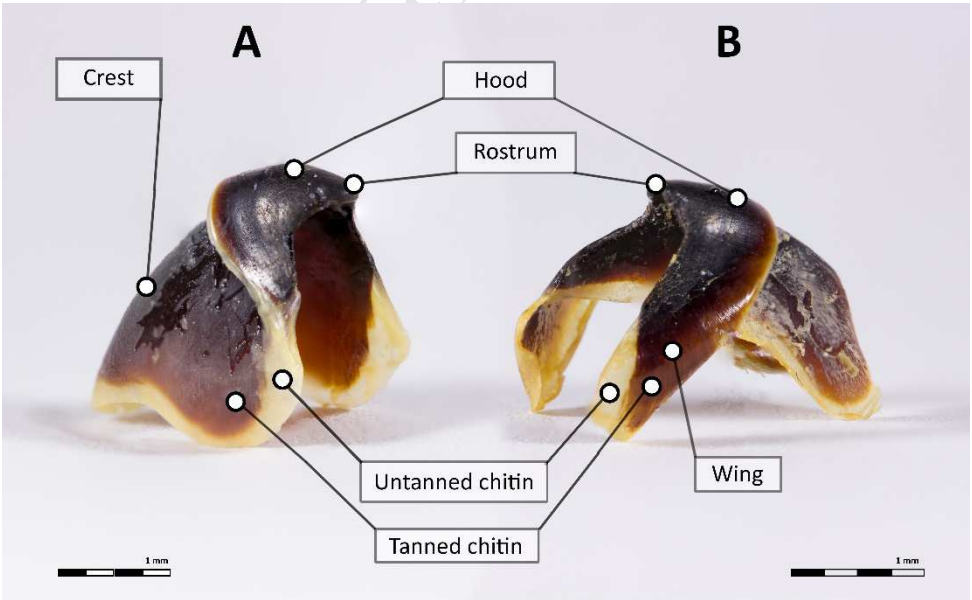

Figure 2. Legend of octopods' upper (A) and lower (B) beaks. Beaks from a *Pareledone turqueti* specimen not used in this study. Measures are: Upper Hood Length (UHL) = 4.98mm; Upper Crest Length (UCL) = 12.53mm and Lower Hood Length (LHL) = 3.55mm.

4

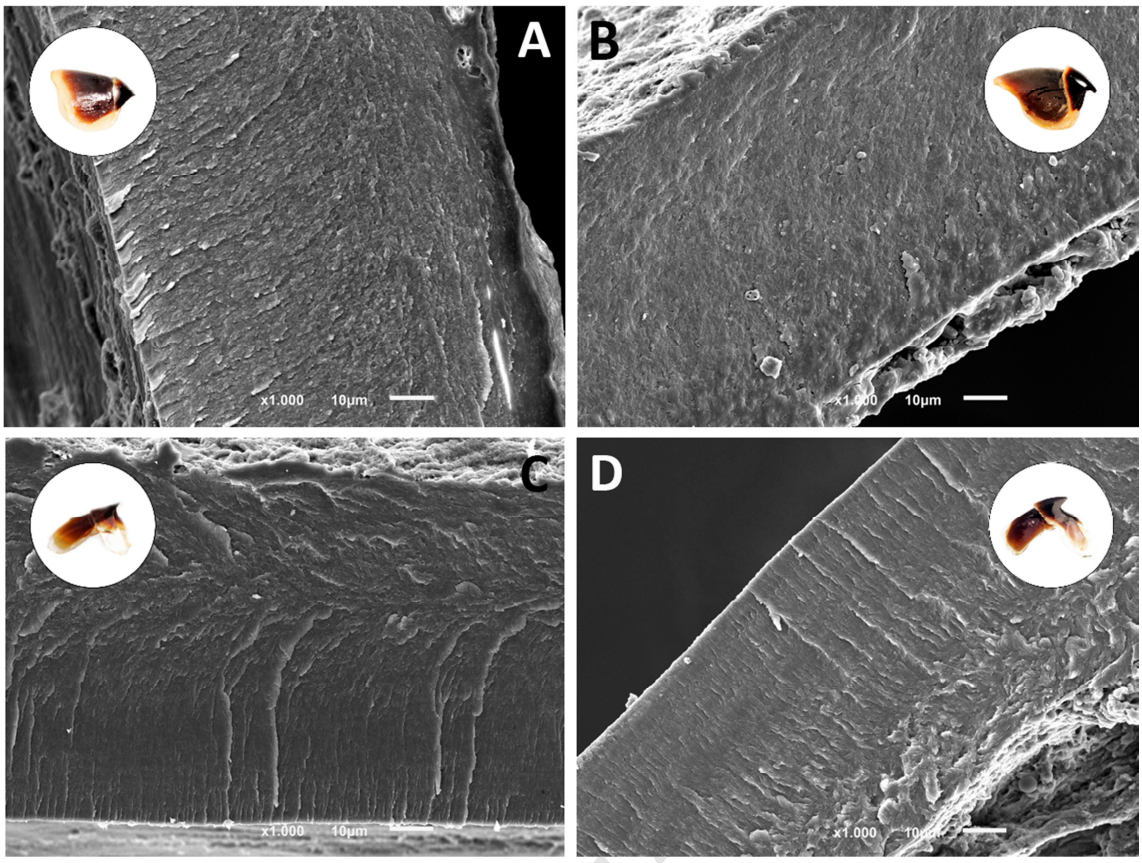

Figure 3. Scanning electron micrographs of longitudinal fractures of the upper and lower beaks of *Adelieledone polymorpha* (A, C) and *Pareledone turqueti* (B, D). Beak images were adapted from Xavier & Cherel (2009).

1

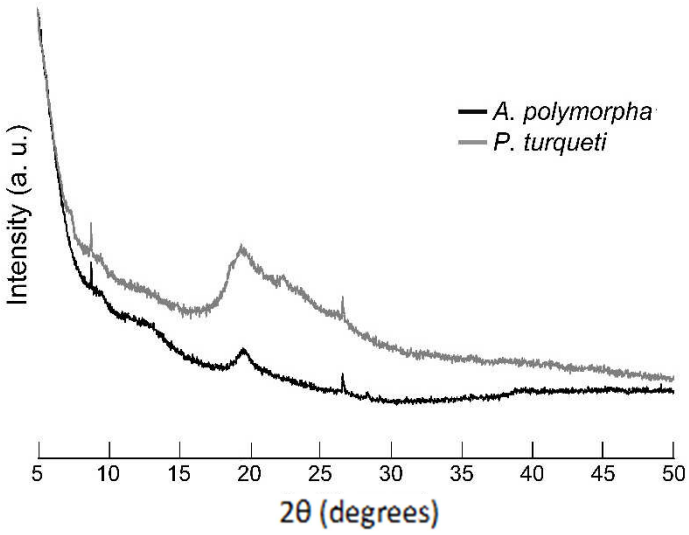

Figure 4. The XRD spectra (dispersion range 2θ of 5-50°) comparing the beak structure of *Adelieledone polymorpha* (M= 145g wt) and *Pareledone turqueti* (M = 57g wt). Beak measures (mm): *A. polymorpha* – Upper Hood Length (UHL) = 3.73, Upper Crest Length (UCL) = 8.60 and Lower Hood Length (LHL) = 3.29; *P. turqueti* – UHL = 4.11, UCL = 11.76 and LHL = 3.81.

2

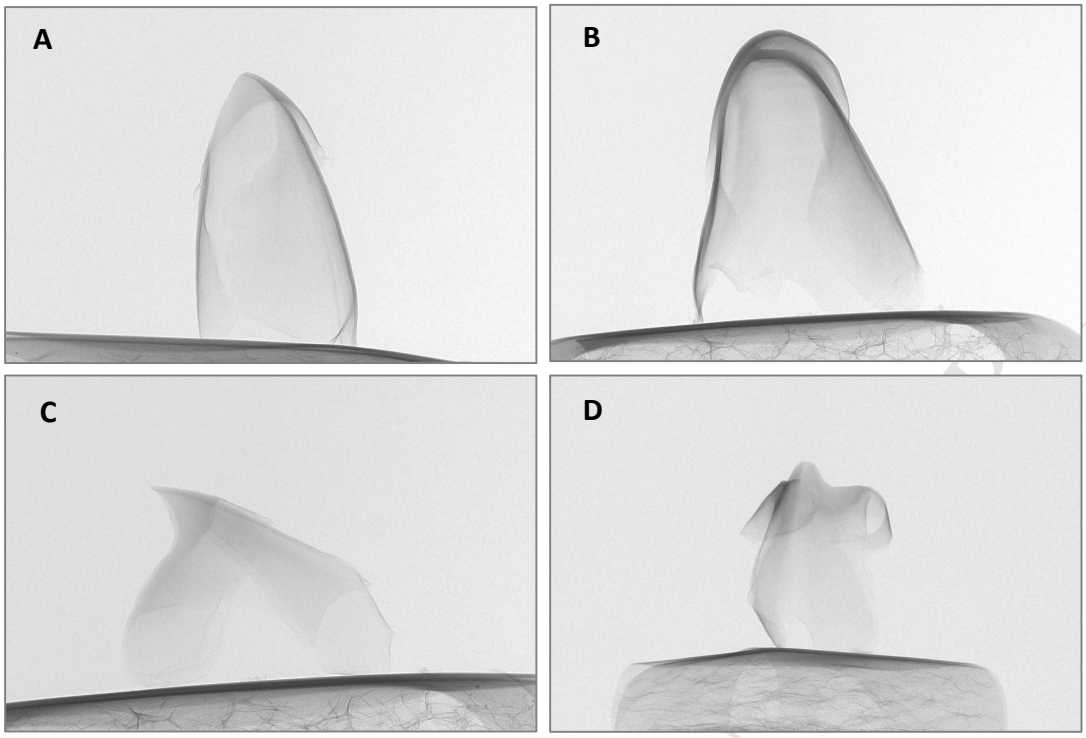

Figure 5. Micro CT scans of the beak structure of *Adelieledone polymorpha* (A – Upper; C – Lower) and *Pareledone turqueti* (B – Upper; D – Lower). Beak measures (mm): *A. polymorpha* – Upper Hood Length (UHL) = 5.83, Upper Crest Length (UCL) = 13.99 and Lower Hood Length (LHL) = 4.52; *P. turqueti* – UHL = 2.59, UCL = 8.65 and LHL = 3.23.

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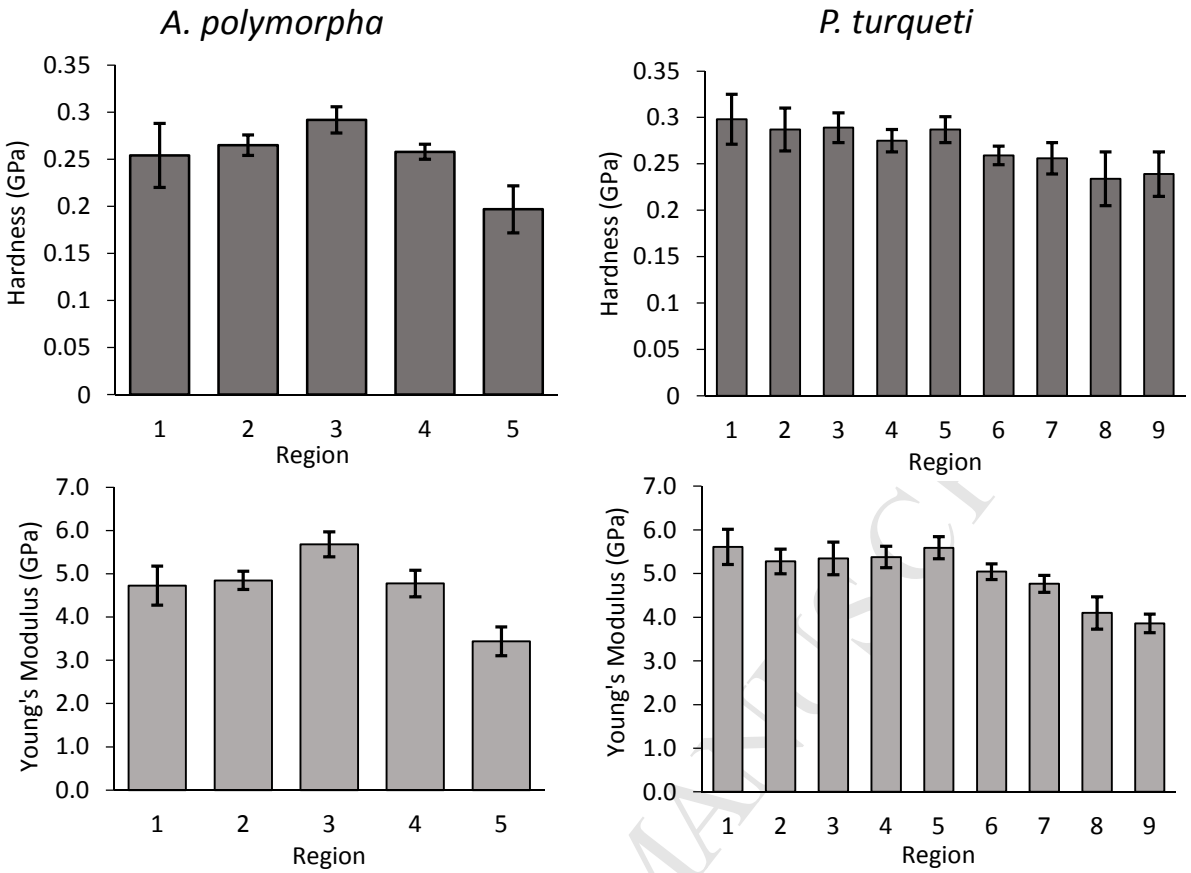

Figure 6. Bar plots for the means and standard deviation values for the hardness (dark grey) and Young's modulus (light grey) registered during the nanoindentation tests performed on the upper beaks of the octopod species, *Adelieledone polymorpha* and *Pareledone turqueti*. The x-axis represents the regions of the beak, being region 1 the closest to the tip of the rostrum. The values are expressed in gigapascal (GPa). *A. polymorpha*: M_{Wet}= 164g, UHL = 3.43mm, UCL = 9.71mm; *P. turqueti*: M_{Wet}= 82g, UHL = 6.25mm and UCL = 14.23mm

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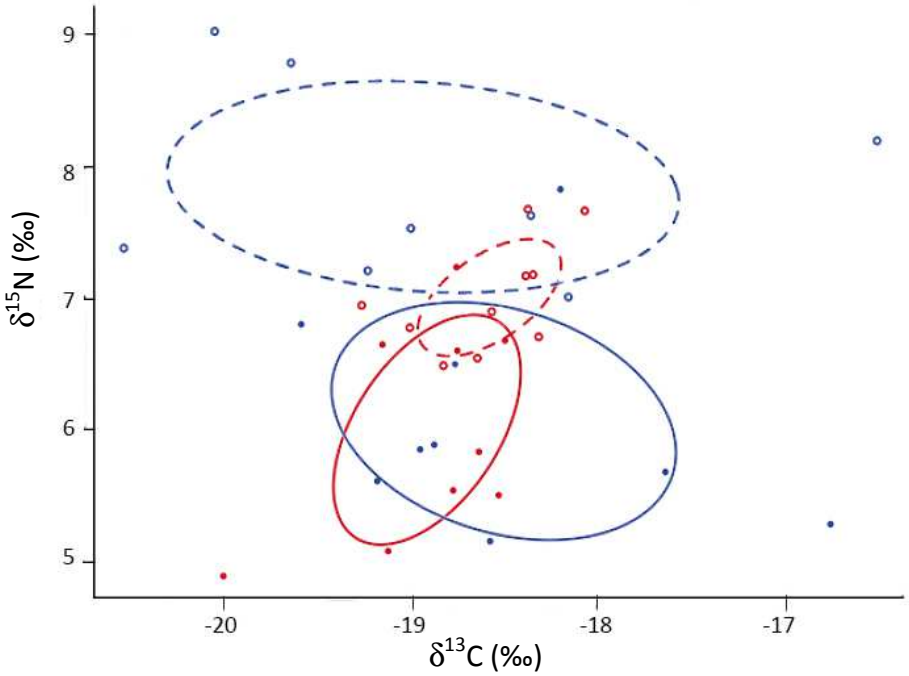

Figure 7. Bivariate $\delta^{13}C$ and $\delta^{15}N$ plot for upper beaks' rostrum (solid lines and filled circles) and crest (dashed lines and empty circles) of *Adelieledone polymorpha* (red) and *Pareledone turqueti* (blue), estimated by the ellipse corrected for the SIBER analysis. Samples of untanned chitin were excluded from this analysis.

2

3

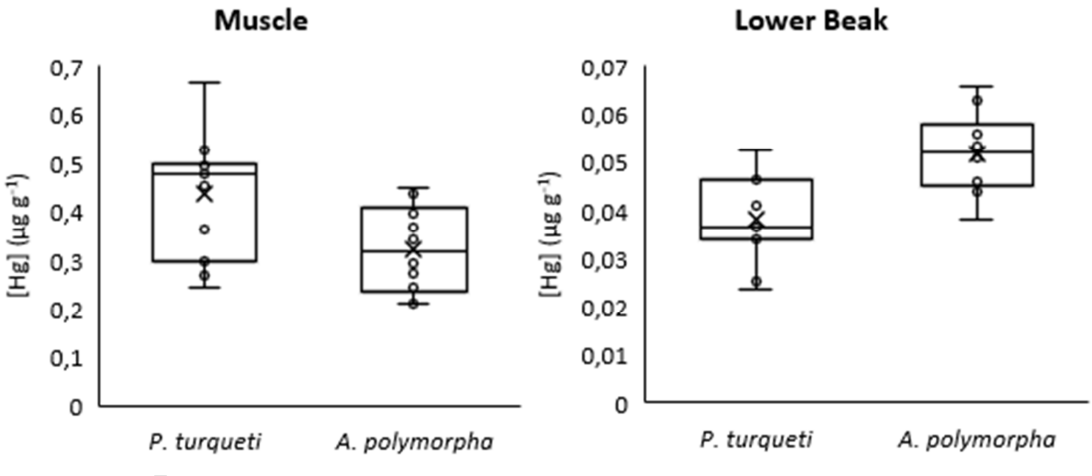

Figure 8. Total mercury total concentrations registered in the muscle and lower beak of *Adelieledone polymorpha* (n = 10) and *Pareledone turqueti* (n = 11). Boxplot shows the mean (cross), median (line), $1^{st}/3^{rd}$ quartile (box), minimum/maximum (whiskers) and concentration values (circles) registered.

4

5

Table 1. Values of upper beak's hood length (UHL), crest length (UCL) and lower beak's hood length (LHL) of the beaks of *Adelieledone polymorpha* and *Pareledone turqueti*. Estimated values for the mass and mantle length were calculated using both known values and estimates given by existent allometric equations (Xavier & Cherel, 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

1

Table 2. 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.

2

Species	n	δ^{13}C (‰) Mean ± SD	δ^{13}C (‰) Min	δ^{13}C (‰) Max	δ^{15}N (‰) Mean ± SD	δ^{15}N (‰) Min	δ^{15}N (‰) Max	C/N mass ratio Mean ± SD	C/N mass ratio Min	C/N mass ratio 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

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