

UNIVERSIDADE DE LISBOA  
FACULDADE DE CIÊNCIAS  
DEPARTAMENTO DE BIOLOGIA ANIMAL



## **Contaminants in deep-sea glass squids (Cranchiidae) from the Eastern Tropical Atlantic oxygen minimum zone**

Ana Patrícia Mil-Homens Rafael

**Mestrado em Ecologia Marinha**

Dissertação orientada por:  
Doutor Rui Rosa, Centro de Ciências do Mar e do Ambiente (MARE) - Pólo de Lisboa,  
Laboratório Marítimo da Guia  
Doutora Joana Raimundo - IPMA - Instituto Português do Mar e da Atmosfera



## ACKNOWLEDGEMENTS

Because we can't accomplish anything by being alone, I wish to show my gratitude to everyone who made this possible.

To Professor Doutor Rui Rosa for giving me the opportunity to work with the deep-sea and with such incredible (yet small) creatures.

Doutora Joana Raimundo for accepting to be my co-advisor, and for all the support throughout this journey with these animals (that we could barely see).

To Uwe Piatkowski, Henk-Jan Hoving and Alexandra Lischka, for making this work possible, by allowing me to use their samples

To Cátia Figueiredo a big thank you for all the disponibility for listening to my questions and for helping me whenever I needed and asked.

To all MSc, PhD and post-doc fellows at Laboratório Marítimo da Guia, for their sympathy and kindness.

To my best friend from high school and neighbor, because even with weeks without meeting, the friendship seems to never end.

To Inês Castanheira, who walk the same path as me, as made this journey to much fun.

To Eduarda Pinto and Francisco Borges for the support, especially during writing.

For last, my family. My parents for the constant support that brought me here, and for letting me choose the path to follow. My brother, who even on the other side of the planet asks if he can help.

## RESUMO

O oceano constitui grande parte do planeta, acomodando cerca de 1 368 milhões Km<sup>3</sup> de água, providenciando mais espaço que todos os habitats terrestres. Apesar da maioria da biodiversidade marinha se encontrar na zona fótica e menos profundas, uma grande variedade vive nas partes mais profundas e sem luz. Em algumas partes do oceano, entre os 10 e os 1 300m, existem zonas de oxigênio mínimo (ZOM). Estes habitats pelágicos apresentam condições estáveis de níveis baixos e persistentes de oxigênio e baixas temperaturas ao longo de vastas áreas. Estes habitats resultam de uma combinação de uma fraca ventilação assim como pouca circulação da água. A maioria das ZOM exibe um perfil de oxigênio semelhante, mas os níveis de oxigênio, espessura e a profundidade de ocorrência podem variar regionalmente. No Atlântico Tropical Oriental, a uma profundidade entre os 200m a 800m, existe uma zona mínima de oxigênio localizada entre o sistema da Corrente Equatorial (a Sul) e a Corrente Equatorial Norte (a Norte). Aqui, o perfil de oxigênio tem dois mínimos a cerca de 70m e 400m de profundidade, com o mínimo mais proeminente entre o Senegal e a Ilha de Cabo Verde.

Os cefalópodes (classe Cephalopoda) podem ser encontrados em todos os oceanos do mundo, desde águas costeiras ao mar profundo, e algumas espécies conseguem viver em condições extremas, como as fontes hidrotermais e as ZOM. A maioria destes moluscos são predadores oportunistas com altas taxas de crescimento, uma única época de reprodução e uma esperança média de vida curta nas zonas costeiras. No mar profundo, estas características biológicas são bastante diferentes, dado que as suas capacidades locomotoras e requisitos metabólicos são mais reduzidos. De uma forma geral, os cefalópodes desempenham um papel fundamental nos ecossistemas marinhos, uma vez que são uma fonte primária de alimento para muitos predadores marinhos, como peixes, mamíferos marinhos ou aves marinhas.

Os cranchídeos (família Cranchiidae) estão entre as lulas mais abundantes do mundo, de grande diversidade morfológica em ambientes pelágicos. A maioria delas têm uma aparência transparente (por isso designadas também por lulas de vidro) dado possuírem grandes cavidades celômicas cheias de fluido amoniacal neutro e de baixa densidade, que lhes confere flutuabilidade neutra. Embora a sua morfologia e anatomia estejam bem estudadas, e de serem muito abundantes, pouco se sabe sobre a sua biologia e ecologia. Muitas das espécies de cranchídeos passam grande parte de sua vida em águas onde quase não há luz ou em completa escuridão. A maioria desses pequenos animais não depende da velocidade para escapar de predadores, mas sim de outras estratégias defensivas, - a sua transparência, que sob condições de iluminação adequadas, as tornam praticamente invisíveis.

A poluição marinha tornou-se um problema muito grave durante as últimas décadas, devido aos seus impactos na biodiversidade e no funcionamento dos ecossistemas. A presença de concentrações acima do normal de contaminantes já foi detetada em vários tipos de habitats marinhos, nomeadamente no mar profundo, onde a preocupação tem vindo a aumentar devido à possibilidade de este, devido à sua dimensão, se poder tornar no maior depósito para tais elementos. A bioacumulação tem sido amplamente estudada em muitos organismos marinhos e demonstrou-se que os moluscos, como os cefalópodes, têm a capacidade de acumular altos níveis de contaminantes. As maiores concentrações determinadas são o carbono, hidrogénio, azoto, oxigénio e enxofre, que são elementos estruturais. Outros elementos encontrados podem ser essenciais, cujo papel biológico é conhecido, como ferro, cobre, zinco, iodo, manganês, selénio ou flúor. Os não essenciais, não têm um papel conhecido nas funções fisiológicas, como ocorre com mercúrio, chumbo e cádmio. Embora existam estudos sobre a acumulação de elementos em cefalópodes, a maioria está focada em espécies comerciais. Entre essas espécies, foram geralmente registados altos níveis de metais, como o cádmio, o cobre e o zinco, tornando os cefalópodes uma potencial via de transporte de contaminantes para níveis tróficos superiores. A absorção de contaminantes por organismos marinhos pode ocorrer a partir da água, incluindo partículas em suspensão, alimentos e sedimentos. Outros fatores, como a disponibilidade de elementos na água,

período de exposição, temperatura, tamanho, sexo, estágio de maturidade e local de captura, também são importantes na acumulação de metais nos tecidos destes organismos. É importante referir que dado que os cefalópodes apresentam uma vida curta e uma alta acumulação de contaminantes, estes organismos podem refletir as condições actuais do ambiente onde vivem e serem utilizados como indicadores de contaminação ambiental. Embora não haja informações sobre contaminantes nos cranchídeos, existe informação sobre outras espécies de cefalópodes, incluindo outras lulas oceânicas. A maioria dos estudos mostrou a capacidade dos cefalópodes de concentrar Zn, Cu e Cd na glândula digestiva mesmo em ambientes com baixa disponibilidade. Neste contexto, o objetivo desta dissertação, foi determinar, pela primeira vez, as concentrações de alguns elementos (V, Cr, Co, Ni, Cu, Zn, As, Se, Cd e Pb) nos tecidos (glândula digestiva, manto e tentáculos) de nove espécies de cranchídeos, da ZOM de Cabo Verde. As lulas foram apanhadas na zona económica exclusiva de Cabo Verde, em oito estações de amostragem, através da utilização de redes pelágicas MOCNESS e IKMT em diferentes profundidades. Os indivíduos capturados foram identificados até à espécie (quando possível) e preservados em azoto líquido a bordo do RV Maria S. Merian e depois transferidas para -80°C no laboratório. Os indivíduos foram dissecados de modo a separar o manto, os tentáculos e a glândula digestiva.

Neste trabalho, foram detectadas diferenças significativas entre espécies de cranchídeos e entre os diferentes tecidos analisados. De uma forma geral, os elementos na glândula digestiva variaram entre os 0,070 e 17  $\mu\text{g g}^{-1}$  peso seco, com as concentrações variando da seguinte forma decrescente: Zn > Cd > As > Cu > V > Se > Co > Cr > Ni > Pb. No manto variaram entre os 0,070 e 28  $\mu\text{g g}^{-1}$  peso seco, com as concentrações variando da seguinte forma decrescente: Zn > Cu > Cr > As > V > Ni > Se > Cd > Pb > Co. Nos tentáculos, os contaminantes variaram entre os 0,13 e os 24  $\mu\text{g g}^{-1}$  peso seco, com as concentrações variando da seguinte forma decrescente: Se > Zn > As > Cu > Cr > Ni > V > Cd > Co > Pb. Estes resultados em comparação com os de outros grupos de cefalópodes (nomeadamente chocos, polvos e outras lulas pelágicas) mostraram que as espécies pelágicas (lulas) apresentavam menores concentrações de Cd do que as espécies bentónicas (polvos) ou nectobentónicas (chocos). Essas diferenças podem ser atribuídas às diferentes concentrações de Cd nas suas presas, uma vez que as presas bentónicas (crustáceos e bivalves) têm maiores concentrações de Cd, do que os peixes que são presas preferenciais de espécies pelágicas. Em relação às espécies nectobentónicas e bentónicas, os sedimentos também podem ser uma via de acumulação - embora a transferência direta seja menor, podem atuar como uma fonte indireta de contaminantes. Por último, as diferenças com os nautilus podem estar relacionadas com diferentes tempos de vida, uma vez que os nautilus vivem entre os 10 e os 15 anos, enquanto os Coleoids (choco, lulas e polvos) vivem entre 1 a 3 anos. Embora a expectativa de vida da maioria dos cranchídeos não seja conhecida, não é provável que os indivíduos estudados atinjam essas idades. É importante notar que os músculos dos cranchídeos apresentaram maior variação e uma maior concentração do que as outras espécies de cefalópodes. Estes resultados não eram esperados e levanta a questão porque é que os cranchídeos concentram elevados níveis de alguns elementos nestes tecidos.

A capacidade dos cefalópodes para concentrar altos níveis de metais sem um sinal de toxicidade, está associada a eficientes processos de desintoxicação que permitem reter os elementos de forma não metabolicamente disponível, limitando assim a sua toxicidade. Neste contexto, a glândula digestiva é um órgão complexo envolvido em várias funções, como digestão, secreção e desintoxicação (entre outros). Por ser considerado um tecido de armazenamento, é também um órgão-chave para a desintoxicação. Não surpreendentemente, mas com algumas exceções (por exemplo, Hg e As), a maioria dos contaminantes são encontrados em concentrações mais elevadas neste tecido. Pensa-se que inicialmente os contaminantes estão ligados a proteínas solúveis, o que implica que as células da glândula digestiva provavelmente estarão envolvidas na desintoxicação dos contaminantes. Esta associação com proteínas citosólicas, inibe as interações tóxicas de iões metálicos com locais de ligação sensíveis (proteínas, moléculas ou estruturas celulares). Além disso, uma vez que os metais como Ag,

Cd, Cu, Hg e Zn têm uma alta afinidade para as metalotioninas, pensa-se que estas desempenham um papel fundamental na homeostasia dos metais essenciais, bem como um papel importante na tolerância dos organismos a elementos não-essenciais.

Estudos anteriores mostraram que não é só o Cd, mas também outros contaminantes, como Co, Cr, Ni e V, são encontrados em concentrações mais elevadas na glândula digestiva do que no músculo. No entanto, nos cranchídeos, e apesar de o Co ter apresentado concentrações mais elevadas na glândula digestiva, o V, Cr e Ni apresentaram valores mais elevados no manto. Quanto às diferenças entre manto e tentáculos, estas podem ser devido a diferentes composições proteicas e respectivos sistemas enzimáticos, podendo explicar os resultados obtidos com os cranchídeos

Em suma, o fato das lulas de vidro, que prosperam em ambientes pelágicos profundos, exibirem concentrações de contaminantes semelhantes aos encontrados nos cefalópodes costeiros (cujos habitats estão sujeitos a uma maior intervenção humana), é inesperado. Estes dados corroboram a ideia corrente de que esses ambientes remotos estão-se a tornar o principal acumulador de contaminantes no planeta.

## ABSTRACT

Trace elements are persistent and have been detected in a wide range of environments, including the deep-sea, where the concern is increasing, as it might act as a global sink for them. One group of cephalopods that thrive in such harsh environments, including the mesopelagic oxygen minimum zones, are the cranchiid (glass) squids. Although their anatomy and morphology is well understood, little is known about their biology and ecology. Elemental bioaccumulation has been greatly studied in many marine organisms and it has been shown that cephalopods have the ability to accumulate high levels of elements. The uptake of trace elements can occur from water, including suspended particulate matter, food and sediments. Because cephalopods display a short life span and high accumulation of trace elements, these characteristics may clearly reflect the ambient life conditions and indicators of environmental contamination. Yet, up to our knowledge, there is no information about trace elements in the deep cranchiid squids. Within this context, the aim of the present dissertation was to determine, for the first time, the concentrations of V, Cr, Co, Ni, Cu, Zn, As, Se, Cd and Pb in the digestive gland, mantle and tentacles of nine different cranchiid squids from the Cape Verde Exclusive Economic Zone in the Eastern Atlantic Ocean. Concomitantly, a comprehensive comparison with trace elements obtained in shallow-living cephalopod species (including cuttlefish, octopods and other squids) was also conducted. In general, trace elements in cranchiid digestive gland ranged between 0.07 and 17  $\mu\text{g}^{-1}$  dw, with the following average concentrations in descending order: Zn > Cd > As > Cu > V > Se > Co > Cr > Ni > Pb. In the mantle ranged between 0.07 and 28  $\mu\text{g g}^{-1}$  dw, with the following average concentrations in descending order: Zn > Cu > Cr > As > V > Ni > Se > Cd > Pb > Co. In the tentacles, trace elements ranged between 0.13 and 24  $\mu\text{g g}^{-1}$  dw, with the following average concentrations in descending order: Se > Zn > As > Cu > Cr > Ni > V > Cd > Co > Pb. The high Cd levels in cranchiid squids may be related to the enriched environment around Cape Verde islands, with dissolved Cd being reinjected in the water column by the upwelling of subsurface waters. Additionally, another route of contamination could be from the Saharian dust deposition, which represents an important source of trace elements (Co, Ni, Zn and Cd) into the Atlantic Ocean. In the present work, it also became evident that benthic cephalopod species showed higher concentrations of Cd than the pelagic conspecifics. These differences could be attributed to distinct trophic ecologies (different trace elements concentrations on their preys). Previous studies have also shown that not only the Cd but also other trace elements, like Co, Cr, Ni and V are found at higher concentrations in the digestive gland (the major storage site and a key organ for detoxification) than in the muscle. These findings were not corroborated here, since while Cd and Co levels were higher concentrations in the digestive gland, V, Cr and Ni have higher

concentration values in the mantle. Last, it is worth noting that the fact that glass (transparent) squids that thrive in deep pelagic environments display trace elements concentrations as high as those found in coastal cephalopods, which live in habitats exposed to enhanced anthropogenic forcing, is quite surprising. These findings corroborate the on-going notion that such remote environments are now the major global sink for contaminants in the planet.

**Key words:** cephalopod, cranchiids, trace elements, Cape Verde,

## INDEX

<b>ACKNOWLEDGEMENTS</b> .....	<b>ii</b>
<b>RESUMO</b> .....	<b>ii</b>
<b>ABSTRACT</b> .....	<b>iv</b>
<b>List of figures and tables</b> .....	<b>vii</b>
<b>List of abbreviations, acronyms and symbols</b> .....	<b>x</b>
<b>1. INTRODUCTION</b> .....	<b>1</b>
1.1. Deep-sea and oxygen minimum zones (OMZs).....	1
1.2. Adaptations to life in OMZs.....	2
1.3. Cephalopods.....	2
1.4. Glass squids (cranchiids).....	3
1.5. Contaminants in cephalopods.....	4
<b>2. MATERIAL AND METHODS</b> .....	<b>5</b>
2.1. Sampling and sample preparation .....	5
2.2. Contaminant concentrations .....	6
2.3. Statistical analysis .....	6
<b>3. RESULTS</b> .....	<b>6</b>
3.1. Vanadium (V).....	7
3.2. Chromium (Cr).....	9
3.3. Cobalt (Co).....	11
3.4. Nickel (Ni).....	13
3.5. Copper (Cu).....	15
3.6. Zinc (Zn) .....	17
3.7. Arsenic (As) .....	19
3.8. Selenium (Se).....	21
3.9. Cadmium (Cd).....	23
3.10. Lead (Pb).....	25
3.11. Differences among cephalopods: a multivariate approach.....	27
<b>4. DISCUSSION</b> .....	<b>29</b>
4.1. Trace elements in cranchiids and other cephalopod groups .....	29
4.2. Trace elements tissue partition .....	29
<b>Annex A</b> .....	<b>37</b>
<b>Annex B</b> .....	<b>39</b>
<b>Annex C</b> .....	<b>46</b>
<b>Annex D</b> .....	<b>53</b>



## List of figures and tables

- Figure 1.1:** Oxygen profile in the eastern tropical North Atlantic (ETNA), with two minima at around 70m and 400m depth (Ryabenko et al., 2012); B) Schematic pattern of the circulation showing the main currents and dynamic features: Canary Current (CC), North Equatorial Current (NEC), North Equatorial Counter Current (NECC), North Equatorial Under Current (NEUC), Poleward Undercurrent (PUC), Mauritania Current (MC), Guinea Undercurrent (GUC), Guinea Dome (GD) and Cape Verde Frontal Zone (CVFZ) (Peña-Izquierdo et al., 2012).....1
- Figure 1.2:** A) *Cranchia scabra* (copyright: 1998 Richard E. Young); B) *Megalocranchia fisheri* (copyright: Richard E. Young); C) *Teuthowenia pellucida* (copyright: Claire Nouvian); D) *Leachia pacifica* (copyright: Roger R. Seapy); E) *Taonius borealis* (copyright: Claire Nouvian).....3
- Figure 2.3:** Map of the sampling stations.....5
- Figure 3.4:** Median, 25 and 75%, percentil, minimum, maximum, outliers and extremes of vanadium (V) concentrations ( $\mu\text{g g}^{-1}$  dw) in cephalopods. A) in the digestive gland of the studied cranchiids (present study); B) in the digestive gland of all studied cranchiids (in the overall) and other cephalopod; C) in the mantle of the studied cranchiids (present study); D) in the mantle of all cranchiids (in the overall) and other cephalopod groups; E) in the tentacles of the studied cranchiids (present study). Numbers in brackets represent the sample size (n). Species or taxonomic groups with only value were not included in the interspecific comparisons (statistical analyses). Different superscript letters represent significant differences ( $P<0.05$ ), among species or orders (lowercase) and among tissues (capital letters).....8
- Figure 3.5:** Median, 25 and 75% percentil, minimum, maximum, outliers and extremes of chromium (Cr) concentrations ( $\mu\text{g g}^{-1}$  dw) in cephalopods. A) in the digestive gland of the studied cranchiids (present study). B) in the digestive gland of all studied cranchiids (in the overall) and other cephalopod groups. C) in the mantle of the studied cranchiids (present study). D) in the mantle of all studied cranchiids (in the overall) and other cephalopod groups. E) in the tentacles of the studied cranchiids (present study). F) in the tentacles of all studied cranchiids (in the overall) and other cephalopod groups. Numbers in brackets represent the sample size (n). Species or taxonomic groups with only value were not included in the interspecific comparisons (statistical analyses). Different superscript letters represent significant differences ( $P<0.05$ ), among species or orders (lowercase) and among tissues (capital letters)..... 10
- Figure 3.6:** Median, 25 and 75% percentil, minimum, maximum, outliers and extremes of cobalt (Co) concentrations ( $\mu\text{g g}^{-1}$  dw) in cephalopods. A) in the digestive gland of the studied cranchiids (present study). B) in the digestive gland of all studied cranchiids (in the overall) and other cephalopod groups. C) in the mantle of the studied cranchiids (present study). D) in the mantle of all studied cranchiids (in the overall) and other cephalopod groups. Numbers in brackets represent the sample size (n). Species or taxonomic groups with only value were not included in the interspecific comparisons (statistical analyses). Different superscript letters represent significant differences ( $P<0.05$ ) among species or orders (lowercase)..... 12
- Figure 3.7:** Median, 25 and 75% percentil, minimum, maximum, outliers and extremes of nickel (Ni) concentrations ( $\mu\text{g g}^{-1}$  dw) in cephalopods. A) in the digestive gland of the studied cranchiids (present study). B) in the digestive gland of all studied cranchiids (in the overall) and other cephalopod groups. C) in the mantle of the studied cranchiids (present study). D) in the mantle of all studied cranchiids (in the overall) and other cephalopod groups. E) in the tentacles of the studied cranchiids (present study). F) in the tentacles of all studied cranchiids (in the overall) and other cephalopod group. Numbers in brackets represent the sample size (n). Species or taxonomic groups

with only value were not included in the interspecific comparisons (statistical analyses). Different superscript letters represent significant differences ( $P < 0.05$ ), among species or orders (lowercase) and among tissues (capital letters)..... **14**

**Figure 3.8:** Median, 25 and 75% percentil, minimum, maximum, outliers and extremes of Copper (Cu) concentrations ( $\mu\text{g g}^{-1}$  dw) in cephalopods. A) in the digestive gland of the studied cranchiids (present study). B) in the digestive gland of all studied cranchiids (in the overall) and other cephalopod groups. C) in the mantle of the studied cranchiids (present study). D) in the mantle of all studied cranchiids (in the overall) and other cephalopod groups. E) in the tentacles of the studied cranchiids (present study). F) in the tentacles of all studied cranchiids (in the overall) and other cephalopod groups . Numbers in brackets represent the sample size (n). Species or taxonomic groups with only value were not included in the interspecific comparisons (statistical analyses). Different superscript letters represent significant differences ( $P < 0.05$ ), among species or orders (lowercase) and among tissues (capital letters)..... **16**

**Figure 3.9:** Median, 25 and 75% percentil, minimum, maximum, outliers and extremes of zinc (Zn) concentrations ( $\mu\text{g g}^{-1}$  dw) in cephalopods. A) in the digestive gland of the studied cranchiids (present study). B) in the digestive gland of all studied cranchiids (in the overall) and other cephalopod groups. C) in the mantle of the studied cranchiids (present study). D) in the mantle of all studied cranchiids (in the overall) and other cephalopod groups. E) in the tentacles of the studied cranchiids (present study). F) in the tentacles of all studied cranchiids (in the overall) and other cephalopod groups. Numbers in brackets represent the sample size (n). Species or taxonomic groups with only value were not included in the interspecific comparisons (statistical analyses). Different superscript letters represent significant differences ( $P < 0.05$ ), among species or orders (lowercase) and among tissues (capital letters)..... **Erro! Marcador não definido.18**

**Figure 3.10:** Median, 25 and 75% percentil, minimum, maximum, outliers and extremes of arsenic (As) concentrations ( $\mu\text{g g}^{-1}$  dw) in cephalopods. A) in the digestive gland of the studied cranchiids (present study). B) in the digestive gland of all studied cranchiids (in the overall) and other cephalopod groups. C) in the mantle of the studied cranchiids (present study). D) in the mantle of all studied cranchiids (in the overall) and other cephalopod groups. E) in the tentacles of the studied cranchiids (present study). F) in the tentacles of all studied cranchiids (in the overall) and other cephalopod groups. Numbers in brackets represent the sample size (n). Species or taxonomic groups with only value were not included in the interspecific comparisons (statistical analyses). Different superscript letters represent significant differences ( $P < 0.05$ ), among species or orders (lowercase) and among tissues (capital letters). ..... **20**

**Figure 3.11:** Median, 25 and 75% percentil, minimum, maximum, outliers and extremes of selenium (Se) concentrations ( $\mu\text{g g}^{-1}$  dw) in cephalopods. A) in the digestive gland of the studied cranchiids (present study). B) in the digestive gland of all studied cranchiids (in the overall) and other cephalopod groups. C) in the mantle of the studied cranchiids (present study). D) in the mantle of all studied cranchiids (in the overall) and other cephalopod groups. Numbers in brackets represent the sample size (n). Species or taxonomic groups with only value were not included in the interspecific comparisons (statistical analyses). Different superscript letters represent significant differences ( $P < 0.05$ ), among species or orders (lowercase)..... **22**

**Figure 3.12:** Median, 25 and 75% percentil, minimum, maximum, outliers and extremes of cadmium (Cd) concentrations ( $\mu\text{g g}^{-1}$  dw) in cephalopods. A) in the digestive gland of the studied cranchiids (present study). B) in the digestive gland of all studied cranchiids (in the overall) and other cephalopod groups. C) in the mantle of the studied cranchiids (present study). D) in the mantle of all studied cranchiids (in the overall) and other cephalopod groups. E) in the tentacles of the studied

cranchiids (present study). F) in the tentacles of all studied cranchiids (in the overall) and other cephalopod groups. Numbers in brackets represent the sample size (n). Species or taxonomic groups with only value were not included in the interspecific comparisons (statistical analyses). Different superscript letters represent significant differences ( $P < 0.05$ ), among species or orders (lowercase) and among tissues (capital letters)..... **24**

**Figure 3.13:** Median, 25 and 75% percentil, minimum, maximum, outliers and extremes of lead (Pb) concentrations ( $\mu\text{g g}^{-1}$  dw) in cephalopods. A) in the digestive gland of the studied cranchiids (present study). B) in the digestive gland of all studied cranchiids (in the overall) and other cephalopod groups. C) in the mantle of the studied cranchiids (present study). D) in the mantle of all studied cranchiids (in the overall) and other cephalopod groups. E) in the tentacles of the studied cranchiids (present study). F) in the tentacles of all studied cranchiids (in the overall) and other cephalopod groups. Numbers in brackets represent the sample size (n). Species or taxonomic groups with only value were not included in the interspecific comparisons (statistical analyses). Different superscript letters represent significant differences ( $P < 0.05$ ), among species or orders (lowercase) and among tissues (capital letters). ..... **26**

**Figure 3.14:** Principal component analyses of Cu, Zn, Cd and Pb in the digestive gland of cephalopods orders..... **27**

**Figure 3.15:** Principal component analyses of Cu, Zn, Cd and Pb in the mantle of cephalopods orders.....**28**

**Figure 3.16:** Principal component analyses of Cu, Zn and Cd in the tentacles of cephalopods orders.....**28**

## **List of abbreviations, acronyms and symbols**

OMZ – Oxygen minimum zone

ETNA – Eastern tropical North Atlantic

AAIW – Atlantic Central Water and Antarctic Intermediate Water

NACW – North Atlantic Central Water

SACW – South Atlantic Central Water

V – Vanadium

Cr – Chromium

Co – Cobalt

Ni – Nickel

Cu – Copper

Zn – Zinc

As – Arsenic

Se – Selenium

Cd – Cadmium

Pb – Lead

EEZ – Exclusive economic zone

MOCNESS – Multiple Opening and Closing Net, with an Environmental Sensing System

IKMT – Isaac Kid Midwater

ICP-MS – Inductively coupled plasma mass spectrometry

Dw – Dry weight

PCA – Principal component analyses

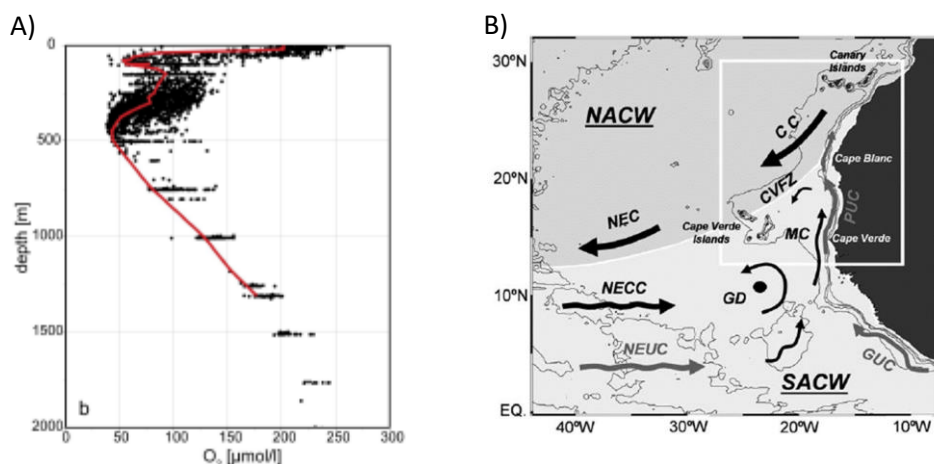
DML - Dorsal mantle length

# 1. INTRODUCTION

## 1.1. Deep-sea and oxygen minimum zones (OMZs)

The ocean is the largest habitat on earth, constituting nearly three-quarters of the earth's surface (Warrant and Locket, 2004). Most of it lies beyond the shallow margins of the continental shelves, with an average depth of 3800 Km (Gage and Tyler, 1991). Accommodating around 1 368 million Km<sup>3</sup> of water, the world's oceans provide a living space several times wider than terrestrial habitats, including airspace and countryside. Although most marine biota live in the shallower depths, where there is light, a remarkable variety also lives in the vast deep, where darkness prevail (Warrant and Locket, 2004). Trace elements are persistent and have been detected in a wide range of environments (Subotić et al., 2013; Bai et al., 2015), including in the deep-sea, where the concern is increasing, as it might act as a global sink for them (Turekian and Imbrie, 1966; Chester and Messiha-Hanna, 1970; Cronin et al., 1998; Kress et al., 1998; Mormede and Davies, 2003).

In some deep-sea habitats, oxygen deficiency is permanent (Levin, 2003). In fact, oxygen minimum zones (OMZ's) can be found at intermediate depths in most of the world' ocean (Stramma et al., 2008), from shelf to upper bathyal zones (10–1300 m) (Levin, 2003). OMZ's are pelagic habitats with stable conditions of persistent low oxygen level and low temperature at intermediate depths over vast areas. These habitats result from a combination of slow ocean ventilation, stagnant circulation and enhanced respiration (Stramma et al., 2008). Most OMZ's exhibit an identical oxygen profile, yet the oxygen levels, OMZ thickness and the depth of occurrence may vary regionally. Generally, the vertical profile of the dissolved oxygen concentration shows a sharp drop in oxygen from the surface to the upper boundary. Beneath there is a zone of continuous low oxygen. The lower OMZ boundary shows a steadier increase in oxygen levels with water depth. The thickness is determined by circulation and oxygen content of the region (Levin, 2003). As one moves horizontally from the centre of these regions to the outside, the thickness of the layer reduces and the minimum quantity of oxygen level rises, since the exchange of oxygen is better (Childress and Seibel, 1998). In the eastern tropical North Atlantic (ETNA), at a range depth range of 200m to 800m exists an oxygen minimum zone located between the Equatorial Current system in the south and the North Equatorial Current in the north (Stramma et al., 2008). Here, the oxygen profile has two minima at around 70m and 400m depth. The shallowest minimum is more prominent between Senegal and the Cape Verde Island. The deeper minimum is more notorious south Cape Verde (Ryabenko et al., 2012).



**Figure 1.1:** A) Oxygen profile in the eastern tropical North Atlantic (ETNA), with two minima at around 70m and 400m depth (Ryabenko et al., 2012); B) Schematic pattern of the circulation showing the main currents and dynamic features: Canary Current (CC), North Equatorial Current (NEC), North Equatorial Counter Current (NECC), North Equatorial Under Current (NEUC), Poleward Undercurrent (PUC), Mauritania Current (MC), Guinea Undercurrent (GUC), Guinea Dome (GD) and Cape Verde Frontal Zone (CVFZ) (Peña-Izquierdo et al., 2012).

The North Atlantic OMZ is composed by Atlantic Central Water and Antarctic Intermediate Water (AAIW). In the eastern tropical Atlantic Ocean, there are two types of Central water, North and South Atlantic Central Water (NACW and SACW, respectively), with Cape Verde Islands separating them. There is a slop boundary between NACW and SACW rising from south to north, with SACW lying on top of NACW. Therefore, near Cape Verde Islands, the lower OMZ is more influenced by NACW than in the upper OMZ layers. There are also differences in oxygen concentration, mainly because the ventilation is weaker at 300 to 600m depth when compared to the strong oxygen supply at 150 to 300 m depth (Stramma et al., 2016). The ETNA OMZ has the lowest oxygen values south and east of the Cape Verde Islands, near the African continent, being a hypoxic OMZ with oxygen concentrations dropping below  $\sim 60$  to  $120 \mu\text{mol kg}^{-1}$  (Stramma et al., 2008, 2016)

## 1.2. Adaptations to life in OMZs

The deep-sea ecosystems present the animals with major challenges that require specific adaptations to different adversities. Some of these challenges are: high hydrostatic pressure, low temperature and light and scarce food supply (dependent on parcels of nutrients from above) (Somero, 1992). In OMZs, these animals are presented with one more challenge – low oxygen levels. In general, the animals who live in these conditions have common adaptations like mechanisms with highly effective oxygen removal; for example, several species of OMZ-living fish and cephalopod species have larger and more developed gills which increase the respiratory surface and diffusion capacity of the oxygen (Seibel et al., 1997; Childress and Seibel, 1998). Moreover, cephalopods have a hemocyanin with higher affinity for oxygen relative to more active squids (Seibel and Childress, 2000). In general, OMZ animals also have the ability to regulate their routine oxygen consumption rates, to the lowest oxygen level that they found in the ocean. Also, the metabolic rates of pelagic fishes, crustaceans and cephalopods is much lower than those of surface-living species (even when the temperature is taken is account) (Childress and Seibel, 1998; Seibel and Childress, 2000). However, low metabolism is not a specific adaptation to the low oxygen concentrations. Other animals living at similar depths but with higher oxygen levels, also have low metabolic rates, although being less tolerant to hypoxia (Seibel and Childress, 2000).

Another option to survive in these conditions is vertical migrations, where the individuals move to areas with better conditions during a period of time (for example to eat) and then go back to the OMZ were they reduce their levels of activity, “lethargy” which can translate to lower metabolic rates (Rosa and Seibel, 2008). All these mechanisms of adaptation have different degrees, depending on the complexity of the animal and how severe are the habitat conditions (temperature, downwelling, light, pressure and zooplankton biomass) (Childress and Seibel, 1998). Another characteristic found in deep-sea fishes and cephalopods is the well-developed eyes and the bioluminescent displays, that are used to attract prey or confuse predators (Seibel et al., 2000).

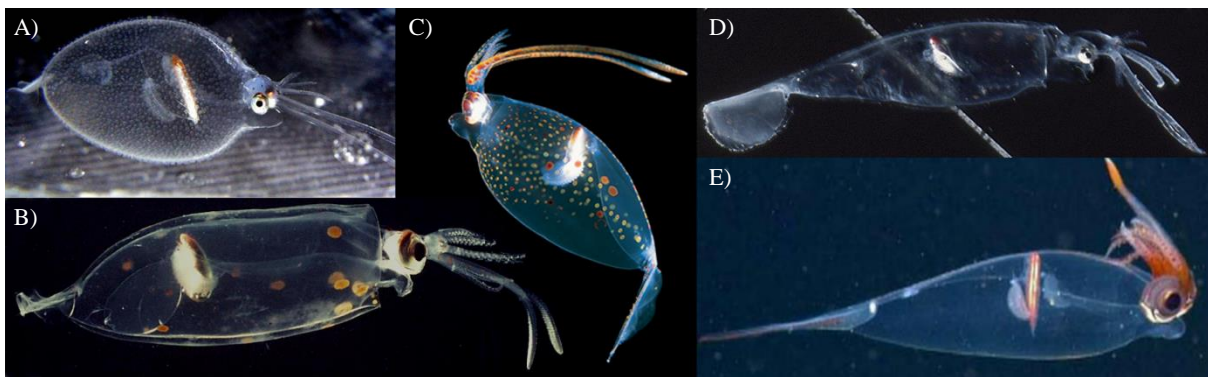
## 1.3. Cephalopods

Cephalopods (class Cephalopoda) are the most complex group in the Mollusca phylum, including exclusive marine animals. They can be found in all the oceans of the world, with the exception of the Black Sea. Their distribution goes from the Artic sea to the Antarctic Ocean (Roper and Jereb, 2010), from shallow coastal waters to the deep sea, with some species living under extreme conditions such as near hydrothermal vents. They can be benthic (octopuses), nektobenthic (cuttlefishes), neritic and pelagic (mainly squids) (Bustamante et al., 2002a, 2006; Bustamante et al., 2008; Roper and Jereb, 2010), occupying a major niche in the trophic chains (Bustamante et al., 1998b). Cephalopods are opportunistic predators with high growth rates, a single breeding season and a shot life span (Jereb and Roper, 2010). In the cephalopods, we can find two types of movements, fin swimming and jet propulsion. The surface living cephalopods have the need for high speed movement, since their

predator/prey interactions are visually cued, and not sit-and-wait strategy that is prevalent in the deep-sea (Seibel et al., 1997). In opposition, the deep-sea cephalopods primary use fin swimming. This choice is mostly based in energy usage, since jet propulsion is more energy costly than fin swimming (Seibel et al., 2000). Because of the conditions these animals live in, their interactions are likely to take place over short distances, making strong locomotor abilities not a necessity (Seibel et al., 1997). Since they also do not have speed as a priority (sit-and-wait predation strategies are prevalent), the use of fin swimming reduces the cost of movement (Seibel et al., 2000). Cephalopods play a key role in many marine ecosystems, representing an essential link in marine trophic chains, since they are a primary food source for many marine predators such as fish, marine mammals or seabirds (Martin and Flegal, 1975; Paco Bustamante et al., 1998a; Bustamante et al., 2002; Panutrakul et al., 2007).

#### 1.4. Glass squids (cranchiids)

As in other cephalopods groups, salinity is considered the most limiting factor in squids' distribution. Their depth range extends from intertidal to over 5 000 m, with many oceanic species undergoing diel vertical migrations. The abundance of squids varies, depending on genera, habitat and season. They can live as isolated individuals, small schools with a few dozen individuals, or vast schools of neritic and oceanic species with millions of specimens (Roper and Jereb, 2010). The cranchiids are among the most numerous squids in the world. These planktonic squids belong to the Cranchiidae family, a pelagic family with a high degree of morphological diversity (Voss, 1980; Voss and Voss, 1983).



**Figure 1.2:** A) *Cranchia scabra* (copyright: 1998 Richard E. Young) ; B) *Megalocranchia fisheri* (copyright: Richard E. Young); C) *Teuthowenia pellucida* (copyright: Claire Nouvian) ; D) *Leachia pacifica* (copyright: Roger R. Seapy); E) *Taonius borealis* (copyright: Claire Nouvian).

A characteristic of this family is the fact that the mantle is fused to the funnel at its posterolateral corner along the acutely diverging lines (Voss and Voss, 1983). Besides the characteristics of the mantle, these squids have large coelomic cavities filled with neutrally buoyant ammoniacal fluid of low density. This fluid enables the squids to “soar” in the water column and to move using predominantly rapid flapping of small fins. Their morphologies and anatomy are well studied, yet despite the abundance, little is known about their biology (Arkhipkin, 1996). It’s suggested that tropical cranchiids spawn at great depth, and that after hatching, small paralarvae ascend to epipelagic waters. For the first four to five months of their ontogenesis they feed and grow quickly in the epipelagic waters, until they move into deep waters for maturation and then spawning (Arkhipkin, 1996; Voss et al., 1992). Most of the cranchiids species undergo ontogenetic changes in morphology, displaying also several forms of sexual dimorphism within the family. These changes can involve changing eye shape and position, fin shape, increased pigmentation, development of photophores on arm tips as well as various modifications of arm structure, including loss of tentacles (Voss, 1980). Many of the cranchiids species spend much of their life in waters where there’s almost no light or in complete darkness. Most of these small animals don’t rely on speed to escape predator, but in different defensive strategies, such as concealment. Cranchiids rely primarily on their transparency, which under proper lighting condition, many animals

are practically invisible. Yet, not everything can be made transparent. Some organs as the retina eye, the ink sac and the digestive gland appear opaque. In order to solve this problem, the cranchiids have developed ways to decrease the effect of this organs. The digestive gland is compacted into a narrow, conical visceral nucleus, which in turn, externally covered by a reflective layer. The eyes are reduced in cross-section and have a similar reflective layer. Besides these adaptations the cranchiids also have the ability to control the orientation of their body and eyes, so they can reduce the size of their own silhouette against surface illumination as well as their shadow (Seapy and Young, 1986; Seibel et al., 1997).

#### 1.5. Contaminants in cephalopods

Marine pollution has been an important issue during the last decades with possible impacts in habitat loss and biodiversity (Georgantelis et al., 2001). Elemental bioaccumulation has been greatly studied in many marine organisms and it has been shown that molluscs, such as cephalopods, have the ability to accumulate high levels of several elements, some essential to metabolic functions and some nonessential (Martin and Flegal, 1975; Finger and Smith, 1987; Georgantelis et al., 2001; Bustamante et al., 2006; Ahdy et al., 2007; Panutrakul et al., 2007; Raimundo et al., 2008, 2010c, 2014). The highest concentrations determined are carbon, hydrogen, nitrogen, oxygen and sulphur, which are structural elements. Essential elements, have known biological role, such as iron, copper, zinc, iodine, manganese, selenium or fluorine. Non-essential elements do not have a known role in the physiological functions, such as occurs with mercury, lead and cadmium (Lourenço et al., 2009) Although there are studies on element accumulation in cephalopods, most are focused in commercial species such as the cuttlefish *Sepia officinalis* (Miramand and Bentley, 1992), the octopuses *Octopus vulgaris* (Raimundo et al., 2004) and some other species of squids (Martin and Flegal, 1975). Among these species, high levels of metals have generally been recorded, such as cadmium, copper and zinc (Bustamante et al., 2000) making the cephalopods a potential threat for higher trophic levels (Bustamante et al., 2002b, 2006, 2008). Bioaccumulation processes are strongly influenced by metal availability (Raimundo et al., 2004). The uptake of trace elements by marine organisms can occur from water, including suspended particulate matter, food and sediments. Uptake via water can take place across the whole-body surface, in addition to the gills. Food may also be a significant source of metals to organisms, if not the primary source, (Bustamante et al., 2006, 2008; Raimundo et al., 2004). Other factors such as level of elements in the water, exposure period, temperature, size, sex, maturity stage and place of capture, may also be relevant, and lead to different metals accumulation in cephalopod tissues (Bustamante et al., 2006, 2008; Pernice et al., 2009; Raimundo et al., 2004, 2014). Because cephalopods display a short life span and high trace element accumulation, these characteristics may clearly reflect the ambient life conditions and proposed them as indicators of environmental contamination (Bustamante et al., 2000; Raimundo et al., 2014).

Although there is no information about trace elements in the cranchiids, there are information about other species of cephalopods including other oceanic squids. Since several elements are considered to be toxic for the biota, they would have to evolve to counteract the possible toxicity. The cephalopods are able to grow and reproduce with very high metal concentrations. In their case, detoxification strategy involves storage mechanisms of these elements, which appears to be an efficient strategy (Finger and Smith, 1987; Bustamante et al., 2000;). The accumulation of higher levels of elements in the digestive gland, than the other tissues, suggests that most of the detoxification process occurs in this organ (Bustamante et al., 1998a, 2002a; Miramand et al., 2006; Murthy et al., 2008; Pernice et al., 2009; Raimundo et al., 2010a, 2014). This detoxification system allows for both essential and non-essential elements storage in a nontoxic form (Bustamante et al., 2002a, 2006; Raimundo et al., 2008; Pernice et al., 2009). A well-documented detoxification strategy implicates the association of metals to metallothioneins (known to be present in marine invertebrates), which plays an important role in the absorption, metabolism, homeostasis and storage of both essential and non-essential elements



(Raimundo et al., 2010a). Essential elements, like Zn and Cu, are regulated in organisms by homeostatic mechanisms, however non-essential metals, like Cd, may substitute them (Bustamante et al., 1998a; Raimundo et al., 2005). Most studies have highlighted the cephalopods ability to concentrate Zn, Cu and Cd in the digestive gland even in environments with low availability (Bustamante et al., 2002b; Raimundo et al., 2005; Pernice et al., 2009).

Based on all this, the aim of the present dissertation was to determined, for the first time, the concentrations of Vanadium (V), Chromium (Cr), Cobalt (Co), Nickel (Ni), Cooper (Cu), Zinc (Zn), Arsenic (As), Selenium (Se), Cadmium (Cd) and Lead (Pb) in the digestive gland, mantle and tentacles of cranchiid squids (*Cranchia scabra*, *Galiteuthis armata*, *Helicocranchia pfefferi*, *Leachia atlantica*, *Leachia* sp., *Liocranchia reinhardtii*, *Megalocranchia oceanica*, *Teuthowenia maculate*, *Teuthowenia* sp.) from the oxygen minimum zone within Cape Verde Exclusive Economic Zone (EEZ). Moreover, a comprehensive comparison with trace elements obtained in other oceanic and shallow-living cephalopods species was conducted.

## 2. MATERIAL AND METHODS

### 2.1. Sampling and sample preparation

Cranchiids squids were collected in the Cape Verde EEZ, more specifically in eight different sampling stations (Figure 2.1) during an expedition of the Maria S. Merian Research/Survey Vessel, from November to December of 2015. The sampling was done with two MOCNESS net (1 – with a 1m<sup>2</sup> opening carrying three nets of 2 mm mesh size specifically for krill plus six nets of 335 µm for macrozooplankton and larger mesozooplankton; 10 – wich has a 10m<sup>2</sup> net opening and was equipped with five nets of 1.5 mm mesh size targeting micronekton) opening down to 1000 m, and IKMT net at 500 m. All specimens were identified and immediately frozen in liquid nitrogen on board, and then storage at -80°C for further analyses. In the lab, the individuals (comprising a total number of 9 species - *Cranchia scabra*, *Galiteuthis armata*, *Helicocranchia pfefferi*, *Leachia atlantica*, *Leachia* sp., *Liocranchia reinhardtii*, *Megalocranchia oceanica*, *Teuthowenia maculate*, *Teuthowenia* sp.; and a total of 63 specimens and all smaller than 50mm; see detail information in the annex A, table A1) were dissected to separate in mantle, tentacles and digestive gland; and then all samples were freeze-dried.

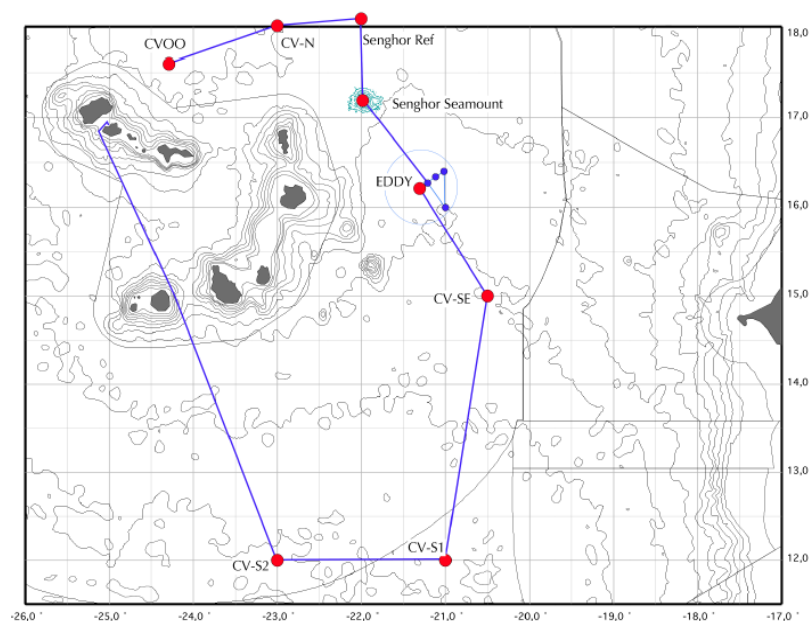


Figure 2.3: Map of the sampling stations

## 2.2. Trace elements concentrations

To determine the trace elements in the different tissues of the cranchiid squids, the samples were digested with distilled HNO<sub>3</sub> (65% v/v) and H<sub>2</sub>O<sub>2</sub> (30% v/v) in a Teflon bomb at different temperatures, according to the method described in Ferreira et al (1990). After the digestion, the samples were measured to 10ml (tentacles and mantle) and 15ml (digestive gland) with Milli-Q water. All lab ware was cleaned with HNO<sub>3</sub> (20%) for two days and rinsed with Milli-Q water to avoid contamination.

Procedural blanks were prepared using the same analytical procedure and reagents, accounting for less than 1% of the total metal in samples. The accuracy of the analytical methods was assessed by the analysis of certified reference material, DORM-4 (fish protein), 1566a (oyster tissue), DOLT-2 (dogfish Liver) and TORT-2 (lobster hepatopancreas), Certified reference materials and blanks were run together with samples. Obtained and certified values were not statistically different ( $p > 0.05$ ). The concentrations of V, Cr, Co, Ni, Cu, Zn, As, Se, Cd and Pb were determined by quadrupole ICP-MS (Thermo Elemental, X-Series). Trace elements detection limits were 0.076  $\mu\text{g g}^{-1}$  for V, 0.16  $\mu\text{g g}^{-1}$  for Cr, 0.033  $\mu\text{g g}^{-1}$  for Co, 0.12  $\mu\text{g g}^{-1}$  for Ni, 0.35  $\mu\text{g g}^{-1}$  for Cu, 0.0078  $\mu\text{g g}^{-1}$  for Zn, 0.057  $\mu\text{g g}^{-1}$  for As, 0.23  $\mu\text{g g}^{-1}$  for Se, 0.012  $\mu\text{g g}^{-1}$  for Cd and 0.010  $\mu\text{g g}^{-1}$  for Pb. All the results are given as medians and ranges in microgram per gram of tissue dry weight ( $\mu\text{g g}^{-1}$  dw). It's worth noting that the number of samples analyzed per tissue (always summarized within brackets in the figures) vary considerable due those detection limits.

## 2.3. Statistical analyses

Kruskal–Wallis tests were applied to the data in order to detect differences between the trace elements among cranchiid species. When differences were found the Dunn test was applied to verify where the differences were (using the software R version 3.3.2). To investigate possible differences among other cephalopod groups (see annex C, tables C1, C2 and C3, for digestive gland, mantle and tentacle data respectively) a humidity factor of 1.25 (equivalent to 80% of humidity (Bustamante et al., 2006; Osman et al., 2014) was used to uniformize such data. Principal component analyses (PCA) was also applied in cranchiid and other cephalopods tissues using the more studied trace elements found in the literature (namely Cu, Zn, Cd and Pb in the digestive gland and mantle, and Cu, Zn and Cd in the tentacles). PCAs were performed using the STATISTICA 6.0 Statistical Software System.

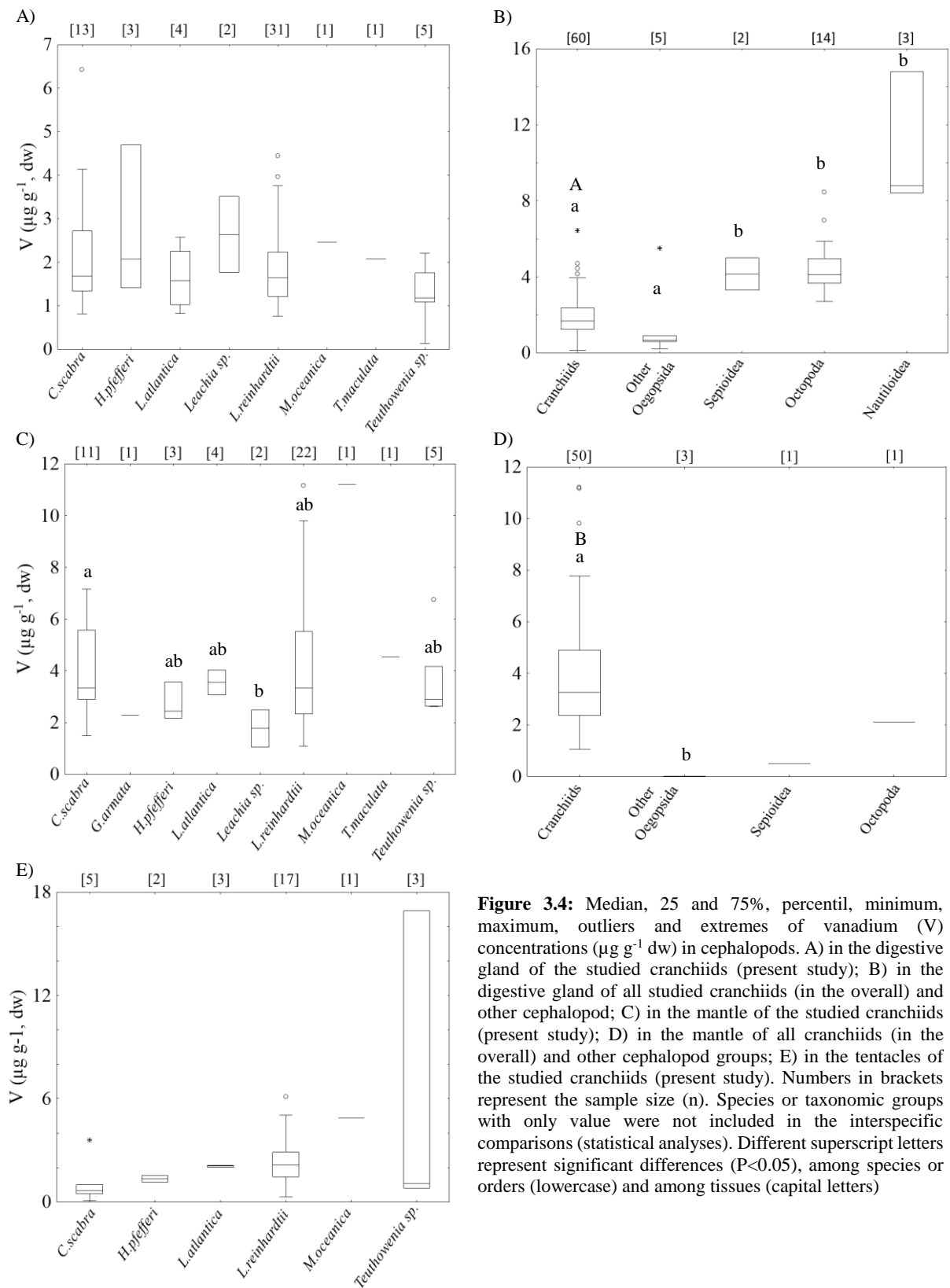
## 3. RESULTS

The figures 3.4 to 3.13 show the concentrations of the analyzed elements (V, Cr, Co, Ni, Cu, Zn, As, Se, Cd, Pb, respectively) in the digestive gland, mantle and tentacles of the studied cranchiid species (panels A, C and E, respectively) and of the other oceanic and shallow-living counterparts (panels B, D and F). Trace elements in the digestive gland ranged between 0.070 and 17  $\mu\text{g g}^{-1}$  dw, with the following average concentrations in descending order: Zn > Cd > As > Cu > V > Se > Co > Cr > Ni > Pb. In the mantle ranged between 0.070 and 28  $\mu\text{g g}^{-1}$  dw, with the following average concentrations in descending order: Zn > Cu > Cr > As > V > Ni > Se > Cd > Pb > Co. In the tentacles, trace elements ranged between 0.13 and 24  $\mu\text{g g}^{-1}$  dw, with the following average concentrations in descending order: Se > Zn > As > Cu > Cr > Ni > V > Cd > Co > Pb.

### 3.1. Vanadium (V)

Regarding V concentrations in the digestive gland, there were no significant differences among cranchiid species (Figure 3.4A; KW test, H: 5.83,  $p > 0.05$ ), with median values varying between  $1.2 \mu\text{g g}^{-1}$  in *Teuthowenia* sp. and  $2.6 \mu\text{g g}^{-1}$  in *Leachia* sp. In the overall, with total median value of  $1.68 \mu\text{g g}^{-1}$ , cranchiid concentrations were significantly lower than nautilus, octopods and cuttlefish, but similar to those of other oegopsid squids (Figure 3.4B; KW test, H: 35.83,  $p < 0.05$ ). In the mantle, with median values varying between  $1.8 \mu\text{g g}^{-1}$  in *Leachia* sp. and  $11. \mu\text{g g}^{-1}$  in *Megalocranchia oceanica*, there were some significant differences among cranchiids (Figure 3.4C; KW test, H: 8.09,  $p < 0.05$ ), with *Cranchia scabra* showing higher V concentrations than *Leachia* sp ( $p < 0.05$ , Dunn test). Cranchiid concentrations (with a total median value of  $3.3 \mu\text{g g}^{-1}$  dw), were significantly higher than other oegopsid squids (Figure 3.4D; KW test, H: 12.14,  $p < 0.05$ ). In the tentacles, there were no significant differences among cranchiids (Figure 3.4E; KW test, H: 6.4243,  $p > 0.05$ ), with median values varying between  $0.67 \mu\text{g g}^{-1}$  in *Cranchia scabra* and  $4.87 \mu\text{g g}^{-1}$  in *Megalocranchia oceanica*.

Regarding differences among tissues, although with some species-specific variations, in the overall the V concentrations were significantly higher in the mantle than in the digestive gland and tentacles (KW test, H: 34.64,  $p < 0.05$ ). The levels in the latter two tissues were quite similar.

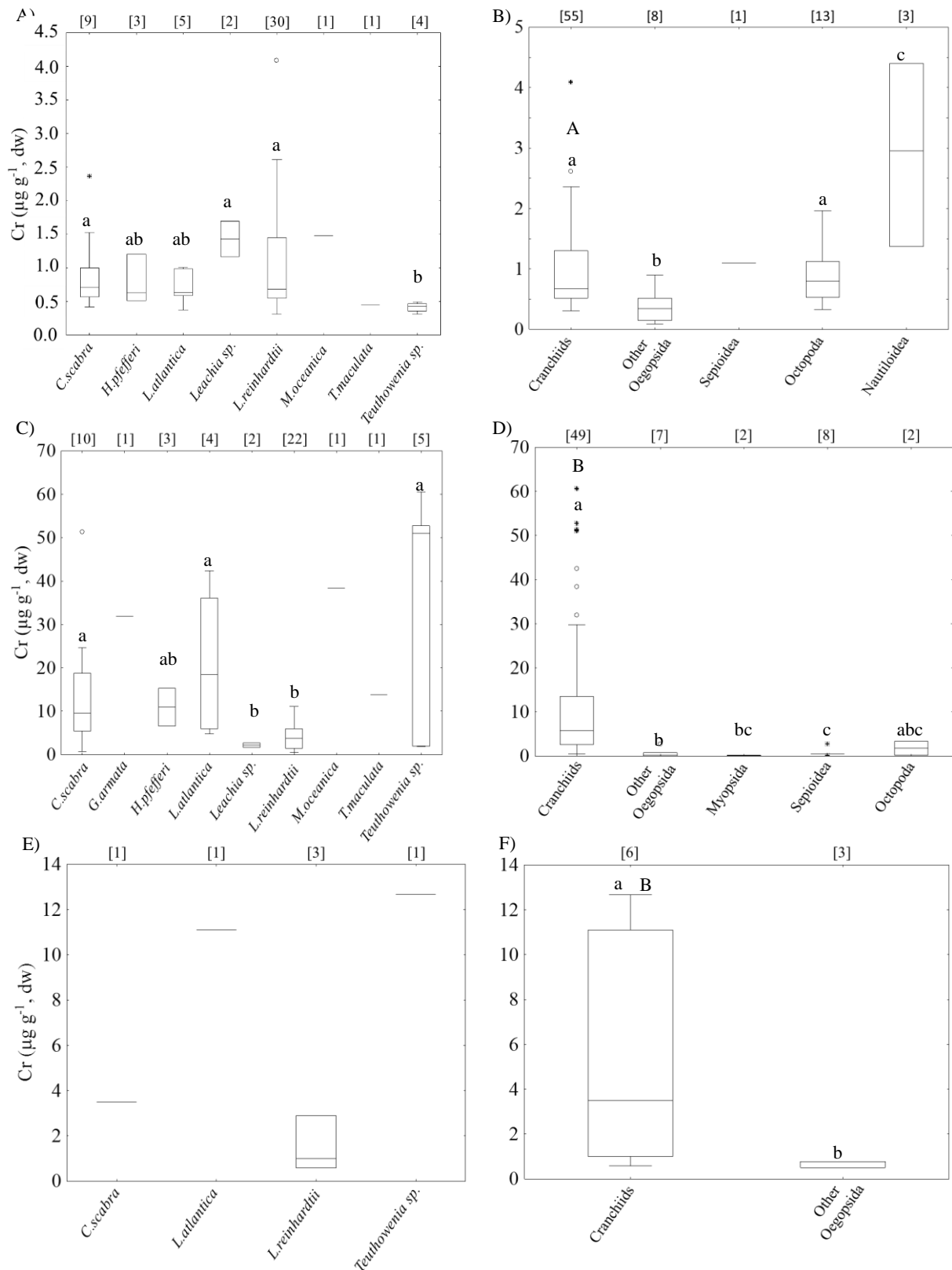


**Figure 3.4:** Median, 25 and 75%, percentil, minimum, maximum, outliers and extremes of vanadium (V) concentrations ( $\mu\text{g g}^{-1}$  dw) in cephalopods. A) in the digestive gland of the studied cranchiids (present study); B) in the digestive gland of all studied cranchiids (in the overall) and other cephalopod; C) in the mantle of the studied cranchiids (present study); D) in the mantle of all cranchiids (in the overall) and other cephalopod groups; E) in the tentacles of the studied cranchiids (present study). Numbers in brackets represent the sample size (n). Species or taxonomic groups with only value were not included in the interspecific comparisons (statistical analyses). Different superscript letters represent significant differences ( $P < 0.05$ ), among species or orders (lowercase) and among tissues (capital letters)

### 3.2. Chromium (Cr)

The median values of Cr concentration in the cranchiids digestive gland varied between  $0.43 \mu\text{g g}^{-1}$  in *Teuthowenia* sp. and  $1.5 \mu\text{g g}^{-1}$  in *Megalocranchia oceanica*. There were significant differences among cranchiid species (Figure 3.5A; KW test, H: 10.79,  $p < 0.05$ ), with *Teuthowenia* sp. presenting the lowest values in comparison to *Cranchia scabra*, *Leachia* sp. and *Liocranchia reinhardtii* ( $p < 0.05$ , Dunn test). Cranchiids concentrations were significantly lower (total median value of  $0.66 \mu\text{g g}^{-1}$ ) than those observed in nautilus, slightly higher than those from other oegopsids squids, but similar to octopods (figure 3.5B; KW test, H: 15.92,  $p < 0.05$ ). There were also significant differences among cranchiids in the mantle tissue (Figure 3.5C; KW test, H: 16.68,  $p < 0.05$ ), with median values varying between  $2.2 \mu\text{g g}^{-1}$  in *Leachia* sp. and  $51 \mu\text{g g}^{-1}$  in *Teuthowenia* sp. The total median value of cranchiid concentrations was  $5.8 \mu\text{g g}^{-1}$ , which was significantly higher than those from all the other orders (figure 3.5D; KW test, H: 33.52,  $p < 0.05$ ). In the tentacles, most samples analyzed showed levels below the detection limit; for the other, the median values varied between  $1.00 \mu\text{g g}^{-1}$  in *Liocranchia reinhardtii* and  $13 \mu\text{g g}^{-1}$  in *Teuthowenia* sp. In the overall, cranchiid concentrations (total median value of  $3.2 \mu\text{g g}^{-1}$ ) were significantly higher than those observed for other oegopsids squids (Figure 3.5F; KW test, H: 3.27,  $p < 0.05$ ).

Regarding elemental partitioning, although species-specific, Cr concentrations were significantly lower in the digestive than in mantle and tentacles. No statistical differences were observed between muscle tissues (KW test, H: 58.52,  $p < 0.05$ ).

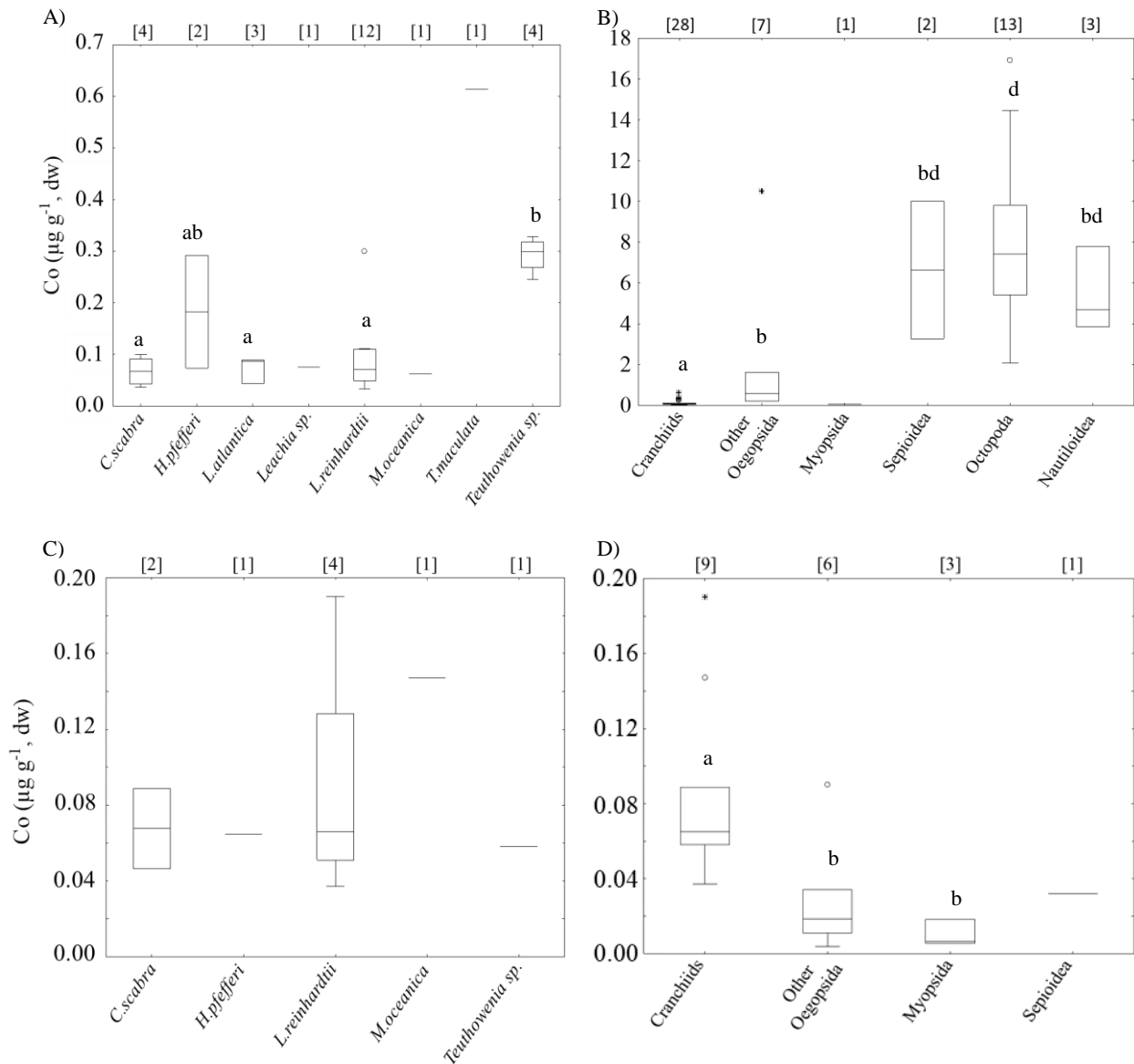


**Figure 3.5:** Median, 25 and 75 percentil, minimum, maximum, outliers and extremes of chromium (Cr) concentrations ( $\mu\text{g g}^{-1}$  dw) in cephalopods. A) in the digestive gland of the studied cranchiids (present study). B) in the digestive gland of all studied cranchiids (in the overall) and other cephalopod groups. C) in the mantle of the studied cranchiids (present study). D) in the mantle of all studied cranchiids (in the overall) and other cephalopod groups. E) in the tentacles of the studied cranchiids (present study). F) in the tentacles of all studied cranchiids (in the overall) and other cephalopod groups. Numbers in brackets represent the sample size (n). Species or taxonomic groups with only value were not included in the interspecific comparisons (statistical analyses). Different superscript letters represent significant differences ( $P < 0.05$ ), among species or orders (lowercase) and among tissues (capital letters).

### 3.3. Cobalt (Co)

In the digestive gland, Cobalt median values varied between  $0.060 \mu\text{g g}^{-1}$  in *Megalocranchia oceanica* and  $0.61 \mu\text{g g}^{-1}$  in *Teuthowenia maculata*. When comparing the species, there were significant differences among cranchiids (Figure 3.6A; KW test, H: 11.84,  $p < 0.05$ ), with *Teuthowenia* sp. showing higher Co concentrations than *Cranchia scabra*, *Leachia atlantica* and *Liocranchia reinhardtii* ( $p < 0.05$ , Dunn test). Significantly lower median levels of Co were registered in cranchiid ( $0.81 \mu\text{g g}^{-1}$ ) in comparison with nautilus, octopods and cuttlefish, and slightly lower than in other oegopsids (Figure 3.6B; KW test, H: 39.16,  $p < 0.05$ ). In the mantle, there were no significant differences among cranchiids (Figure 3.6C; KW test, H: 1.97,  $p > 0.05$ ), with median values ranging between  $0.060 \mu\text{g g}^{-1}$  in *Teuthowenia* sp. and  $0.15 \mu\text{g g}^{-1}$  in *Megalocranchia oceanica*. Among the cephalopod species, cranchiid concentrations (with a total median value of  $0.070 \mu\text{g g}^{-1}$ ) were significantly higher than those from the other orders (Figure 3.6D; KW test, H: 10.51,  $p < 0.05$ ). Only two species of cranchiids presented Co concentrations in the tentacles above the detection limit, and only one individual per each, *Liocranchia reinhardtii* ( $0.053 \mu\text{g g}^{-1}$ ) and *Megalocranchia oceanica* ( $0.16 \mu\text{g g}^{-1}$ ).

Regarding Co concentrations in the tissues no significant differences (KW test, H: 0.94,  $p > 0.05$ ) were obtained.



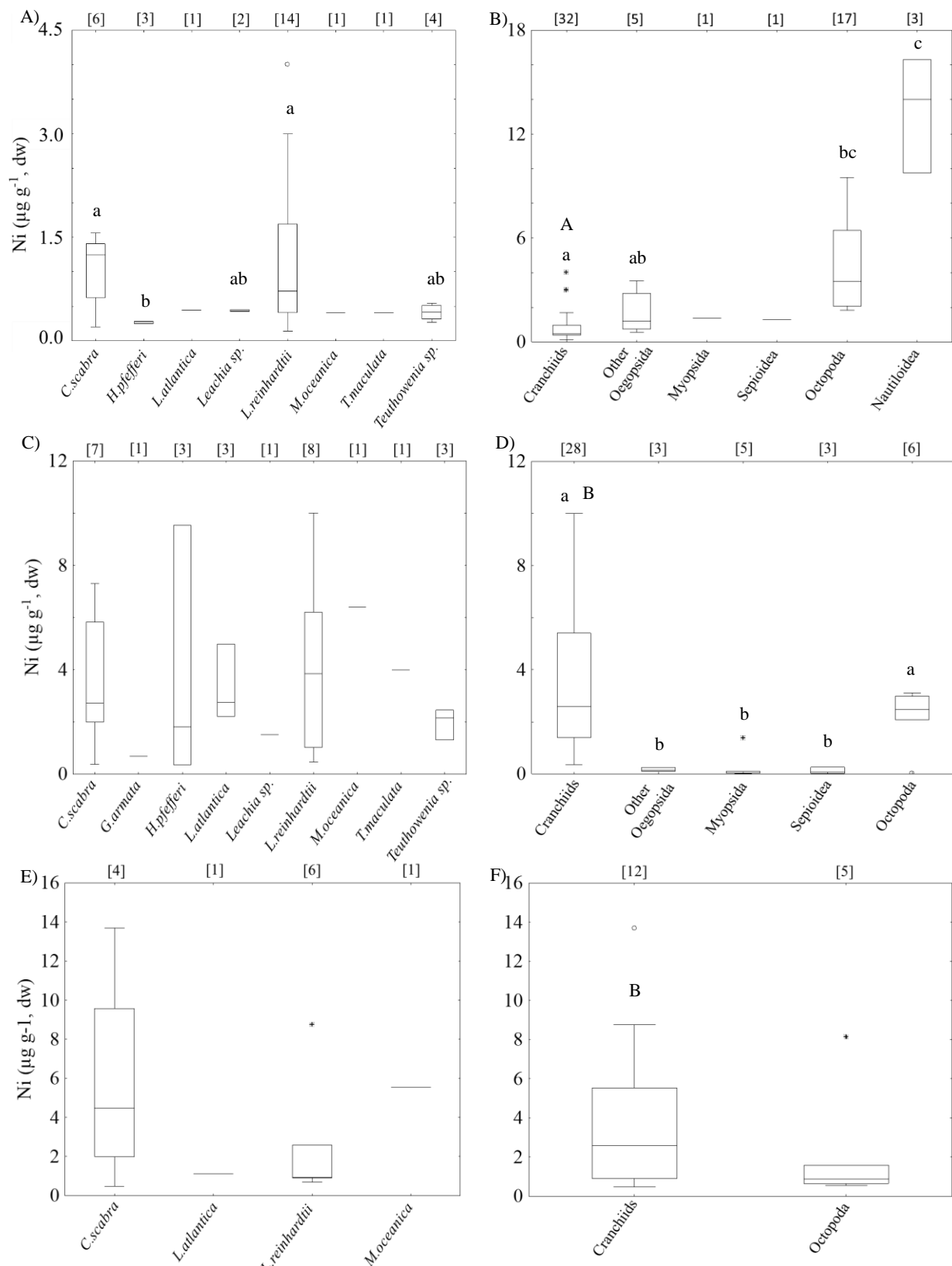
**Figure 3.6:** Median, 25 and 75% percentil, minimum, maximum, outliers and extremes of cobalt (Co) concentrations ( $\mu\text{g g}^{-1}$  dw) in cephalopods. A) in the digestive gland of the studied cranchiids (present study). B) in the digestive gland of all studied cranchiids (in the overall) and other cephalopod groups. C) in the mantle of the studied cranchiids (present study). D) in the mantle of all studied cranchiids (in the overall) and other cephalopod groups. Numbers in brackets represent the sample size (n). Species or taxonomic groups with only value were not included in the interspecific comparisons (statistical analyses). Different superscript letters represent significant differences ( $P < 0.05$ ) among species or orders (lowercase).



### 3.4. Nickel (Ni)

Regarding digestive gland Ni concentrations, the median values varied between  $0.26 \mu\text{g g}^{-1}$  in *Helicocranchia pfefferi* and  $1.2 \mu\text{g g}^{-1}$  in *Cranchia scabra*. Significant differences were obtained among cranchiid species (Figure 3.7A; KW test, H: 11.84,  $p < 0.05$ ), with *Helicocranchia pfefferi* showing lower concentrations than *Cranchia scabra* and *Liocranchia reinhardtii* ( $p < 0.05$ , Dunn test). In the overall, with total median value of  $0.47 \mu\text{g g}^{-1}$ , cranchiid concentrations were significantly lower than nautilus and octopods, but is similar to those of other oegopsids (Figure 3.7B; KW test, H: 37.08,  $p < 0.05$ ). No significant differences were registered in the mantle of the various cranchiids (Figure 3.7C; KW test, H: 5.37,  $p > 0.05$ ), with median values varying between  $0.68 \mu\text{g g}^{-1}$  in *Helicocranchia pfefferi* and  $6.4 \mu\text{g g}^{-1}$  in *Megalocranchia oceanica*. In the overall, with total median value of  $2.6 \mu\text{g g}^{-1}$ , cranchiid concentrations were significantly higher than other oegopsids and myopsids squids and cuttlefish, but is similar to those of octopus (Figure 3.7D; KW test, H: 21.08,  $p < 0.05$ ). In the tentacles, there were no significant differences among cranchiids (Figure 3.7E; KW test, H: 1.57,  $p > 0.05$ ), with median values varying between  $0.94 \mu\text{g g}^{-1}$  in *Liocranchia reinhardtii* and  $5.5 \mu\text{g g}^{-1}$  in *Megalocranchia oceanica*. With a total median value of  $2.6 \mu\text{g g}^{-1}$ , cranchiid concentrations were similar to those in octopuses (Figure 3.7F; KW test, H: 1.16,  $p > 0.05$ ).

Regarding differences among tissues, although with some species-specific variations, in the overall, the Ni concentrations in the digestive gland were significantly lower concentration than those in the mantle and tentacles (KW test, H: 26.02,  $p < 0.05$ ). The levels in the latter two tissues were quite similar.



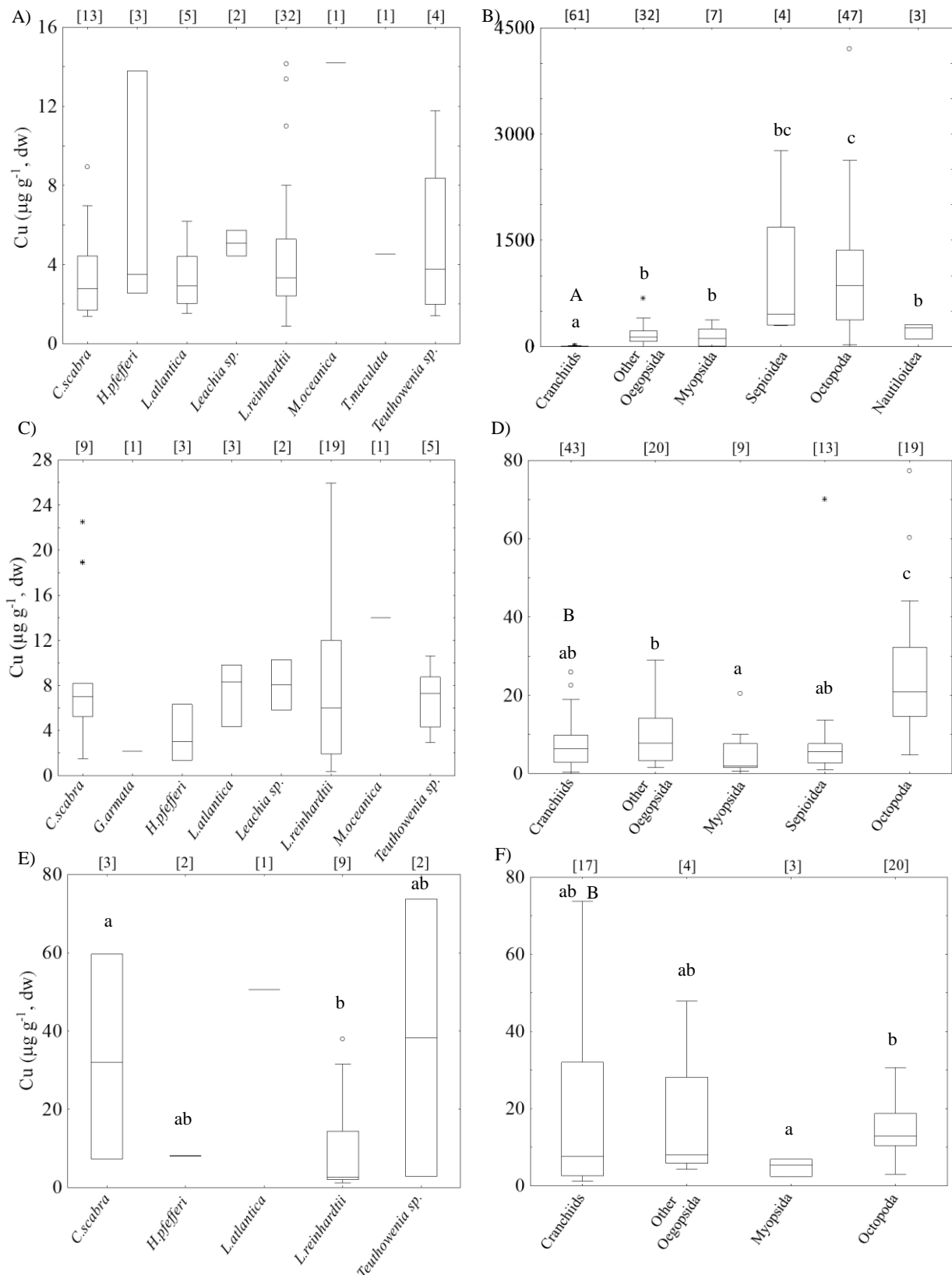
**Figure 3.7:** Median, 25 and 75% percentil, minimum, maximum, outliers and extremes of nickel (Ni) concentrations ( $\mu\text{g g}^{-1}$  dw) in cephalopods. A) in the digestive gland of the studied cranchiids (present study). B) in the digestive gland of all studied cranchiids (in the overall) and other cephalopod groups. C) in the mantle of the studied cranchiids (present study). D) in the mantle of all studied cranchiids (in the overall) and other cephalopod groups. E) in the tentacles of the studied cranchiids (present study). F) in the tentacles of all studied cranchiids (in the overall) and other cephalopod group. Numbers in brackets represent the sample size (n). Species or taxonomic groups with only value were not included in the interspecific comparisons (statistical analyses). Different superscript letters represent significant differences ( $P < 0.05$ ), among species or orders (lowercase) and among tissues (capital letters).

### 3.5. Copper (Cu)

Regarding digestive gland Cu concentrations, there were no significant differences among cranchiid species (Figure 3.8A; KW test, H: 6.70,  $p > 0.05$ ), with median values ranging between  $2.8 \mu\text{g g}^{-1}$  in *Cranchia scabra* and  $14 \mu\text{g g}^{-1}$  in *Megalocranchia oceanica*. Significantly lower Cu concentrations were observed in the cranchiids digestive gland (with a total median value of  $3.4 \mu\text{g g}^{-1}$ ) than those in the other orders (Figure 3.8B; KW test, H: 119.25,  $p < 0.05$ ). In the mantle, significant differences among cranchiids were detected (Figure 3.8C; KW test, H: 5.11,  $p > 0.05$ ), with median values varying between  $2.2 \mu\text{g g}^{-1}$  in *Galiteuthis armata* and  $14 \mu\text{g g}^{-1}$  in *Megalocranchia oceanica*. Copper concentration were slightly lower in cranchiids mantle (with a total median value of  $6.32 \mu\text{g g}^{-1}$ ) than in octopuses and other oegopsids squids, but similar to those of myopsids squids and cuttlefish (Figure 3.8D; KW test, H: 31.64,  $p < 0.05$ ). Copper concentrations in the tentacles present median values varying between  $2.6 \mu\text{g g}^{-1}$  in *Liocranchia reinhardtii* and  $50 \mu\text{g g}^{-1}$  in *Leachia atlantica*. Significant differences among cranchiids (Figure 8C; KW test, H: 5.34,  $p > 0.05$ ), with *Liocranchia reinhardtii* being different than *Cranchia scabra* ( $p < 0.05$ , Dunn test).

Copper concentrations determined in cranchiid tentacles (with a total median value of  $8.0 \mu\text{g g}^{-1}$ ) were similar to those described for other orders (Figure 3.8F; KW test, H: 5.38,  $p > 0.05$ ).

Although with some species-specific variations, in the overall Cu concentrations were significantly lower in the digestive gland than in the mantle and tentacles (KW test, H: 9.06,  $p < 0.05$ ). Similar levels were registered in both muscle tissues.

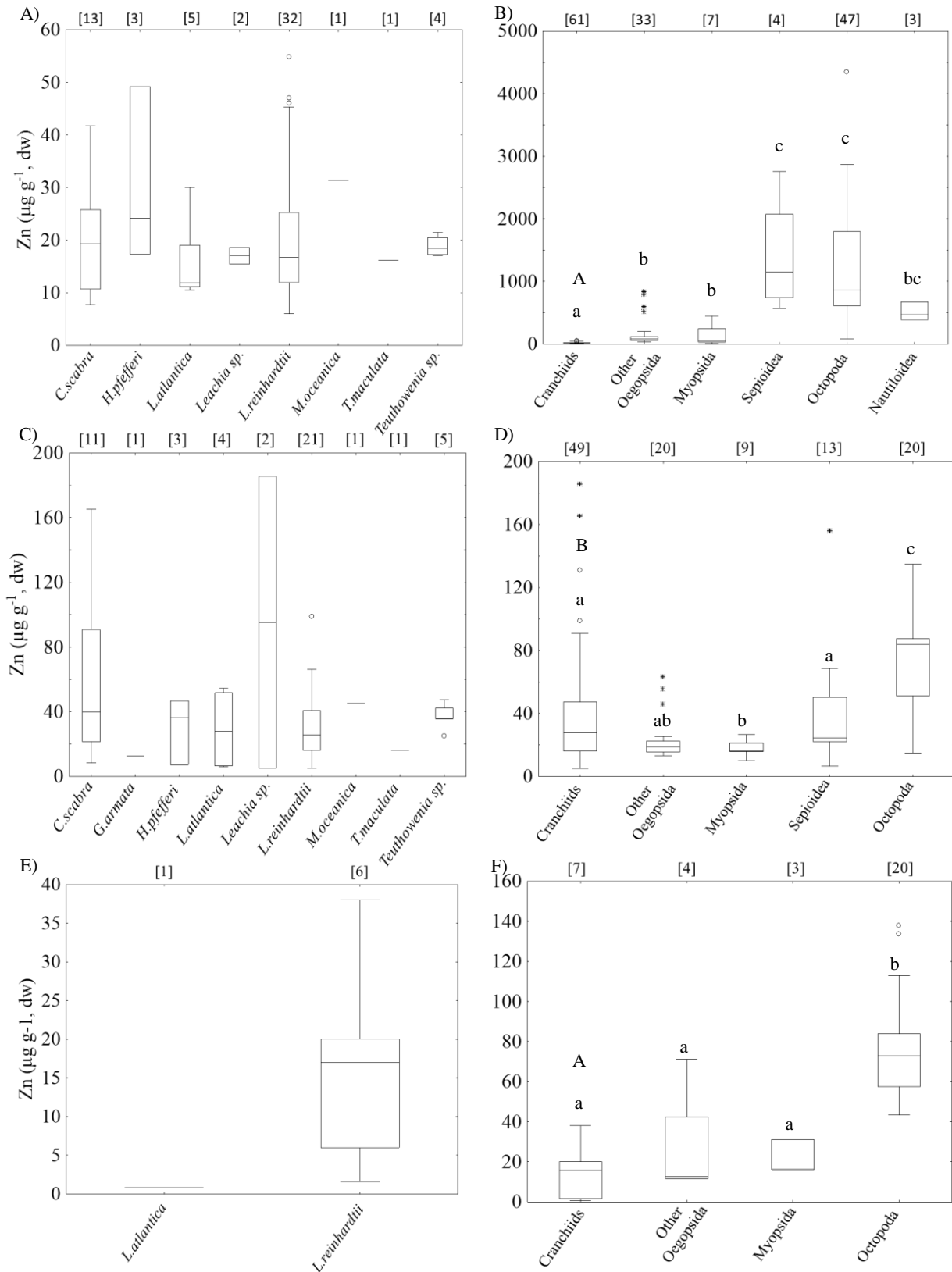


**Figure 3.8:** Median, 25 and 75% percentil, minimum, maximum, outliers and extremes of Copper (Cu) concentrations ( $\mu\text{g g}^{-1}$  dw) in cephalopods. A) in the digestive gland of the studied cranchiids (present study). B) in the digestive gland of all studied cranchiids (in the overall) and other cephalopod groups. C) in the mantle of the studied cranchiids (present study). D) in the mantle of all studied cranchiids (in the overall) and other cephalopod groups. E) in the tentacles of the studied cranchiids (present study). F) in the tentacles of all studied cranchiids (in the overall) and other cephalopod groups. Numbers in brackets represent the sample size (n). Species or taxonomic groups with only value were not included in the interspecific comparisons (statistical analyses). Different superscript letters represent significant differences ( $P < 0.05$ ), among species or orders (lowercase) and among tissues (capital letters).

### 3.6. Zinc (Zn)

There were no significant differences in the digestive gland zinc concentrations among cranchiid species (Figure 3.9A; KW test, H: 4.32,  $p > 0.05$ ), with median values varying between  $12 \mu\text{g g}^{-1}$  in *Liocranchia atlantica* and  $31 \mu\text{g g}^{-1}$  in *Megalocranchia oceanica*. In the overall, with total median value of  $17 \mu\text{g g}^{-1}$ , cranchiid concentrations were significantly lower than those of the other orders (Figure 3.9B; KW test, H: 126.81,  $p < 0.05$ ). In the mantle, there were no significant differences among cranchiids (figure 3.9C; KW test, H: 4.39,  $p > 0.05$ ), with median values varying between  $13 \mu\text{g g}^{-1}$  in *Galiteuthis armata* and  $95 \mu\text{g g}^{-1}$  in *Leachia* sp. In the overall, with a total median value of  $28 \mu\text{g g}^{-1}$ , cranchiid concentrations were significantly lower than octopus and slightly higher than myopsids squids, but similar to those of other oegopsid squids and cuttlefish (Figure 3.9D; KW test, H: 29.91,  $p < 0.05$ ). In the mantle (Figure 3.9E), cranchiids median values varying between  $0.80 \mu\text{g g}^{-1}$  in *Leachia atlantica* and  $17 \mu\text{g g}^{-1}$  in *Liocranchia reinhardtii*. In the overall, with total median value of  $16 \mu\text{g g}^{-1}$ , cranchiid concentrations were significantly lower than octopuses but similar to those of other oegopsids and myopsids squids (Figure 3.9F; KW test, H: 21.60,  $p < 0.05$ ).

Zinc concentrations were also significantly different among tissues (KW test, H: 11.04,  $p < 0.05$ ), with the mantle having higher concentration values than those in the digestive gland and the tentacles. The levels in the latter two tissues were quite similar.

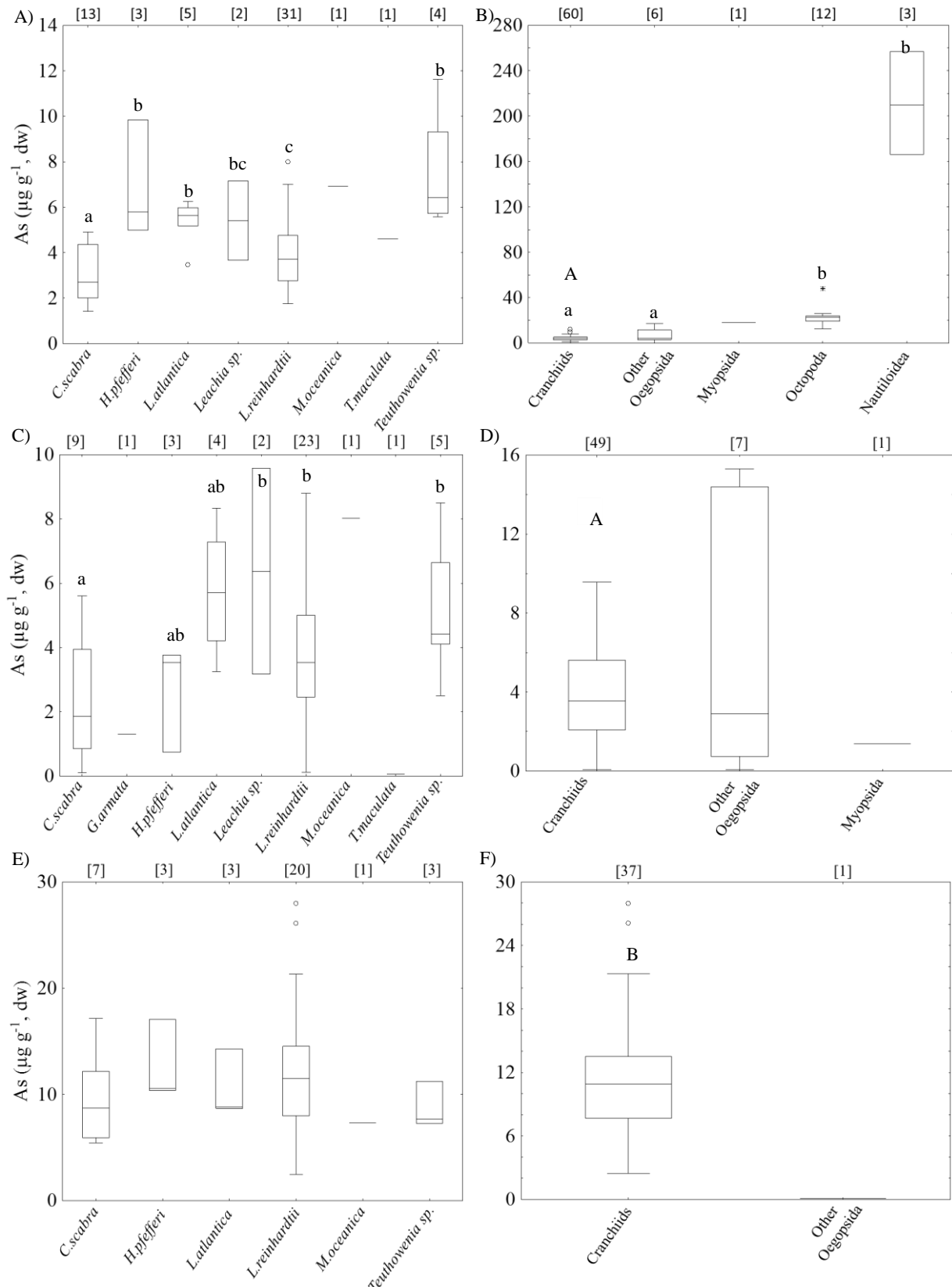


**Figure 3.9:** Median, 25 and 75% percentil, minimum, maximum, outliers and extremes of zinc (Zn) concentrations ( $\mu\text{g g}^{-1}$  dw) in cephalopods. A) in the digestive gland of the studied cranchiids (present study). B) in the digestive gland of all studied cranchiids (in the overall) and other cephalopod groups. C) in the mantle of the studied cranchiids (present study). D) in the mantle of all studied cranchiids (in the overall) and other cephalopod groups. E) in the tentacles of the studied cranchiids (present study). F) in the tentacles of all studied cranchiids (in the overall) and other cephalopod groups. Numbers in brackets represent the sample size (n). Species or taxonomic groups with only value were not included in the interspecific comparisons (statistical analyses). Different superscript letters represent significant differences ( $P < 0.05$ ), among species or orders (lowercase) and among tissues (capital letters).

### 3.7. Arsenic (As)

Regarding As concentrations in the digestive gland, there were some significant differences among cranchiid species (Figure 10A; KW test, H: 423.94,  $p < 0.05$ ). With the lower median value, *Cranchia scabra* ( $2.7 \mu\text{g g}^{-1}$ ) is different than all the other species. *Liocranchia reinhardtii* show differences with the all the other species except for *Leachia* sp. ( $p < 0.05$ , Dunn test). *Megalocranchia oceanica* has the highest media value with  $6.93 \mu\text{g g}^{-1}$ . In the overall, with total median value of  $4.1 \mu\text{g g}^{-1}$ , cranchiid concentrations were significantly lower than in nautilus and octopus, but similar to those of other squids (Figure 3.10B; KW test, H: 38.27,  $p < 0.05$ ). In the mantle, the median values varied between  $0.060 \mu\text{g g}^{-1}$  in *Teuthowenia maculata* and  $8.0 \mu\text{g g}^{-1}$  in *Megalocranchia oceanica*. There were some significant differences among cranchiids (Figure 10B; KW test, H: 15.86,  $p < 0.05$ ), with *Cranchia scabra* having lower concentration than *Leachia* sp., *Liocranchia reinhardtii* and *Teuthowenia* sp ( $p < 0.05$ , Dunn test). In the overall, similar As concentrations were observed between cranchiid (with a total median value of  $3.5 \mu\text{g g}^{-1}$ ) and squid species from previous studies (Figure 3.10D; KW test, H: 1.03,  $p > 0.05$ ). In the tentacles, there were no significant differences among cranchiids (Figure 3.10E; KW test, H: 3.02,  $p > 0.05$ ), with median values varying between  $7.3 \mu\text{g g}^{-1}$  in *Megalocranchia oceanica* and  $11 \mu\text{g g}^{-1}$  in *Liocranchia reinhardtii*. With a total median value of  $11 \mu\text{g g}^{-1}$ , cranchiid concentrations were significantly higher than those observed for other oegopsid squids (Figure 3.10F; KW test, H: 2.85,  $p < 0.05$ ).

Arsenic concentrations among cranchiid tissues, were, in general, significantly higher in the tentacles than those in the digestive gland and mantle different (KW test, H: 61.42,  $p < 0.05$ ). No differences were observed between mantle and digestive gland.



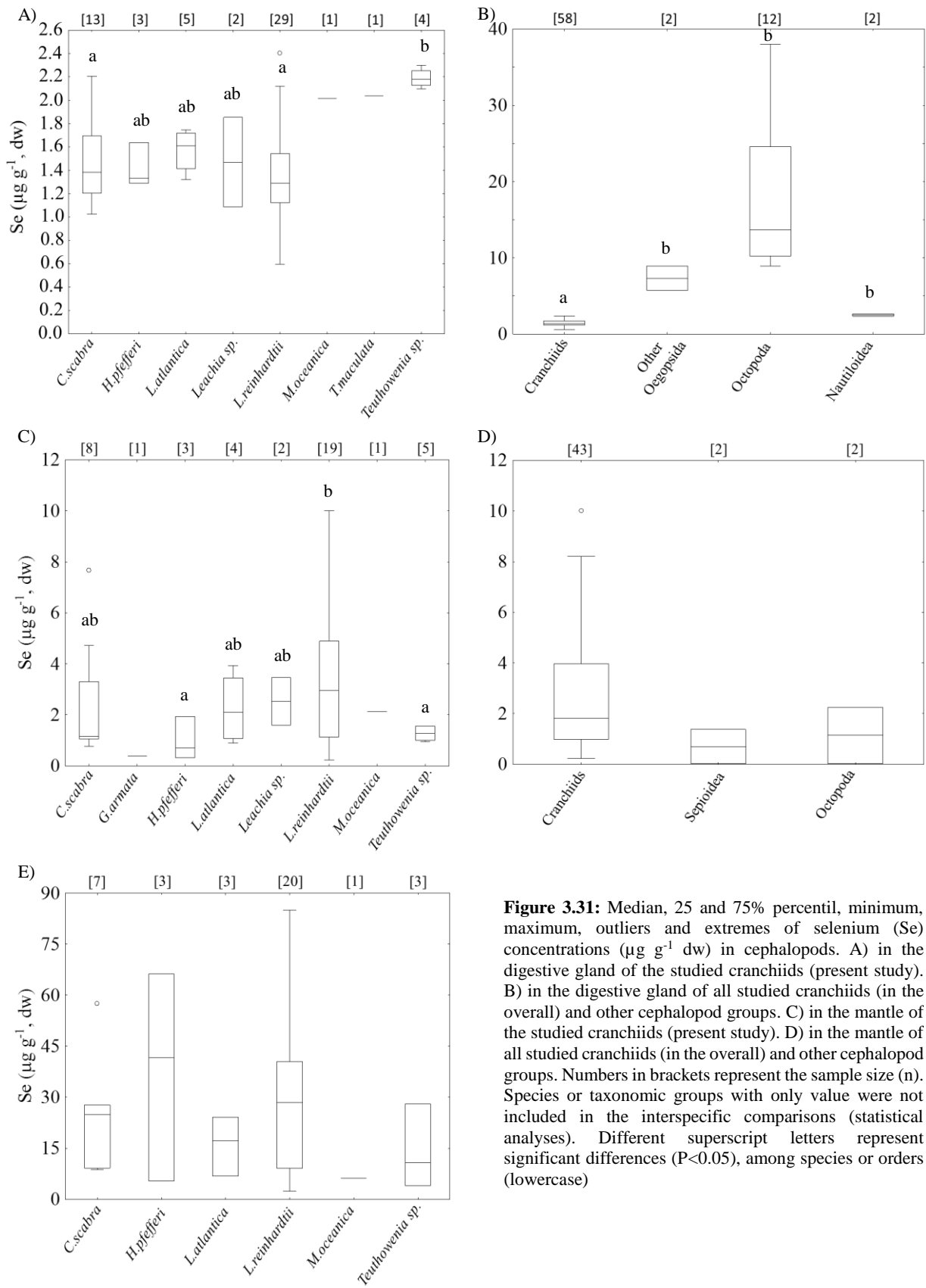
**Figure 3.10:** Median, 25 and 75% percentil, minimum, maximum, outliers and extremes of arsenic (As) concentrations ( $\mu\text{g g}^{-1}$  dw) in cephalopods. A) in the digestive gland of the studied cranchiids (present study). B) in the digestive gland of all studied cranchiids (in the overall) and other cephalopod groups. C) in the mantle of the studied cranchiids (present study). D) in the mantle of all studied cranchiids (in the overall) and other cephalopod groups. E) in the tentacles of the studied cranchiids (present study). F) in the tentacles of all studied cranchiids (in the overall) and other cephalopod groups. Numbers in brackets represent the sample size (n). Species or taxonomic groups with only value were not included in the interspecific comparisons (statistical analyses). Different superscript letters represent significant differences ( $P < 0.05$ ), among species or orders (lowercase) and among tissues (capital letters).



### 3.8. Selenium (Se)

Regarding digestive gland selenium concentrations, there were significant differences among cranchiid species (Figure 11A; KW test, H: 15.53,  $p < 0.05$ ), with *Teuthowenia* sp. (with the highest mean value of  $2.2 \mu\text{g g}^{-1}$ ) being different than *Cranchia scabra* and *Liocranchia reinhardtii*, that has the lowest median value ( $1.3 \mu\text{g g}^{-1}$ ) ( $p < 0.05$ , Dunn test). With a total median value of  $1.4 \mu\text{g g}^{-1}$ , cranchiid concentrations showed significant differences, with significantly lower values than those obtained in octopus and myopsids, and slightly lower than nautilus (Figure 3.11B; KW test, H: 37.42,  $p < 0.05$ ). In the mantle, the median values varied varying between  $0.40 \mu\text{g g}^{-1}$  in *Galiteuthis armata* and  $2.9 \mu\text{g g}^{-1}$  in *Liocranchia reinhardtii*. There were some significant differences among cranchiids (Figure 11B; KW test, H: 9.145,  $p < 0.05$ ), with *Liocranchia reinhardtii* being different than *Helicocranchia pfefferi* and *Teuthowenia* sp ( $p < 0.05$ , Dunn test). Cranchiid concentrations (with a total median value of  $1.8 \mu\text{g g}^{-1}$ ) were similar of those of octopus and cuttlefish (Figure 3.11D; KW test, H: 31.64,  $p > 0.05$ ). In the tentacles, there were no significant differences among cranchiids (Figure 3.11E; KW test, H: 3.8,  $p > 0.05$ ), with median values varying between  $6.1 \mu\text{g g}^{-1}$  in *Megalocranchia oceanica* and  $41.68 \mu\text{g g}^{-1}$  in *Helicocranchia pfefferi*.

Elemental tissue composition varied with species, but generally Se concentrations were significantly higher in the tentacles than those in digestive gland and mantle (KW test, H: 75.25,  $p < 0.05$ ), that presented similar levels.

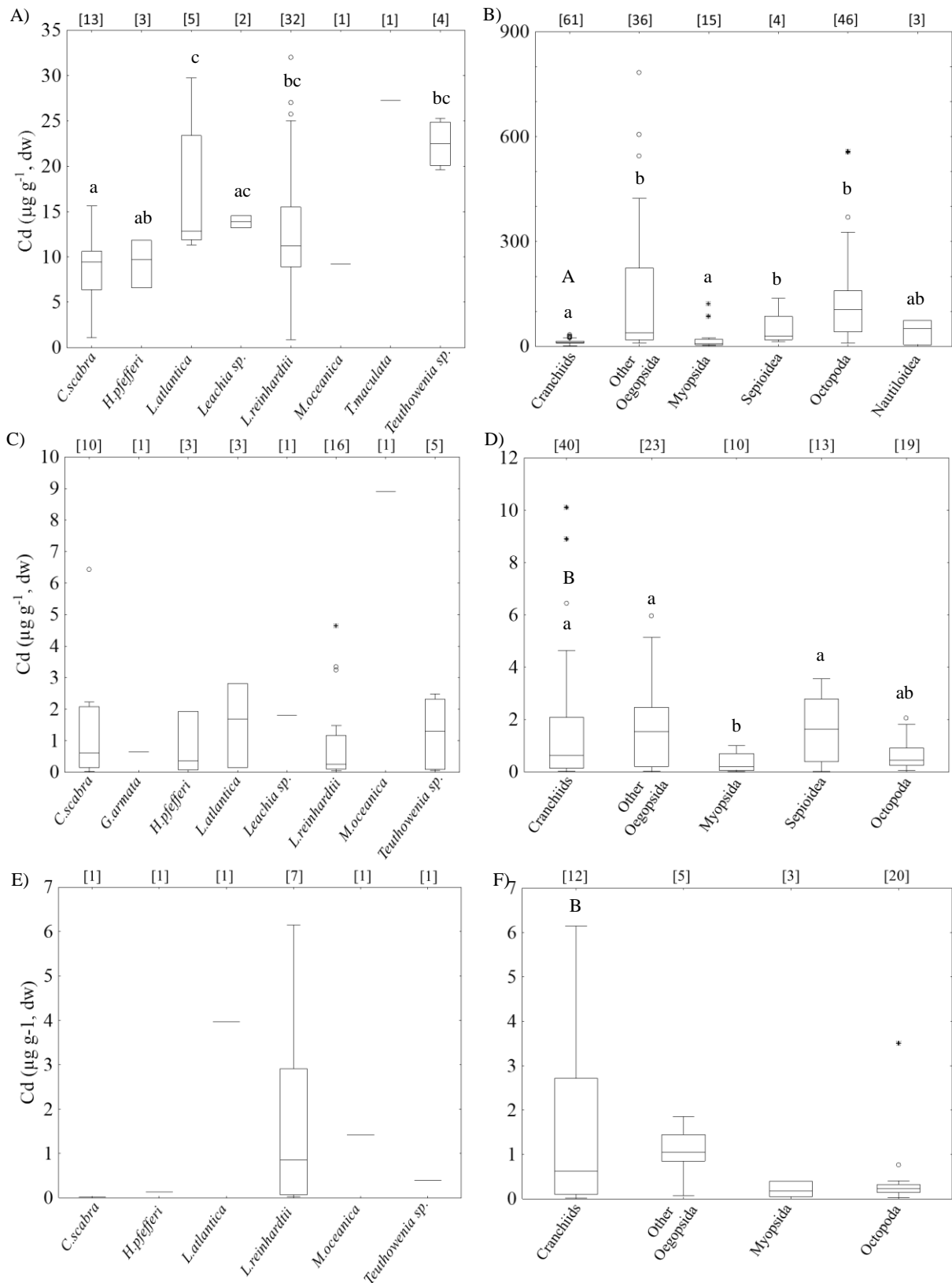


**Figure 3.31:** Median, 25 and 75% percentil, minimum, maximum, outliers and extremes of selenium (Se) concentrations ( $\mu\text{g g}^{-1}$  dw) in cephalopods. A) in the digestive gland of the studied cranchiids (present study). B) in the digestive gland of all studied cranchiids (in the overall) and other cephalopod groups. C) in the mantle of the studied cranchiids (present study). D) in the mantle of all studied cranchiids (in the overall) and other cephalopod groups. Numbers in brackets represent the sample size (n). Species or taxonomic groups with only value were not included in the interspecific comparisons (statistical analyses). Different superscript letters represent significant differences ( $P < 0.05$ ), among species or orders (lowercase)

### 3.9. Cadmium (Cd)

Cadmium median values in the digestive gland, varied between  $9.2 \mu\text{g g}^{-1}$  in *Megalocranchia oceanica* and  $27 \mu\text{g g}^{-1}$  in *Teuthowenia maculata*. When tested for statistical differences among species, some were found (Figure 12A; KW test, H: 13.49,  $p > 0.05$ ). *Cranchia scabra* is different than *Teuthowenia* sp., *Liocranchia reinhardtii* and *Leachia atlantica*, which in turn is different than *Helicocranchia pfefferi* ( $p < 0.05$ , Dunn test). With a total median value of  $11 \mu\text{g g}^{-1}$ , cranchiid concentrations were significantly lower than in octopus and cuttlefish and slightly lower than other oegopsid squids, but similar to those of myopsida and nautilus (Figure 3.12B; KW test, H: 86.46,  $p < 0.05$ ). In the mantle, there were no significant differences among cranchiids (Figure 3.12C; KW test, H: 5.03,  $p > 0.05$ ), with median values varying between  $0.26 \mu\text{g g}^{-1}$  in *Liocranchia reinhardtii* and  $8.9 \mu\text{g g}^{-1}$  in *Megalocranchia oceanica*. With a total median value of  $0.61 \mu\text{g g}^{-1}$ , cranchiid concentrations were slightly higher than those of octopus and myopsid squids, but similar than those of other oegopsid squids and cuttlefish (Figure 3.12D; KW test, H: 31.64,  $p < 0.05$ ). The median values of cranchiid tentacles varied between  $0.020 \mu\text{g g}^{-1}$  in *Cranchia scabra* and  $4.0 \mu\text{g g}^{-1}$  in *Leachia atlantica* and were similar to those of other and myopsids squids and octopus (Figure 3.12F; KW test, H: 3.79,  $p > 0.05$ ).

Cd concentrations were significantly different among tissues (KW test, H: 72.00,  $p < 0.05$ ); with the digestive showing higher concentration values than the mantle and tentacles, that presented similar levels.

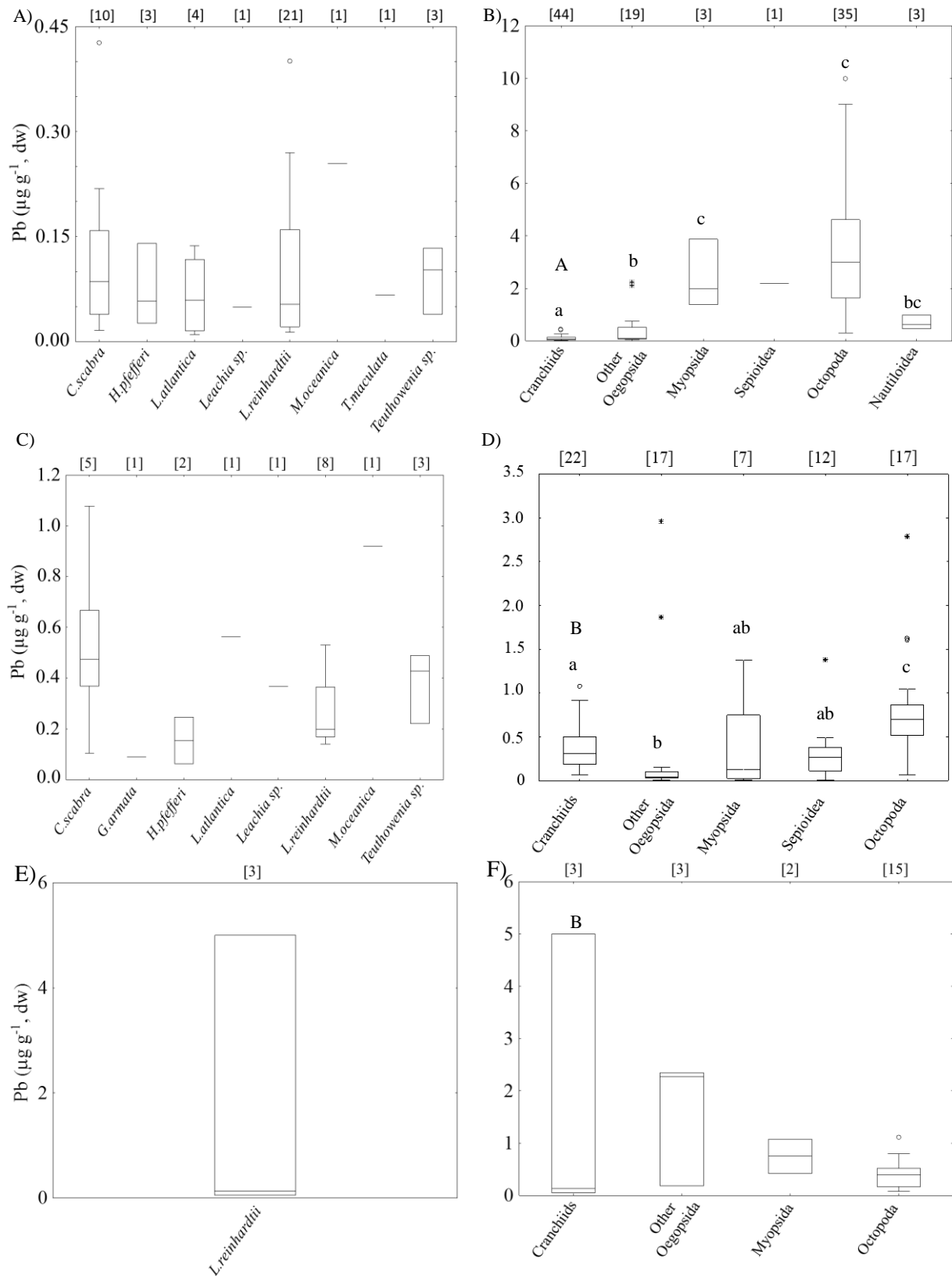


**Figure 3.42:** Median, 25 and 75 percentil, minimum, maximum, outliers and extremes of cadmium (Cd) concentrations ( $\mu\text{g g}^{-1}$  dw) in cephalopods. A) in the digestive gland of the studied cranchiids (present study). B) in the digestive gland of all studied cranchiids (in the overall) and other cephalopod groups. C) in the mantle of the studied cranchiids (present study). D) in the mantle of all studied cranchiids (in the overall) and other cephalopod groups. E) in the tentacles of the studied cranchiids (present study). F) in the tentacles of all studied cranchiids (in the overall) and other cephalopod groups. Numbers in brackets represent the sample size (n). Species or taxonomic groups with only value were not included in the interspecific comparisons (statistical analyses). Different superscript letters represent significant differences ( $P < 0.05$ ), among species or orders (lowercase) and among tissues (capital letters).

### 3.10. Lead (Pb)

There were no significant differences in the digestive gland lead concentrations among cranchiid species (Figure 3.13A; KW test, H: 3.19,  $p > 0.05$ ), with median values varying between  $0.010 \mu\text{g g}^{-1}$  in *Leachia* sp. and  $0.25 \mu\text{g g}^{-1}$  in *Megalocranchia oceanica*. With a total median value of  $0.070 \mu\text{g g}^{-1}$ , cranchiid concentrations values were similar to those of all the other orders (Figure 3.13B; KW test, H: 79.50,  $p > 0.05$ ). In the mantle, there were significant differences among cranchiids (Figure 3.13C; KW test, H: 9.21,  $p < 0.05$ ), with median values varying between  $0.090 \mu\text{g g}^{-1}$  in *Galiteuthis armata* and  $0.92 \mu\text{g g}^{-1}$  in *Megalocranchia oceanica*. With total median value of  $0.31 \mu\text{g g}^{-1}$ , cranchiid concentrations were significantly higher than those of other oegopsid squids and lower than octopus; but similar to those of myopsid squids and cuttlefish (Figure 3.13D; KW test, H: 31.64,  $p < 0.05$ ). In the cranchiid tentacles (Figure 3.13E), only *Liocranchia reinhardtii* showed Pb values above the detection limit, with a median value of  $0.13 \mu\text{g g}^{-1}$  dw. In the overall, such values were similar to those observed in other oegopsid and myopsid squids and octopus (Figure 3.13F; KW test, H: 2.80,  $p > 0.05$ ).

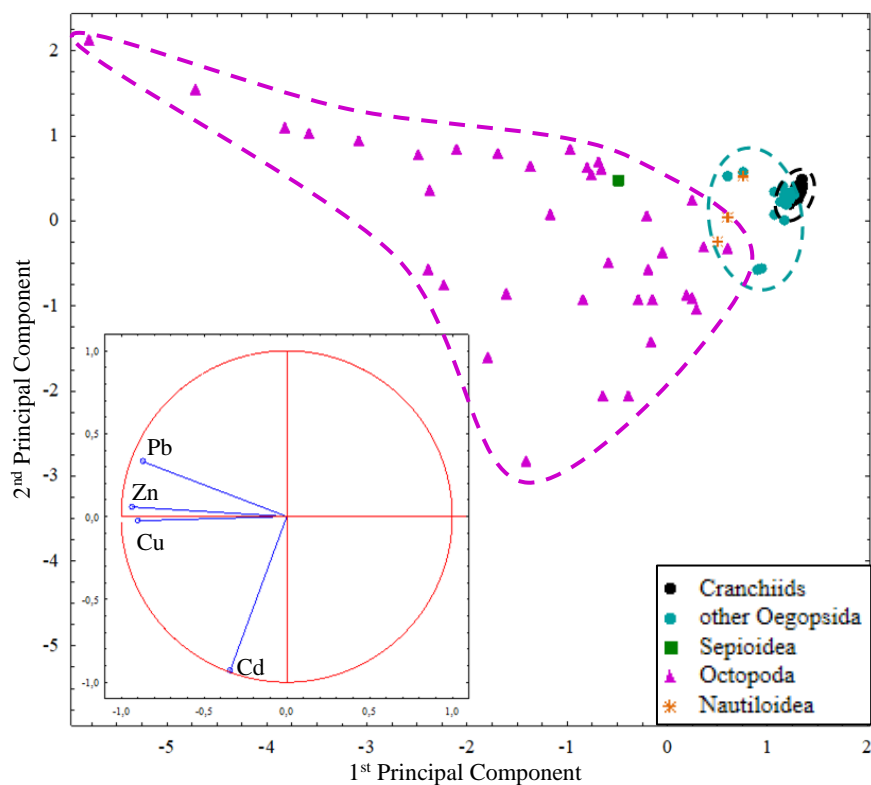
Pb concentrations were significantly different among tissues (KW test, H: 25.13,  $p < 0.05$ ), with the digestive gland revealing lower concentration values than the mantle and tentacles. The levels in the latter two tissues were similar.



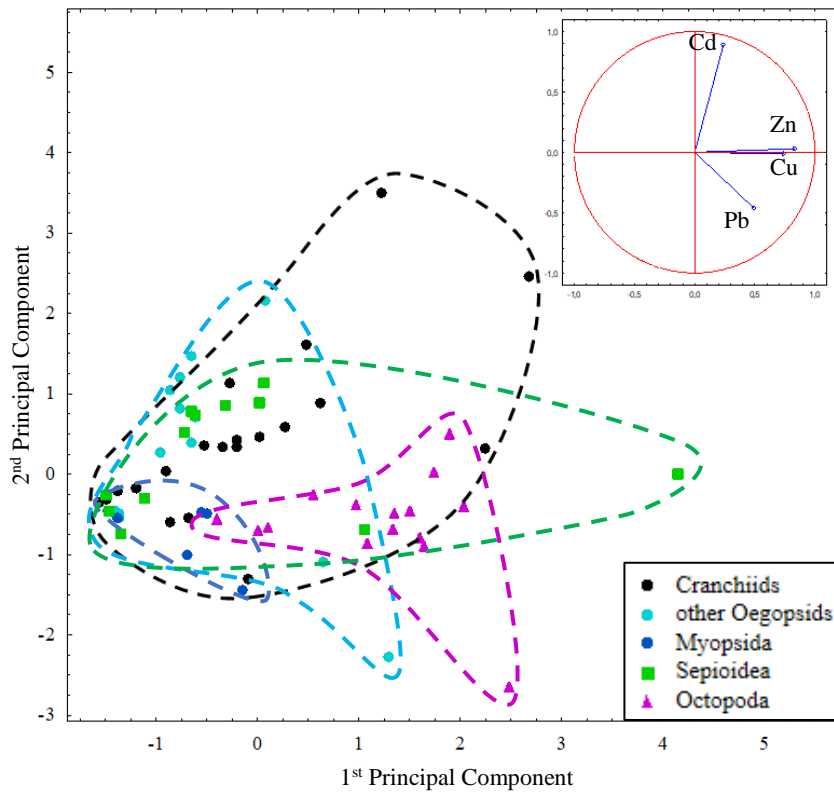
**Figure 3.53:** Median, 25 and 75% percentil, minimum, maximum, outliers and extremes of lead (Pb) concentrations ( $\mu\text{g g}^{-1}$  dw) in cephalopods. A) in the digestive gland of the studied cranchiids (present study). B) in the digestive gland of all studied cranchiids (in the overall) and other cephalopod groups. C) in the mantle of the studied cranchiids (present study). D) in the mantle of all studied cranchiids (in the overall) and other cephalopod groups. E) in the tentacles of the studied cranchiids (present study). F) in the tentacles of all studied cranchiids (in the overall) and other cephalopod groups. Numbers in brackets represent the sample size (n). Species or taxonomic groups with only value were not included in the interspecific comparisons (statistical analyses). Different superscript letters represent significant differences ( $P < 0.05$ ), among species or orders (lowercase) and among tissues (capital letters).

### 3.11. Differences among cephalopods: a multivariate approach

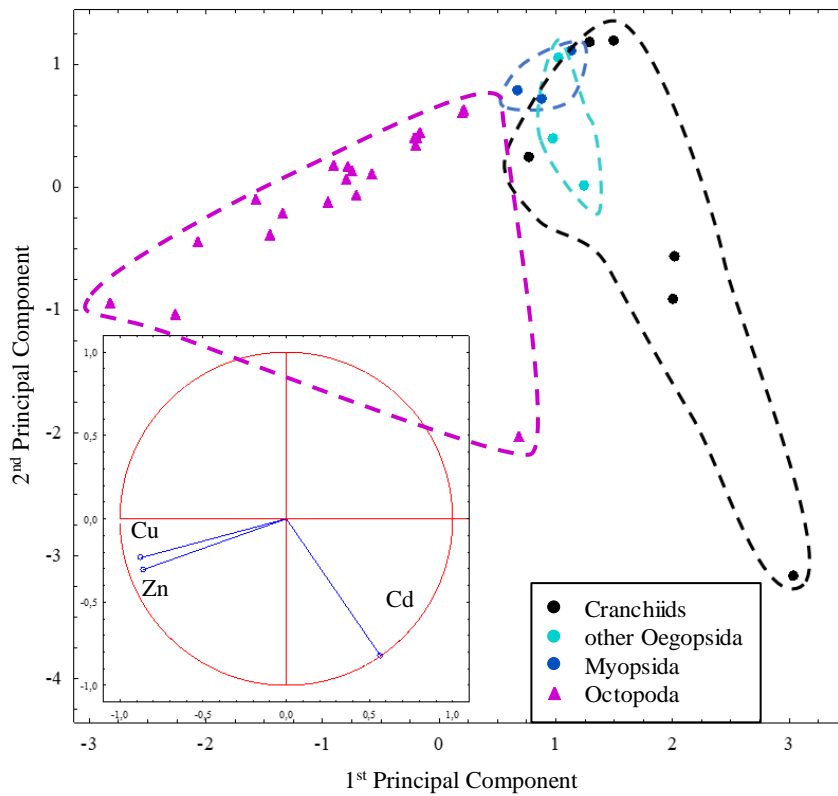
The PCA results (using Cd, Cu, Zn and Pb) in the digestive gland of the different cephalopod groups showed a clear separation of the cranchiids from the other cephalopod groups, mostly along the PC1 (explaining 63.63% of variance). PC2 only explained 24.46 % of the variance (Figure 3.14). Such separation was not obtained in the mantle (Figure 3.15; PC1 - 38.56% of variance; PC2 - 25.24 % of the variance). In the tentacles, the separation of cranchiids from the octopod group was also evident mostly along PC1 (Figure 3.16; PC1 – 60.89% of variance; PC2 – 27.51% of variance)



**Figure 3.64:** Principal component analyses of Cu, Zn, Cd and Pb in the digestive gland of different cephalopods orders



**Figure 3.75:** Principal component analyses of Cu, Zn, Cd and Pb in the mantle of different cephalopod orders



**Figure 3.86:** Principal component analyses of Cu, Zn and Cd in the tentacles of different cephalopod orders



## 4. DISCUSSION

There is an increasing concern on the presence of trace elements in the deep-sea, as it might act as a global sink for them (Turekian and Imbrie, 1966; Chester and Messiha-Hanna, 1970; Cronin et al., 1998; Kress et al., 1998; Mormede and Davies, 2003). With the land resources becoming exhausted, the exploitation of the marine environment increases, so also does extraction of deep-sea minerals. Concomitantly, as the population grows, the amount of litter increases (Ramirez-Ilodra et al., 2011), which ends up sinking into the abyss, by a variety of sources and via different pathways (Thiel, 2015).

### 4.1. Trace elements in cranchiids and other cephalopod groups

As observed in previous studies with coastal species (Bustamante et al., 2002b; Raimundo et al., 2005; Pernice et al., 2009), it was not surprising to detect high concentrations of Cd in cranchiid species. In fact, besides the ability that cephalopods have to retain Cd, the concentrations on our squids could be explained by enriched environment around Cape Verde islands. The oceanographic conditions in this area allows the enrichment of such water masses (Horner et al., 2013), with dissolved Cd being reinjected in the water column by the upwelling (Auger et al., 2015). Another source could be from the Saharian dust deposition, which represents a source of trace elements, like Co, Ni, Zn and Cd, into the Atlantic Ocean (Ridame et al., 2011). Moreover, in the present interspecific comparisons, it became apparent that benthic species showed higher concentrations of Cd than the pelagic species. These differences could be attributed to the different Cd concentrations on their preys, since benthic preys (crustaceans and bivalves), have higher Cd concentrations, than the fish that are the preferential prey of pelagic species (Penicaud et al., 2017). The concentrations in oceanic species could also be explained by their feeding habits, as they eat pelagic crustaceans (rich in Cd) as well as other cephalopod species, including cannibalism (Santos and Haimovici, 1997). In relation to nectobenthic and benthic species, the sediments may also have to be taken in account - although direct transfer is known to be low, they can act as an indirect source of contaminants (Bustamante et al., 2002b, 2004; Penicaud et al., 2017). Last, the differences with the Nautilus group may be related with their different life span, since they live 10 to 15 years while the Coleoids (cuttlefish, squids and octopods) live between 1 to 3 years (Bustamante et al., 2000). Although the life expectancy of most cranchiids is not known, is not likely that the studied individuals reach such ages. As said before, the metabolic rates of deep pelagic cephalopods are lower than those of surface living species (Childress and Seibel, 1998; Seibel et al., 2000), which can also explain some of the differences from the coastal living ones. Moreover, the differences among oegopsids (our cranchiids and other species) can also be related with the occupancy of different ecological niches and trophic levels. In fact, cranchiids are placed in a much lower trophic level than the other studied oegopsids (Budko et al., 2015), which can explain their general lower concentrations of the trace elements. Last, it is important to note that the muscles in the cranchiids showed more variation as well as some higher concentration than the other cephalopod species. These results were not expected and raises the question of why the cranchiids concentrate such high concentration in these tissues.

### 4.2. Trace elements tissue partition

The capacity of cephalopods to concentrate high levels of trace elements is associated to detoxification processes that allows to transforme these elements in a non-metabolically-available form, thereby limiting their toxicity (Bustamante et al., 1998 ab, 2000, 2008; Raimundo et al., 2005; Penicaud et al., 2017). Within this context, the digestive gland is a complex organ involved in several functions, such as digestion, secretion and detoxification (among others) (Penicaud et al., 2017). Being considered a

major storage tissue, is consequently a key organ for detoxification (Smith et al., 1984; Ueda et al., 1985; Miramand and Bentley, 1992; Bustamante et al., 1998b, 2000, 2003; Raimundo et al., 2005). Not surprisingly, but with some exceptions (e.g. Hg and As), most trace elements were found at higher concentrations in the digestive gland tissue (Bustamante et al., 2008, 2006b; Kojadinovic et al., 2011). It is argued that trace elements are initially bound to soluble proteins, trapped in solid proteo-mineral structures, implying that the cells from the digestive gland are likely to be involved in trace element detoxification. This association with cytosolic proteins, inhibits the toxic interactions of metallic ions with sensitive binding sites such as cell structures. The digestive gland cells contain two type of structures which can participate in trace element detoxification: “boules”, which corresponds to the typical vacuoles (thought to be involved in intracellular digestion and enzyme secretion); and brown bodies, which are large vacuoles containing cellular debris (thought to have an excretory role). Moreover, since metals like Ag, Cd, Cu, Hg and Zn have a high affinity for metallothioneins, they are thought to play a key role in the homeostasis of essential metals, as well as an important role in the trace element tolerance of organisms (Penicaud et al., 2017).

Previous studies have shown that Cd concentrations were always higher in the digestive gland than in other tissues, despite being different species from different places, with different Cd concentrations. The same tissue partition was observed in the present dissertation. As said before, previous studies have also shown that not only the Cd but also other trace elements, like Co, Cr, Ni and V are found at higher concentrations in the digestive gland than in the muscle (Bustamante et al., 1998 ab, 2000, 2008; Raimundo et al., 2005). These findings were not corroborated in the present work with cranchiids. In fact, while Co had higher concentrations in the digestive gland, V, Cr and Ni had higher concentration values in the mantle. Regarding the differences between mantle and tentacles, Kariya et al. (1986) pointed out that tissue differences in protein composition in octopus may explain partition of trace elements between those two tissues. Such differences in tissue biochemical composition and the associated enzyme systems (Smith et al., 1984) may also explain the present cranchiid findings.

Once again, the cephalopods showed the importance of their digestive gland in the storage of trace elements, especially Cd. Although our species showed different tissue distribution for the majority of the elements when compared with the other cephalopod species, it still showed the capacity of this group to accumulate trace elements for their environment.

Last, it is worth noting that the fact that glass (transparent) squids that thrive in deep pelagic environments display trace elements concentrations as high as those found in coastal cephalopods, which live in habitats exposed to enhanced anthropogenic forcing, is quite surprising. These findings corroborate the on-going notion that such remote environments are now the major global sink for contaminants in the planet.

## REFERENCES

- Ahdy, H.H.H., Abdallah, A.M.A., Tayel, F.T., 2007. Assessment of Heavy Metals and Nonessential Content of Some Edible and Soft Tissues. *Egypt. J. Aquatic Res.* 33, 85–97.
- Arkhipkin, A., 1996. Age and growth of planktonic squids *Cranchia scabra* and *Liocranchia reinhardtii* (Cephalopoda, Cranchiidae) in epipelagic waters of the central-east Atlantic. *J. Plankton Res.* 18, 1675–1683.
- Auger, P.A., Machu, E., Gorges, T., Grima, N., Waeles, M., 2015. Comparative study of potential transfer of natural and anthropogenic cadmium to plankton communities in the North-West African upwelling. *Science Total Environ.* 505, 870–888, doi:10.1016/j.scitotenv.2014.10.045
- Ayas, D., Ozogul, Y., 2011. The Effects of Season and Sex in the Metal Levels of Mature Common Cuttlefish (*Sepia officinalis*) in Mersin Bay , Northeastern Mediterranean. *J. Food Sci.* 76, 21–24. doi:10.1111/j.1750-3841.2011.02152.x
- Bai, J., Zhao, Q., Lu, Q., Wang, J., Reddy, K.R., 2015. Effects of freshwater input on trace element pollution in salt marsh soils of a typical coastal estuary, China. *J. Hydrol.* 520, 186–192. doi:10.1016/j.jhydrol.2014.11.007
- Budko, D.F., Demina, L.L., Martynova, D.M., Gorshkova, O.M., 2015. Trace Elements in Organisms of Different Trophic Groups in the White Sea. *Mar. Oceanol.* 55, 730–741. doi:10.1134/S0001437015050021
- Bustamante, P., Caurant, F., Fowler, S.W., Miramand, P., 1998a. Cephalopods as a vector for the transfer of cadmium to top marine predators in the north-east Atlantic Ocean. *Sci. Total Environ.* 220, 71–80.
- Bustamante, P., Cherel, Y., Caurant, F., Miramand, P., 1998b. Cadmium, copper and zinc in octopuses from Kerguelen Islands, Southern Indian Ocean. *Polar Biol.* 19, 264–271. doi:10.1007/s003000050244
- Bustamante, P., Grigiono, S., Boucher-Rodoni, R., Caurant, F., Miramand, P., 2000. Bioaccumulation of 12 trace elements in the tissues of the *Nautilus macromphalus* from New Caledonia. *Mar. Pollut. Bull.* 40, 688–696.
- Bustamante, P., Cosson, R.P., Gallien, I., Caurant, F., 2002a. Cadmium detoxification processes in the digestive gland of cephalopods in relation to accumulated cadmium concentrations. *Mar. Environ. Res.* 53, 227–241.
- Bustamante, P., Teyssié, J., Fowler, S.W., Cotret, O., Danis, B., Miramand, P., Warnau, M., 2002b. Biokinetics of zinc and cadmium accumulation and depuration at different stages in the life cycle of the cuttlefish *Sepia officinalis*. *Mar. Ecol. Prog. Ser.* 231, 167–177. doi:10.3354/meps231167
- Bustamante, P., Teyssié, J.L., Danis, B., Fowler, S.W., Miramand, P., Cotret, O., Warnau, M., 2004. Uptake, transfer and distribution of silver and cobalt in tissues of the common cuttlefish *Sepia officinalis* at different stages of its life cycle. *Mar. Ecol. Prog. Ser.* 269, 185–195. doi:10.3354/meps269185
- Bustamante, P., Lahaye, V., Durnez, C., Churlaud, C., Caurant, F., 2006. Total and organic Hg concentrations in cephalopods from the North Eastern Atlantic waters : Influence of geographical origin and feeding ecology. *Sci. Total Environ.* 368, 585–596. doi:10.1016/j.scitotenv.2006.01.038
- Bustamante, P., González, A.F., Rocha, F., Miramand, P., Guerra, A., 2008. Metal and metalloid concentrations in the giant squid *Architeuthis dux* from Iberian waters. *Mar. Environ. Res.* 66, 278–287. doi:10.1016/j.marenvres.2008.04.003

- Chester, R., Messiha-Hanna, R.G., 1970. Trace element partition patterns in North Atlantic deep-sea sediments. *Geochim. Cosmochim. Acta* 34, 1121–1128. doi:10.1016/0016-7037(70)90166-3
- Childress, J.J., Seibel, B.A., 1998. Life at stable low oxygen levels: adaptations of animals to oceanic oxygen minimum layers. *J. Exp. Biol.* 201, 1223–1232.
- Chouvelon, T., Cherel, Y., Caurant, F., Paula, M., Bustamante, P., Chouvelon, T., 2011. Species and ontogenic-related differences in d13C and d15N values and Hg and Cd concentrations of cephalopods. *Mar. Ecol. Prog. Ser.* 433, 107–120.
- Craig, S., Overnell, J., 2003. Metals in squid, *Loligo forbesi*, adults, eggs and hatchlings. No evidence for a role for Cu- or Zn-metallothionein. *Comp. Biochem. Physiol. Part C* 134, 311–317.
- Cronin, M., Davies, I.M., Newton, A., Pirie, J.M., Topping, G., Swan, S., 1998. Trace metal concentrations in deep sea fish from the North Atlantic. *Mar. Environ. Res.* 45, 225–238. doi:10.1016/S0141-1136(98)00024-5
- David Smith, J., Plues, L., Heyraud, M., Cherry, R.D., 1984. Concentrations of the elements Ag, Al, Ca, Cd, Cu, Fe, Mg, Mn, Pb and Zn, and the radionuclides 210Pb and 210Po in the digestive gland of the squid *Nototodarus gouldi*. *Mar. Environ. Res.* 13, 55–68. doi:10.1016/0141-1136(84)90014-X
- Dornele, P.R., Lailson-Brito, J., Santos, R.A., Costa, P.A.S., Malm, O., Azevedo, A.F., Torres, J.P.M., 2007. Cephalopods and cetaceans as indicators of offshore bioavailability of cadmium off Central South Brazil Bight. *Environ. Pollut.* 148, 352–359. doi:10.1016/j.envpol.2006.09.022
- Duysak, Ö., Dural, M., 2015. Heavy Metal Concentrations in Tissues of Short-Finned Squid *Illex coindetii* (Cephalopoda: Ommastrephidae) (Vérany, 1839) in Iskenderun Bay, North-Eastern Mediterranean. *Pakistan J. Zool.* 47, 447–453.
- Falandysz, J., 1988. Trace metals in squid *Illex argentine*, *European Food and Research & Technology*, 187, 359–361.
- Falandysz, J., 1991. Concentrations of Trace Metals in Various Tissues of the Squid *Loligo opalescens* and Their Redistribution after Canning. *J. Sci. Food Agric.* 54, 79–87.
- Farraj, S. Al, El-Gendy, A.H., Alyahya, H., El-Hedeny, M., 2011. Heavy Metals Accumulation in the Mantle of the Common Cuttlefish *Sepia pharaonis* from the Arabian Gulf. *Aust. J. Basic Appl. Sci.* 5, 897–905.
- Ferreira, A.M., Cortesão, C., Castro, O.G., Vale, C., 1990. Accumulation of metals and organochlorines in tissues of the oyster *Crassostrea angulata* from the Sado estuary, Portugal. *Sci. Total Environ.* 85, 627–639.
- Finger, J.M., Smith, J.D., 1987. Molecular association of Cu, Zn, Cd and 210 Po in the digestive gland of the squid *Nototodarus gouldi*. *Mar. Biol.* 95, 87–91.
- Gage, J.D., Tyler, P.A., 1991. Deep-sea biology: a natural history of organisms at the deep-sea floor. xvi, 504p. (Cambridge University Press. *Journal of the Marine Biological Association of the United Kingdom*, 71(3))
- Georgantelis, D., Papavergou, E., Katsouyannopoulos, V., 2001. Determination of Trace Metals in Canned Cephalopods, in: 7th International Conference on Environmental Science and Technology Ermoupolis, Syros Island, Greece. pp. 109–113.
- Gerpe, M.S., Moreno, J.E.A., Moreno, V.J., Patat, M.L., 2000. Cadmium, zinc and copper accumulation in the squid *Illex argentinus* from the Southwest Atlantic Ocean. *Mar. Biol.* 136, 1039–1044.

- Ghiretti-Magaldi, A., Giuditta, A., Ghiretti, F., 1958. Pathways of terminal respiration in Marine Invertebrates I. The respiratory system in cephalopods. *J. Cell. Physiol.* 389–429.
- Horner, T.J., Lee, R.B.Y., Henderson, G.M., Rosalind, E.M., Horner, T.J., Lee, R.B.Y., Henderson, G.M., Rickaby, R.E.M., 2013. Nonspecific uptake and homeostasis drive the oceanic cadmium cycle. *PNAS* 110, 2500–2505. doi:10.1073/pnas.1213857110
- Ichihashi, H., Nakamura, Y., Kannan, K., Tsumura, A., Yamasaki, S., 2001. Multi-Elemental Concentrations in Tissues of Japanese Common Squid (*Todarodes pacificus*). *Arch. Environ. Contam. Toxicol.* 490, 483–490. doi:10.1007/s002440010275
- Ichimashi, H., Kohno, H., Kannan, K., Tsumura, A., Yamasaki, S.-I., 2001. Multielemental Analysis of Purpleback Flying Squid Using High Resolution Inductively Coupled Plasma-Mass Spectrometry (HR ICP-MS). *Environ. Sci. Technol* 35, 3103–3108.
- Jereb, P., Roper, C.F.E. & Vecchione, M. 2010. Introduction. In P. Jereb & C.F.E. Roper, eds. *Cephalopods of the world. An annotated and illustrated catalogue of species known to date. Volume 2. Myopsid and Oegopsid Squids. FAO Species Catalogue for Fishery Purposes. No. 4, Vol. 2. Rome, FAO.* pp. 1–11
- Jinadasa, B.K.K.K., 2014. Concentration of Trace Metals in the Squids (*Loligo duvauceli*, *Sepioteuthis lessoniana*) and cuttlefish (*Sepia latimanus*) from the North-Western Coast of Sri Lanka. *J. Aquat. Sci.* 2, 5–10. doi:10.12691/jas-2-2-1
- Kariya, Y., Ochiai, Y., Hashimoto, K., 1986. Protein Components and Ultrastructure of the arm and Mantle Muscles of Octopus *Octopus vulgaris*. *Bull. Japanese Soc. Sci. Fish.* 52, 131–138.
- Kojadinovic, J., Jackson, C.H., Cherel, Y., Jackson, G.D., Kojadinovic, J., Jackson, C.H., Cherel, Y., Jackson, G.D., Bustamante, P., 2011. Multi-elemental concentrations in the tissues of the oceanic squid *Todarodes filippovae* from Tasmania and the southern Indian Ocean. *Ecotoxicol. Environmetal Saf.* 74, 1238–1249.
- Kress, N., Hornung, H., Herut, B., 1998. Concentrations of Hg, Cd, Cu, Zn, Fe and Mn in deep sea benthic fauna from the southeastern Mediterranean sea: A comparison study between fauna collected at a pristine area and at two waste disposal sites. *Mar. Pollut. Bull.* 36, 911–921. doi:10.1016/S0025-326X(98)00069-1
- Levin, L.A., 2003. Oxygen minimum zone benthos: adaptation and community response to hypoxia. *Oceanogr. Mar. Biol. an Annu. Rev.* 41, 1–45.
- Lourenço, H.M., Anacleto, P., Afonso, C., Ferraria, V., Martins, M.F., Carvalho, M.L., Lino, A.R., Nunes, M.L., 2009. Elemental composition of cephalopods from Portuguese continental waters. *Food Chem.* 113, 1146–1153. doi:10.1016/j.foodchem.2008.09.003
- Martin, J.H., Flegal, R., 1975. High copper concentrations in squid livers in association with elevated levels of silver, cadmium, and zinc. *Mar. Biol.* 30, 51–55. doi:10.1007/BF00393752
- Miramand, P., Guary, J.C., 1980. High concentrations of some heavy metals in tissues of the mediterranean octopus. *Bull. Environ. Contam. Toxicol.* 24, 783–788. doi:10.1007/BF01608189
- Miramand, P., Bentley, D., 1992. Concentration and distribution of heavy metals in tissues of two cephalopods, *Eledone cirrhosa* and *Sepia officinalis*, from the French coast of the English Channel. *Mar. Biol.* 114, 407–414. doi:10.1007/BF00350031
- Miramand, P., Bustamante, P., Bentley, D., Kouéta, N., 2006. Variation of heavy metal concentrations (Ag, Cd, Co, Cu, Fe, Pb, V, and Zn) during the life cycle of the common cuttlefish *Sepia officinalis*. *Sci. Total Environ.* 361, 132–143. doi:10.1016/j.scitotenv.2005.10.018

- Mormede, S., Davies, I.M., 2003. Horizontal and vertical distribution of organic contaminants in deep-sea fish species. *Chemosphere* 50, 563–574. doi:10.1016/S0045-6535(02)00493-9
- Murthy, L.N., Panda, S.K., Madhu, V.R., Asokan, P.K., 2008. Cadmium in the purpleback flying squid *Sthenoteuthis oualaniensis* (Lesson, 1830) along northwest coast of India. *J. Mar. Biol.* 50, 191–195.
- Nho, E.Y., Khan, N., Choi, J.Y., Kim, J.S., Park, K.S., Kim, K.S., 2016. Determination of Toxic Metals in Cephalopods From South Korea. *Anal. Lett.* 49, 1578–1588. doi:10.1080/00032719.2015.1107082
- Osman, I.H., Gabr, H.R., El-Etreby, S.G., Mohammed, S.Z., 2014. Feeding biology and biochemical composition of the lessepsian migrant *Octopus aegina* (Cephalopoda: Octopodidae). *Egypt. J. Aquat. Biol. Fish* 18, 15–27.
- Panutrakul, S., Jayasinghe, R.P.P.K., Chookong, C., 2007. Heavy Metal Contents in Purpleback Squid (*Sthenoteuthis oualaniensis*) from the Bay of Bengal. *Ecosystem-Based Fish. Manag. Bay Bengal* 233–244.
- Peña-Izquierdo, J., Pelegrí, J.L., Pastor, M. V, Castellanos, P., Emelianov, M., Gasser, M., Salvador, J., Vázquez-domínguez, E., 2012. The continental slope current system between Cape Verde and the Canary Islands. *Sci. Mar.* 76, 65–78. doi:10.3989/scimar.03607.18C
- Penicaud, V., Lacoue-labarthe, T., Bustamante, P., 2017. Metal bioaccumulation and detoxification processes in cephalopods: A review review. *Environ. Res.* 155, 123–133. doi:10.1016/j.envres.2017.02.003
- Pernice, M., Boucher, J., Boucher-rodoni, R., Joannot, P., Bustamante, P., 2009. Ecotoxicology and Environmental Safety Comparative bioaccumulation of trace elements between *Nautilus pompilius* and *Nautilus macromphalus* (Cephalopoda: Nautiloidea) from Vanuatu and New Caledonia. *Ecotoxicol. Environ. Saf.* 72, 365–371. doi:10.1016/j.ecoenv.2008.04.019
- Pierce, G., Stowasser, G., Hastie, L., Bustamante, P., Pierce, G., Stowasser, G., Hastie, L., Geographic, P.B., 2008. Geographic, seasonal and ontogenetic variation in cadmium and mercury concentrations in squid (Cephalopoda: Teuthoidea) from UK waters. *Ecotoxicol. Environ. Saf.* 70, 422–432.
- Raimundo, J., Caetano, M., Vale, C., 2004. Geographical variation and partition of metals in tissues of *Octopus vulgaris* along the Portuguese coast. *Sci. Total Environ.* 325, 71–81.
- Raimundo, J., Pereira, P., Vale, C., Caetano, M., 2005. Fe, Zn, Cu and Cd concentrations in the digestive gland and muscle tissues of *Octopus vulgaris* and *Sepia officinalis* from two coastal areas in Portugal. *Ciencias Mar.* 31, 243–251.
- Raimundo, J., Vale, C., Duarte, R., Moura, I., 2008. Sub-cellular partitioning of Zn, Cu, Cd and Pb in the digestive gland of native *Octopus vulgaris* exposed to different metal concentrations (Portugal). *Sci. Total Environ.* 390, 410–416. doi:10.1016/j.scitotenv.2007.10.029
- Raimundo, J., Costa, P.M., Vale, C., Costa, M.H., Moura, I., 2010a. Metallothioneins and trace elements in digestive gland, gills, kidney and gonads of *Octopus vulgaris*. *Comp. Biochem. Physiol. - C Toxicol. Pharmacol.* 152, 139–146. doi:10.1016/j.cbpc.2010.03.009
- Raimundo, J., Costa, P.M., Vale, C., Costa, M.H., Moura, I., 2010b. DNA damage and metal accumulation in four tissues of feral *Octopus vulgaris* from two coastal areas in Portugal. *Ecotoxicol. Environ. Saf.* 73, 1543–1547. doi:10.1016/j.ecoenv.2010.07.034

- Raimundo, J., Vale, C., Duarte, R., Moura, I., 2010c. Association of Zn, Cu, Cd and Pb with protein fractions and sub-cellular partitioning in the digestive gland of *Octopus vulgaris* living in habitats with different metal levels. *Chemosphere* 81, 1314–1319. doi:10.1016/j.chemosphere.2010.08.029
- Raimundo, J., Vale, C., Rosa, R., 2014. Trace element concentrations in the top predator jumbo squid (*Dosidicus gigas*) from the Gulf of California. *Ecotoxicol. Environ. Saf.* 102, 179–186. doi:10.1016/j.ecoenv.2014.01.026
- Ramirez-Ilodra, E., Tyler, P.A., Baker, M.C., Bergstad, O.A., Clark, M.R., Escobar, E., Levin, L.A., 2011. Man and the Last Great Wilderness: Human Impact on the Deep Sea. *PLoS One* 1–35.
- Ridame, C., Moal, M. Le, Guieu, C., Ternon, E., Biegala, I.C., Helguen, S.L., 2011. Nutrient control of N<sub>2</sub> fixation in the oligotrophic Mediterranean Sea and the impact of Saharan dust events. *Biogeosciences* 8, 2773–2783. doi:10.5194/bg-8-2773-2011
- Rjeibi, M., Metian, M., Hajji, T., Guyot, T., Rafika, C., Bustamante, P., Rjeibi, M., Metian, M., Hajji, T., Guyot, T., Rafika, B.C., 2015. Seasonal Survey of Contaminants (Cd and Hg) and Micronutrients (Cu and Zn) in Edible Tissues of Cephalopods from Tunisia: Assessment of Risk and Nutritional Benefits. *J. Food Sci. Educ.* 80, 199–206.
- Ryabenko, E., Kock, A., Bange, H.W., Altabet, M.A., Wallace, D.W.R., 2012. Contrasting biogeochemistry of nitrogen in the Atlantic and Pacific Oxygen Minimum Zones. *Biogeosciences* 9, 203–215. doi:10.5194/bg-9-203-2012
- Santos, R.A., Haimovici, M., 1997. Reproductive biology of winter-spring spawners of *Illex argentinus* (Cephalopoda : Ommastrephidae) off southern Brazil. *Sci. Mar.* 61, 53–64.
- Seapy, R.R., Young, R.E., 1986. Concealment in epipelagic pterotracheid heteropods (Gastropoda) and cranchiid squids (Cephalopoda). *J. Zool. Ser. A* 210, 137–147. doi:10.1111/j.1469-7998.1986.tb03626.x
- Seibel, B.A., Thuesen, E. V., Childress, J.J., Gorodezky, L.A., 1997. Decline in pelagic cephalopod metabolism with habitat depth reflects differences in locomotory efficiency. *Biol. Bull.* 192, 262–278. doi:10.2307/1542720
- Seibel, B.A., Childress, J.J., 2000. Metabolism of benthic octopods (Cephalopoda) as a function of habitat depth and oxygen concentration. *Deep. Res. Part I Oceanogr. Res. Pap.* 47, 1247–1260. doi:10.1016/S0967-0637(99)00103-X
- Somero, G.N., 1992. Biochemical ecology of deep-sea animals. *Experientia* 48, 537–543.
- Storelli, M.M., 2008. Potential human health risks from metals (Hg, Cd, and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption: Estimation of target hazard quotients (THQs) and toxic equivalents (TEQs). *Food Chem. Toxicol.* doi:10.1016/j.fct.2008.05.011
- Stramma, L., Brandt, P., Schafstall, J., Schott, F., Fischer, J., Körtzinger, A., 2008. Oxygen minimum zone in the North Atlantic south and east of the Cape Verde Islands. *J. Geophys. Res. Ocean.* 113, 1–15. doi:10.1029/2007JC004369
- Stramma, L., Czeschel, R., Tanhua, T., Brandt, P., Visbeck, M., Giese, B.S., 2016. The flow field of the upper hypoxic eastern tropical North Atlantic oxygen minimum zone. *Ocean Sci.* 12, 153–167. doi:10.5194/os-12-153-2016
- Subotić, S., Spasić, S., Višnjić-Jeftić, Ž., Hegediš, A., Krpo-Ćetković, J., Mićković, B., Skorić, S., Lenhardt, M., 2013. Heavy metal and trace element bioaccumulation in target tissues of three edible predatory fish species from Bovan Reservoir (Serbia). *Ecotoxicol. Environ. Saf.* 23, 1884–1891. doi:10.1016/j.ecoenv.2013.08.020

- Thanonkaew, A., Benjakul, S., Visessanguan, W., 2006. Chemical composition and thermal property of cuttlefish (*Sepia pharaonis*) muscle. *J. Food Compos. Anal.* 19, 127–133. doi:10.1016/j.jfca.2005.04.008
- Thiel, H., 2015. Anthropogenic impacts on the deep sea in: *Ecosystems of the Deep Oceans*. Elsevier.
- Turekian, K.K., Imbrie, J., 1966. The distribution of trace elements in deep-sea sediments of the Atlantic Ocean. *Earth Planet. Sci. Lett.* 1, 161–168.
- Ueda, T., Nakahara, M., Ishii, T., Suzuki, Y., Suzuki, H., 1979. Amounts of Trace Elements in Marine Cephalopods. *J.Radia.Res.* 20, 338–342.
- Voss, N., 1980. A generic revision of the Cranchiidae (Cephalopoda; Oegopsida). *Bull. Mar. Sci.* 30, 365–412.
- Voss, N.A., Voss, R.S., 1983. Phylogenetic relationships in the cephalopod family cranchiidae (Oegopsida). *Malacologia* 23, 397–426. doi:10.1007/s12526-013-0197-9
- Voss, N.A., Stephen, S.J., Dong, Z., 1992. Family Cranchiidae, Prosch 1849, in: MJ, S., Roper, C.F.E., Mangold, K., Clarke, M., von Boletzky, S. (Eds.), *Larval and Juvenile Cephalopods: A Manual for Their Identification*. *Smithson Contrib Zool*, pp. 187–210.
- Voss, N.A., Voss, R.S., 1983. Phylogenetic relationships in the cephalopod family cranchiidae (Oegopsida). *Malacologia* 23, 397–426. doi:10.1007/s12526-013-0197-9
- Warrant, E.J., Locket, N.A., 2004. Vision in the deep sea. *Biol. Rev.* 79, 671–712. doi:10.1017/s1464793103006420
- Wu, Y.Y., Shen, Y., Huang, H., Yang, X.Q., Zhao, Y.Q., 2016. Trace Element Accumulation and Tissue Distribution in the Purpleback Flying Squid *Sthenoteuthis oualaniensis* from the Central and Southern South China Sea. *Biol Trace Res.* doi:10.1007/s12011-016-0751-y



## Annex A

The table presented in this Annex correspond to the information about the collected cranchiid specimens.

### List of tables:

**Table A1:** Collection information on cranchiids used in this study; Species, Station, Gear, Depth [m], Latitude, Longitude, Dorsal mantle length (DML) [mm]

**Table A1:** Collection information on the cranchiids used in this study; Species, Station, Gear, Depth [m], Latitude, Longitude, Dorsal mantle length (DML) [mm]

Species	Station	Gear	Depth [m]	Latitude	Longitude	DML [mm]
<i>Cranchia scabra</i>	Senghor Summit	IKMT	0-90	17,10.52	21,57.6	15
<i>Cranchia scabra</i>	Senghor Summit	IKMT	0-90	17,10.52	21,57.6	9
<i>Cranchia scabra</i>	Eddy Core	MOC-1	0-200	17,4.63	24,54.43	12
<i>Cranchia scabra</i>	Eddy Core	MOC-1	50-0	16,8.81	21,20.52	7
<i>Cranchia scabra</i>	Eddy Core	MOC-1	50-0	16,8.81	21,20.52	6
<i>Cranchia scabra</i>	Eddy Core	MOC-10	0-1000	16,8.81	21,20.52	7
<i>Cranchia scabra</i>	Eddy Core	MOC-10	0-1000	16,8.81	21,20.52	14
<i>Cranchia scabra</i>	CVSE	MOC-1	100-50	14,56.62	20,32.03	9
<i>Cranchia scabra</i>	CVSE	MOC-10	0-1000	14,56.62	20,32.03	6
<i>Cranchia scabra</i>	CVSE	MOC-10	0-1000	14,56.62	20,32.03	4
<i>Cranchia scabra</i>	CVSE	MOC-10	0-1000	14,56.62	20,32.03	6
<i>Cranchia scabra</i>	CVSE	MOC-10	0-1000	14,56.62	20,32.03	5
<i>Cranchia scabra</i>	CVS2	MOC-1	0-200	11,58.58	23,1.46	6
<i>Galiteuthis armata</i>	CVSE	MOC-10	0-1000	14,56.62	20,32.03	20
<i>Helicocranchia pfefferi</i>	CVSE	MOC-10	0-1000	14,56.62	20,32.03	22
<i>Helicocranchia pfefferi</i>	CVS1	MOC-1	0-200	12,0.19	20,59.91	13
<i>Helicocranchia pfefferi</i>	CVS2	MOC-10	400-100	11,58.58	23,1.46	13
<i>Leachia atlantica</i>	Senghor Slope Se	MOC-10	0-1000	17,4.63	24,54.43	20
<i>Leachia atlantica</i>	Senghor Slope Se	MOC-1	100-50	17,4.63	24,54.43	7
<i>Leachia atlantica</i>	Senghor Slope Se	MOC-1	50-0	17,4.63	24,54.43	10
<i>Leachia atlantica</i>	Eddy Core	MOC-1	50-0	16,8.81	21,20.52	12
<i>Leachia sp.</i>	Senghor Hang NW	MOC-10	100-0	17,11.01	22,2.48	9
<i>Leachia sp.</i>	Senghor Hang NW	MOC-10	100-0	17,11.01	22,2.48	27
<i>Lechia atlantica</i>	Senghor Slope Se	MOC-10	0-1000	17,4.63	24,54.43	23
<i>Liocranchia reinhardtii</i>	CN Senghor Ref 585	MOC-10	0-200	18,5.086	21,59.94	50
<i>Liocranchia reinhardtii</i>	Senghor Summit	MOC-1	25-50	17,9.49	21,57.59	9
<i>Liocranchia reinhardtii</i>	Senghor Summit	MOC-1	50-25	17,9.49	21,57.59	7
<i>Liocranchia reinhardtii</i>	Senghor NW	MOC-1	0-200	17,10.97	22,2.50	6
<i>Liocranchia reinhardtii</i>	Senghor Summit	MOC-1	0-25	17,10.52	21,57.6	10
<i>Liocranchia reinhardtii</i>	Senghor Slope Se	MOC-10	0-1000	17,4.63	24,54.43	7
<i>Liocranchia reinhardtii</i>	Senghor Slope Se	MOC-10	0-1000	17,4.63	24,54.43	7
<i>Liocranchia reinhardtii</i>	Senghor Slope Se	MOC-10	0-1000	17,4.63	24,54.43	4
<i>Liocranchia reinhardtii</i>	Senghor Slope Se	MOC-10	0-1000	17,4.63	24,54.43	4
<i>Liocranchia reinhardtii</i>	Senghor Slope Se	MOC-10	100-0	17,4.63	24,54.43	6
<i>Liocranchia reinhardtii</i>	Senghor Slope Se	MOC-1	0-200	17,4.63	24,54.43	4
<i>Liocranchia reinhardtii</i>	Senghor Slope Se	MOC-1	100-0	17,4.63	24,54.43	6
<i>Liocranchia reinhardtii</i>	Eddy Core	MOC-1	0-200	16,8.81	21,20.52	10
<i>Liocranchia reinhardtii</i>	Eddy Core	MOC-1	50-0	16,8.81	21,20.52	7
<i>Liocranchia reinhardtii</i>	Eddy Core	MOC-1	50-0	16,8.81	21,20.52	6
<i>Liocranchia reinhardtii</i>	Eddy Core	MOC-1	100-50	16,8.81	21,20.52	4
<i>Liocranchia reinhardtii</i>	Eddy Core	MOC-10	100-0	16,8.81	21,20.52	9
<i>Liocranchia reinhardtii</i>	Eddy Core	MOC-10	100-0	16,8.81	21,20.52	7
<i>Liocranchia reinhardtii</i>	Eddy Core	MOC-1	50-0	16,8.81	21,20.52	6
<i>Liocranchia reinhardtii</i>	Eddy Core	MOC-1	50-0	16,8.81	21,20.52	5
<i>Liocranchia reinhardtii</i>	Eddy Core	MOC-10	100-0	16,8.81	21,20.52	9
<i>Liocranchia reinhardtii</i>	Eddy Core	MOC-10	100-0	16,8.81	21,20.52	9
<i>Liocranchia reinhardtii</i>	Eddy Core	MOC-1	50-0	16,8.81	21,20.52	7

<i>Liocranchia reinhardtii</i>	CVSE	MOC-1	0-200	14,56.62	20,32.03	7
<i>Liocranchia reinhardtii</i>	CVSE	MOC-10	0-1000	14,56.62	20,32.03	25
<i>Liocranchia reinhardtii</i>	CVS1	MOC-10	1000-603	12,0.19	20,59.91	7
<i>Liocranchia reinhardtii</i>	CVS2	MOC-1	50-0	11,58.58	23,1.46	9
<i>Liocranchia reinhardtii</i>	CVS2	MOC-10	0-1000	11,58.58	23,1.46	4
<i>Liocranchia reinhardtii</i>	CVS2	MOC-10	0-1000	11,58.58	23,1.46	6
<i>Liocranchia reinhardtii</i>	CVS2	MOC-10	0-1000	11,58.58	23,1.46	7
<i>Liocranchia reinhardtii</i>	CVS2	MOC-10	0-1000	11,58.58	23,1.46	5
<i>Liocranchia reinhardtii</i>	CVS2	MOC-10	0-1000	11,58.58	23,1.46	6
<i>Megalocranchia oceanica</i>	CV00	MOC-1	0-200	17,35.30	24,17.16	34
<i>Teuthowenia maculata</i>	CVS2	MOC-10	0-1000	11,58.58	23,1.46	7
<i>Teuthowenia sp.</i>	Eddy Core	MOC-10	0-1000	16,8.81	21,20.52	10
<i>Teuthowenia sp.</i>	Eddy Core	MOC-1	50-0	16,8.81	21,20.52	10
<i>Teuthowenia sp.</i>	Eddy Core	MOC-1	0-200	16,8.81	21,20.52	3
<i>Teuthowenia sp.</i>	Eddy Core	MOC-1	0-200	16,8.81	21,20.52	4
<i>Teuthowenia sp.</i>	CVSE	MOC-10	0-1000	14,56.62	20,32.03	5,5

## **Annex B**

The tables present in this Annex correspond to the trace elements concentrations in the different analyzed tissues

### **List of tables**

Table B1: Trace elements concentration ( $\mu\text{g g}^{-1}$  dw) on the cranchiids digestive gland

Table B2: Trace elements concentration ( $\mu\text{g g}^{-1}$  dw) on the cranchiids mantle

Table B3: Trace elements concentration ( $\mu\text{g g}^{-1}$  dw) on the cranchiids tentacles

**Table B1:** Trace elements concentration ( $\mu\text{g g}^{-1}$  dw) on the cranchiids digestive gland

Species	Order	Family	V	Cr	Co	Ni	Cu	Zn	As	Se	Cd	Pb
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	2,7259	-	0,0839	-	8,9277	41,6640	4,8734	2,2057	15,6249	-
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	2,7178	1,5238	-	1,3176	6,9724	41,7594	4,6670	1,6947	6,3950	0,4265
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	1,6549	0,9967	0,0496	0,6246	1,6875	13,5537	3,6466	1,1055	13,1266	0,2187
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	1,2658	0,7095	0,0994	0,1990	2,6161	21,5167	4,3667	1,2531	9,7509	0,1582
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	1,8027	-	-	1,4035	4,4453	25,8037	2,6976	1,2090	1,3436	0,1023
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	4,1412	0,6829	-	-	3,2381	19,3356	4,9133	1,7014	9,4604	0,0879
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	1,6852	-	-	1,5595	4,6785	28,1233	2,7595	1,3831	1,4716	0,0825
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	0,8160	0,9360	0,0361	-	2,7790	9,5863	1,4184	1,0510	10,9879	0,0546
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	1,6797	-	-	1,1674	3,6007	21,4008	2,1444	1,9576	1,0866	0,0388
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	0,8390	0,4176	-	-	1,3789	10,6827	1,7275	1,4117	10,5293	0,0181
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	6,4199	0,5548	-	-	1,7015	7,6911	2,0118	1,0243	9,0868	0,0161
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	1,3411	2,3568	-	-	1,5485	12,1595	1,8714	1,2058	10,6233	-
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	2,3598	0,5674	-	-	1,5235	7,7935	2,1357	1,5315	9,1853	-
<i>Helicocranchia pfefferi</i>	Oegopsida	Cranchiidae	2,0757	1,2005	0,2919	0,2757	13,7926	49,1830	9,8493	1,6375	11,8332	0,1399
<i>Helicocranchia pfefferi</i>	Oegopsida	Cranchiidae	4,6996	0,5111	0,0738	0,2493	3,5075	24,2158	5,7956	1,2882	9,6956	0,0579
<i>Helicocranchia pfefferi</i>	Oegopsida	Cranchiidae	1,4128	0,6285	-	0,2782	2,5669	17,3467	4,9828	1,3338	6,5975	0,0259
<i>Leachia atlantica</i>	Oegopsida	Cranchiidae	2,5758	1,0056	0,0866	0,4438	4,4206	29,9887	5,9717	1,7452	29,7558	0,1367
<i>Leachia atlantica</i>	Oegopsida	Cranchiidae	-	0,5838	0,0893	-	2,0230	19,0608	6,2464	1,7185	23,3916	0,0976
<i>Leachia atlantica</i>	Oegopsida	Cranchiidae	1,2352	0,9808	0,0435	-	6,1825	10,5007	5,1783	1,6098	12,8417	0,0209
<i>Leachia atlantica</i>	Oegopsida	Cranchiidae	1,9316	0,6340	-	-	2,9325	11,8491	5,6433	1,4131	11,8795	0,0100
<i>Leachia atlantica</i>	Oegopsida	Cranchiidae	0,8236	0,3689	-	-	1,5252	11,0960	3,4618	1,3209	11,3029	-
<i>Leachia sp.</i>	Oegopsida	Cranchiidae	3,5146	1,1679	0,0748	0,4225	4,4438	18,6125	7,1526	1,0870	14,5611	0,0494
<i>Leachia sp.</i>	Oegopsida	Cranchiidae	1,7650	1,6944	-	0,4513	5,7335	15,4534	3,6653	1,8534	13,1952	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,5000	1,8000	-	4,0000	5,0000	26,0000	8,0000	2,4000	32,0000	0,4000
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,0000	0,6000	0,0700	-	11,0000	47,0000	7,0000	-	25,0000	0,2700
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	2,4644	0,9347	0,0724	0,5898	14,1434	43,7107	5,5034	2,0148	9,7123	0,2261
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	2,6744	1,9032	-	0,4718	7,8093	18,1961	3,4743	1,4124	13,4462	0,2239
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,9897	-	-	1,6864	4,9879	29,8202	3,1259	1,8528	1,5030	0,1693
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,3000	1,3000	0,3000	3,0000	8,0000	46,0000	5,0000	-	27,0000	0,1600
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	2,0054	0,6985	-	-	3,6314	11,5842	3,6120	1,5428	11,0960	0,1373
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	3,9614	0,6515	-	-	7,1629	17,7335	4,4777	1,1244	6,4231	0,1021
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	2,0727	-	0,0606	0,9688	3,1737	17,9436	1,7935	0,5949	0,8933	0,1008
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	3,7562	2,6100	0,0956	1,5218	3,2481	38,6491	3,7898	2,1190	15,1157	0,0725
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,4000	1,5159	-	0,1634	4,5782	45,3041	3,6199	1,5648	10,4289	0,0528
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,9036	0,3848	0,0337	-	3,3591	17,3802	4,0169	1,2445	11,8483	0,0467

<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	0,8771	0,5647	0,0347	-	5,5708	12,6855	3,2935	1,4275	10,7011	0,0360
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	0,9506	0,5499	0,0503	-	2,4717	23,0188	4,2704	1,2774	23,1693	0,0291
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	0,9063	0,3799	-	-	3,2560	16,0647	4,1567	1,1665	17,8540	0,0247
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	4,4491	0,5796	-	-	1,9014	13,0930	5,1295	1,1221	14,0874	0,0210
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,2531	0,4343	-	-	3,6410	13,5643	3,7067	1,1228	8,0717	0,0194
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	3,2601	1,4521	-	-	1,6925	5,9679	1,7569	1,0192	6,8412	0,0189
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	-	0,9986	-	-	0,8752	8,6476	3,9783	1,1164	9,7103	0,0177
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,6590	0,5260	0,1114	-	2,1522	13,5865	5,1157	0,9524	15,8819	0,0174
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,6477	1,3572	-	-	1,8907	17,2332	4,7532	1,1573	14,1002	0,0133
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	3,5790	0,3695	0,1112	0,4081	13,3748	54,7983	-	-	25,7488	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,0272	4,0857	-	0,9425	4,1648	11,4351	2,1573	1,1826	16,1844	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	2,2354	1,7471	0,1098	0,7222	2,9349	16,2263	1,7809	1,7880	14,4603	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,2660	1,2993	-	-	4,9619	9,5772	4,5141	1,6336	14,1523	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,2137	0,3235	-	-	2,5274	12,7347	2,8141	1,3136	11,3821	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,5988	0,5879	-	-	2,3580	24,5432	2,7143	1,0491	10,5298	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,9442	0,5419	-	-	2,6250	7,0631	2,4004	1,2926	9,3034	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,6633	1,9832	-	0,4958	3,2848	7,5600	2,8102	0,6828	8,9450	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	0,7861	0,6639	-	-	1,7445	12,2358	5,2863	1,3910	8,8475	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	0,7621	0,3120	-	0,1374	7,0258	8,8402	2,7567	1,3775	7,7513	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,4772	0,6968	0,0489	0,4040	2,3057	18,6571	1,9341	1,2913	6,8972	-
<i>Megalocranchia oceanica</i>	Oegopsida	Cranchiidae	2,4614	1,4781	0,0626	0,3994	14,2076	31,3734	6,9306	2,0126	9,2145	0,2544
<i>Teuthowenia maculata</i>	Oegopsida	Cranchiidae	2,0862	0,4474	0,6139	0,4003	4,5325	16,1388	4,6214	2,0365	27,2992	0,0669
<i>Teuthowenia sp.</i>	Oegopsida	Cranchiidae	1,0829	0,3088	0,3288	0,2660	1,4093	21,4679	11,6317	2,0959	19,6018	0,1338
<i>Teuthowenia sp.</i>	Oegopsida	Cranchiidae	2,2042	0,4068	0,2903	0,4697	4,9665	19,5508	6,9877	2,2043	25,2983	0,1026
<i>Teuthowenia sp.</i>	Oegopsida	Cranchiidae	1,1852	0,4458	0,3074	0,5469	2,5543	17,3172	5,5809	2,1555	24,4509	0,0391
<i>Teuthowenia sp.</i>	Oegopsida	Cranchiidae	1,7539	0,4883	0,2459	0,3628	11,7866	17,1127	5,8641	2,3011	20,5422	-
<i>Teuthowenia sp.</i>	Oegopsida	Cranchiidae	0,1325	-	-	-	-	-	-	-	-	-

**Table B2:** Trace elements concentration ( $\mu\text{g g}^{-1}$  dw) on the cranchiids mantle

<i>Species</i>	<b>Order</b>	<b>Family</b>	<b>Cd</b>	<b>V</b>	<b>Cr</b>	<b>Co</b>	<b>Ni</b>	<b>Cu</b>	<b>Zn</b>	<b>As</b>	<b>Se</b>	<b>Pb</b>
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	0,0297	4,5898	8,9038	-	26,3934	6,3244	39,8364	1,1137	0,9715	1,0779
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	6,4363	1,9223	-	-	7,2944	22,4911	130,8414	12,8099	7,6553	0,6673
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	2,2342	3,2871	18,7737	-	1,9853	7,3076	41,1201	3,9373	0,7738	0,4740
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	2,0783	2,8945	51,3491	-	2,0636	7,0222	38,5239	3,5145	1,1246	0,3684
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	0,4756	3,2509	0,6732	0,0465	0,3810	1,5016	8,3406	0,8452	-	0,1033
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	1,1198	7,1576	6,4506	-	5,8326	5,2373	53,8075	5,6058	4,7265	-
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	0,6225	4,5911	10,0535	-	-	18,9068	11,6654	2,3315	1,2088	-
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	0,5955	3,3368	13,1799	-	3,0347	-	21,3766	1,3766	-	-
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	0,1399	5,5721	3,5103	-	-	1,7745	22,6480	0,3686	-	-
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	0,1397	1,4881	5,4400	0,0887	2,7168	8,1890	90,7411	0,0942	1,1336	-
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	-	6,7839	24,6000	-	-	-	165,1774	-	1,8772	-
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	-	-	-	-	-	-	-	-	-	-
<i>Galiteuthis armata</i>	Oegopsida	Cranchiidae	0,6371	2,2726	31,8921	-	0,6809	2,1595	12,6577	1,2942	0,3987	0,0901
<i>Helicocranchia pfefferi</i>	Oegopsida	Cranchiidae	1,9249	2,1695	99,1508	-	1,8181	6,3215	36,1493	3,5373	1,9432	0,2458
<i>Helicocranchia pfefferi</i>	Oegopsida	Cranchiidae	0,3523	2,4420	15,2872	0,0647	0,3655	1,3498	7,2289	0,7466	0,3241	0,0633
<i>Helicocranchia pfefferi</i>	Oegopsida	Cranchiidae	0,0674	3,5763	6,6165	-	9,5355	3,0050	46,7702	3,7547	0,6873	-
<i>Leachia atlantica</i>	Oegopsida	Cranchiidae	-	4,0330	29,7911	-	2,2032	8,3198	48,9753	3,2437	3,9297	11,9296
<i>Leachia atlantica</i>	Oegopsida	Cranchiidae	2,8098	3,0710	42,4037	-	2,7399	9,8325	54,3898	5,1735	0,8993	0,5631
<i>Leachia atlantica</i>	Oegopsida	Cranchiidae	1,6891	22,4896	4,8158	-	4,9758	4,3377	6,9171	6,2367	2,9581	-
<i>Leachia atlantica</i>	Oegopsida	Cranchiidae	0,1494	34,4792	7,1603	-	-	-	5,9669	8,3265	1,2538	-
<i>Leachia sp.</i>	Oegopsida	Cranchiidae	1,8136	1,0582	1,6978	-	1,5080	5,8419	185,6154	9,5753	1,5886	0,3661
<i>Leachia sp.</i>	Oegopsida	Cranchiidae	-	2,4933	2,7500	-	-	10,2793	4,9158	3,1728	3,4599	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	3,3355	2,3482	-	-	3,3854	12,0178	66,1662	6,4270	3,1956	0,5303
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	4,6430	59,8344	7,7800	-	1,2370	9,1776	58,1872	7,5736	1,7588	0,5005
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	0,4000	2,8000	6,0000	-	16,0000	44,0000	5,0000	6,0000	4,0000	0,2300
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	0,0800	2,2000	4,0000	0,1900	22,0000	12,0000	18,0000	5,0000	10,0000	0,2100
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	0,8447	3,1894	11,0985	0,0370	0,8205	2,8874	16,1865	1,5806	0,2321	0,1860
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	-	2,3335	3,6203	-	-	1,9143	20,6900	2,8713	4,8929	0,1844
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	3,2403	2,1081	0,9243	0,0665	0,4688	8,5383	39,8740	8,7998	2,0125	0,1515
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	0,0400	1,1000	1,4000	-	10,0000	4,0000	49,0000	4,0000	10,0000	0,1400
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	10,0884	7,7832	0,4896	-	-	2,2590	12,3213	3,8997	0,9205	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,4826	6,6377	10,0019	-	4,3228	-	17,1381	0,1227	4,3147	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	0,8472	2,3984	1,4090	-	-	5,9934	27,9435	3,5361	2,2400	-

<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	0,3289	11,1652	4,7679	-	-	16,2824	57,7769	6,9022	4,3412	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	0,1838	1,5433	1,3123	-	-	7,9791	5,5914	0,4554	-	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	0,1340	5,5135	1,8017	-	-	8,9088	19,4043	3,7374	-	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	0,1287	2,4804	0,8437	-	-	15,9181	13,0392	1,1632	1,1218	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	0,1105	1,9224	10,0758	-	-	1,4647	40,6172	2,8801	0,4487	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	0,0842	5,1162	7,5701	-	29,0467	-	212,7438	2,0921	5,0514	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	0,0559	4,1665	5,5507	-	-	0,6906	8,0545	3,3634	1,9803	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	-	9,8077	2,3734	-	-	25,9471	-	2,5732	8,2050	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	-	4,6964	3,2260	-	-	2,7600	25,9840	2,6562	2,9606	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	-	7,0117	5,1549	0,0650	6,2238	0,3578	25,8126	4,8086	0,8856	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	-	4,7000	5,3000	-	6,1817	-	98,8621	3,6954	-	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	-	3,5000	3,3000	-	-	0,9980	27,1295	2,4510	-	-
<i>Megalocranchia oceanica</i>	Oegopsida	Cranchiidae	8,8996	11,2147	38,3680	0,1472	6,3945	14,0317	45,1655	8,0130	2,1354	0,9195
<i>Teuthowenia maculata</i>	Oegopsida	Cranchiidae	-	4,5310	13,7848	-	3,9974	-	15,9972	0,0627	-	-
<i>Teuthowenia sp.</i>	Oegopsida	Cranchiidae	2,4892	2,8876	52,7647	-	2,4523	8,7454	47,4107	4,4242	1,2619	0,4891
<i>Teuthowenia sp.</i>	Oegopsida	Cranchiidae	2,3244	2,6188	60,5787	-	2,1539	7,2796	42,2203	4,1051	0,9489	0,4271
<i>Teuthowenia sp.</i>	Oegopsida	Cranchiidae	1,2931	2,6339	50,9892	0,0582	1,3022	4,3068	24,8693	2,5025	1,0068	0,2212
<i>Teuthowenia sp.</i>	Oegopsida	Cranchiidae	0,0794	4,1746	1,9737	-	35,2539	10,6070	36,0762	6,6440	1,5561	-
<i>Teuthowenia sp.</i>	Oegopsida	Cranchiidae	0,0558	6,7508	1,9161	-	-	2,9452	35,7126	8,5056	1,5546	-

**Table B3:** Trace elements concentration ( $\mu\text{g g}^{-1}$  dw) on the cranchiids tentacles

Species	Order	Family	V	Cr	Co	Ni	Cu	Zn	As	Se	Cd	Pb
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	3,5980	-	-	3,5133	32,0175	-	8,6861	8,6820	-	-
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	0,6663	3,5109	-	13,6882	7,2718	-	12,1567	9,1921	0,0165	-
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	-	-	-	5,4637	-	-	11,7619	25,6719	-	-
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	1,0111	-	-	0,4667	-	-	5,9073	27,7087	-	-
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	0,4710	-	-	-	59,6663	-	17,1395	57,4038	-	-
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	0,0806	-	-	-	-	-	6,9907	24,9972	-	-
<i>Cranchia scabra</i>	Oegopsida	Cranchiidae	-	-	-	-	-	-	5,4211	21,2271	-	-
<i>Helicocranchia pfefferi</i>	Oegopsida	Cranchiidae	1,1567	-	-	-	8,1033	-	10,3547	5,3605	0,1341	-
<i>Helicocranchia pfefferi</i>	Oegopsida	Cranchiidae	1,5218	-	-	-	8,0463	-	10,5610	41,6825	-	-
<i>Helicocranchia pfefferi</i>	Oegopsida	Cranchiidae	-	-	-	-	-	-	17,0639	66,1869	-	-
<i>Leachia atlantica</i>	Oegopsida	Cranchiidae	2,0889	11,0952	-	-	-	-	8,6655	24,1776	-	-
<i>Leachia atlantica</i>	Oegopsida	Cranchiidae	2,1166	-	-	1,1301	50,5448	-	14,2751	17,2624	-	-
<i>Leachia atlantica</i>	Oegopsida	Cranchiidae	2,0223	-	-	-	-	0,7994	8,8063	6,8076	3,9687	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	2,8309	0,5908	0,0526	0,6899	5,4305	38,0098	9,8168	2,3694	0,8590	0,0508
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	4,0000	2,9000	-	-	2,6000	6,0000	5,0000	2,6000	0,0700	5,0000
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,7427	-	-	8,7572	37,9599	-	7,6543	21,0598	-	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	3,0000	1,0000	-	-	1,3000	1,6000	10,0000	22,0000	0,1700	0,1300
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	2,7535	-	-	-	2,6161	18,2948	4,8040	7,4614	6,1511	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,4593	-	-	-	2,0255	20,0368	14,8370	3,4812	2,9162	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	2,3296	-	-	-	1,1996	15,7208	11,2908	4,1698	2,5170	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	6,1040	-	-	-	31,4895	-	26,0958	83,6687	-	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	0,2919	-	-	-	14,3796	-	2,4405	10,9285	-	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	-	-	-	-	-	-	11,7422	44,5974	0,0270	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	0,5209	-	-	2,5881	-	-	12,2507	34,6263	-	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,3570	-	-	0,8965	-	-	8,2815	25,9433	-	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	-	-	-	0,9416	-	-	13,4985	31,1434	-	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	5,0532	-	-	-	-	-	27,9325	85,0318	-	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	-	-	-	-	-	-	21,3133	66,6409	-	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	2,8998	-	-	-	-	-	11,5131	34,6413	-	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	2,1534	-	-	-	-	-	15,2568	42,3812	-	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,6971	-	-	-	-	-	11,4962	38,6206	-	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,4825	-	-	-	-	-	14,2149	30,8726	-	-
<i>Liocranchia reinhardtii</i>	Oegopsida	Cranchiidae	1,0756	-	-	-	-	-	5,8095	25,3640	-	-



<i>Megalocranchia oceanica</i>	Oegopsida	Cranchiidae	4,8683	-	0,1578	5,5380	-	-	7,2999	6,1222	1,4254	-
<i>Teuthowenia sp.</i>	Oegopsida	Cranchiidae	1,0829	-	-	-	2,8134	-	11,2093	4,0016	0,4006	-
<i>Teuthowenia sp.</i>	Oegopsida	Cranchiidae	0,8057	-	-	-	73,7255	-	7,2846	28,0934	-	-
<i>Teuthowenia sp.</i>	Oegopsida	Cranchiidae	16,9020	12,6842	-	-	-	-	7,6912	10,7866	-	-

## **Annex C**

The tables present in this Annex correspond to the trace elements concentrations on cephalopods from the literature.

### **List of tables**

Table C1: Trace elements concentration ( $\mu\text{g g}^{-1}$  dw) on cephalopods digestive gland from literature

Table C2: Trace elements concentration ( $\mu\text{g g}^{-1}$  dw) on cephalopods mantle from literature

Table C3: Trace elements concentration ( $\mu\text{g g}^{-1}$  dw) on cephalopods tentacles from literature

**Table C1:** Trace elements concentration ( $\mu\text{g g}^{-1}$  dw) on cephalopods digestive gland from literature

Species	Order	Family	V	Cr	Co	Ni	Cu	Zn	As	Se	Cd	Pb	Ref
<i>Illex argentinus</i>	Oegopsida	Ommastrephidae	-	-	-	-	77.19	117.53	-	-	606.25	-	
<i>Illex argentinus</i>	Oegopsida	Ommastrephidae	-	-	-	-	92.11	111.33	-	-	423.75	-	
<i>Illex argentinus</i>	Oegopsida	Ommastrephidae	-	-	-	-	291.75	201.378	-	-	328.8	-	Gerpe et al, 2000
<i>Illex argentinus</i>	Oegopsida	Ommastrephidae	-	-	-	-	141.2	125.63	-	-	319.94	-	
<i>Illex argentinus</i>	Oegopsida	Ommastrephidae	-	-	-	-	296.04	186.66	-	-	242.88	-	
<i>Illex argentinus</i>	Oegopsida	Ommastrephidae	-	-	-	-	208.83	104.11	-	-	204.95	-	
<i>Illex argentinus</i>	Oegopsida	Ommastrephidae	-	-	-	-	-	-	-	-	23.13	-	Dorneless et al, 2007
<i>Nototodarus gouldi</i>	Oegopsida	Ommastrephidae	-	-	-	-	230	799	-	-	58	-	Smith et al, 1984
<i>Nototodarus gouldi</i>	Oegopsida	Ommastrephidae	-	-	-	-	258	596	-	-	45	-	
<i>Nototodarus gouldi</i>	Oegopsida	Ommastrephidae	-	-	-	-	363	830	-	-	33	-	Finger and Smith 1987
<i>Ommastrephes bartramii</i>	Oegopsida	Ommastrephidae	-	-	-	-	1.72	513	-	-	782	-	Martin and Flegal 1975
<i>Ommastrephes bartramii</i>	Oegopsida	Ommastrephidae	-	-	-	-	675	97.5	-	-	-	-	Ueda et al, 1979
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	-	-	-	-	-	-	-	-	544.03	-	Murthy et al, 2008
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	-	-	-	-	195	163	-	-	287	-	Martin and Flegal, 1975
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	-	0.09	-	-	186.57	111.12	0.1	-	164.75	-	Wu et al, 2016
<i>Todarodes pacificus</i>	Oegopsida	Ommastrephidae	-	-	0.6	-	7.13	53.75	-	-	-	-	Ueda et al, 1979
<i>Todarodes sagittatus</i>	Oegopsida	Ommastrephidae	-	-	-	-	-	-	-	-	65.3	-	Pierce et al, 2008
<i>Todarodes sagittatus</i>	Oegopsida	Ommastrephidae	-	-	-	-	-	-	-	-	18.6	-	Chouvelon et al, 2011
<i>Todaropsis eblanae</i>	Oegopsida	Ommastrephidae	-	-	-	-	-	-	-	-	25.2	-	Pierce et al, 2008
<i>Illex argentinus</i>	Oegopsida	Ommastrephidae	-	-	-	-	67.5	58.75	-	-	45	0.75	Falandysz, 1988
<i>Illex argentinus</i>	Oegopsida	Ommastrephidae	-	-	-	-	30	41.25	-	-	47.5	0.3	
<i>Illex coindetti</i>	Oegopsida	Ommastrephidae	-	0.6	-	-	257.25	82.85	-	-	9.25	2.21	Duysak and Dural, 2015
<i>Illex coindetti</i>	Oegopsida	Ommastrephidae	-	0.9	-	-	405.13	106.61	-	-	11.89	2.1	
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	-	-	1.63	-	150	43.75	-	-	102.5	0.41	Ichihashi et al, 2001b
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	-	-	-	-	139.5	56.09	-	-	25.01	0.11	
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	-	-	-	-	97.25	57.48	-	-	16.81	0.10	
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	-	-	-	-	27.46	39.33	-	-	17.73	0.09	
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	-	-	-	-	71.51	43.67	-	-	28.05	0.08	
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	-	-	-	-	117.69	53.55	-	-	18.59	0.08	Panutrakul et al, 2007
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	-	-	-	-	62.49	43.8	-	-	15.03	0.07	
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	-	-	-	-	111.51	53.08	-	-	18.48	0.07	
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	-	-	-	-	82.65	69.68	-	-	28.92	0.06	
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	-	-	-	-	75.79	65.98	-	-	25.16	0.05	
<i>Todarodes filippovae</i>	Oegopsida	Ommastrephidae	0.91	0.27	10.5	3.54	-	94.3	11.5	5.75	246	0.52	Kojadinovic et al, 2011
<i>Todarodes filippovae</i>	Oegopsida	Ommastrephidae	0.59	0.14	0.92	0.55	137	88	17.1	8.92	98.5	0.07	

<i>Todarodes pacificus</i>	Oegopsida	Ommastrephidae	0.22	0.42	0.2	1.2	130	78	4.9	-	19	0.64	
<i>Todarodes pacificus</i>	Oegopsida	Ommastrephidae	5.5	0.15	0.31	2.8	11	78	3	-	24	0.24	Ichihashi et al, 2001
<i>Todarodes pacificus</i>	Oegopsida	Ommastrephidae	0.66	0.43	0.2	0.76	140	75	3.2	-	8.7	0.14	
<i>Doryteuthis bleekeri</i>	Myopsida	Loliginidae	-	-	0.06	-	112.5	31.25	-	-	-	-	Ueda et al, 1979
<i>Alloteuthis sp.</i>	Myopsida	Loliginidae	-	-	-	-	-	-	-	-	9.48	-	Pierce et al, 2008
<i>Loligo forbesi</i>	Myopsida	Loliginidae	-	-	-	-	-	-	-	-	12.2	-	
<i>Loligo forbesi</i>	Myopsida	Loliginidae	-	-	-	-	-	-	-	-	8	-	
<i>Loligo forbesi</i>	Myopsida	Loliginidae	-	-	-	-	-	-	-	-	7.4	-	Chouvelon et al, 2011
<i>Loligo forbesi</i>	Myopsida	Loliginidae	-	-	-	-	-	-	-	-	4	-	
<i>Loligo forbesi</i>	Myopsida	Loliginidae	-	-	-	1.375	137.5	5.375	17.875	-	1.875	-	Craig and Overnell 2003
<i>Loligo opalescens</i>	Myopsida	Loliginidae	-	-	-	-	8.37	449	-	-	121.5	-	Martin and Flegal 1975
<i>Loligo opalescens</i>	Myopsida	Loliginidae	-	-	-	-	5.35	247	-	-	85	-	
<i>Loligo plei</i>	Myopsida	Loliginidae	-	-	-	-	-	-	-	-	24.5	-	Dorneless et al, 2007
<i>Loligo vulgaris</i>	Myopsida	Loliginidae	-	-	-	-	-	-	-	-	5.6	-	
<i>Loligo vulgaris</i>	Myopsida	Loliginidae	-	-	-	-	-	-	-	-	4.8	-	Chouvelon et al, 2011
<i>Loligo vulgaris</i>	Myopsida	Loliginidae	-	-	-	-	-	-	-	-	4.4	-	
<i>Loligo opalescens</i>	Myopsida	Loliginidae	-	-	-	-	375	47.5	-	-	20	3.875	
<i>Loligo opalescens</i>	Myopsida	Loliginidae	-	-	-	-	72.5	33.75	-	-	4.5	2	Falandysz, 1991
<i>Loligo opalescens</i>	Myopsida	Loliginidae	-	-	-	-	250	47.5	-	-	17.5	1.375	
<i>Sepia officinalis</i>	Sepiida	Sepiidae	-	-	-	-	293	907	-	-	137	-	Raimundo et al, 2005
<i>Sepia officinalis</i>	Sepiida	Sepiidae	-	-	-	-	2765	2754	-	-	35	-	
<i>Sepia officinalis</i>	Sepiida	Sepiidae	5	1.1	3.27	1.3	315	571	-	-	12.67	-	Miranda and Bentley, 1992
<i>Sepia officinalis</i>	Sepiida	Sepiidae	3.3	-	10	-	600	1400	-	-	25	2.2	Miramand et al, 2006
<i>Graneledone sp</i>	Octopoda	Octopodidae	-	-	-	-	1092	102	-	-	369	-	Bustamante et al, 1998 a
<i>Benthoctopus thielei</i>	Octopoda	Octopodidae	-	-	-	-	42	416	-	-	215	-	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	2550	-	-	-	-	-	Ghiretti-Magaldi et al, 1958
<i>Eledone cirrhosa</i>	Octopoda	Octopodidae	3.3	0.8	2.06	2.5	456	646	-	-	24	-	Miranda and Bentley, 1992
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	4.5	-	-	-	2500	1450	-	-	50	-	Miranda and Guary, 1980
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	28.021	81.05	-	-	-	-	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	-	666.93	-	-	184.69	3.47	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	411.28	666.60	-	-	158.78	3.20	Raimundo and Vale, 2008
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	404.05	582.12	-	-	104.90	2.92	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	91.08	569.05	-	-	95.95	2.43	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	59.73	316.10	-	-	93.89	2.09	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	601	616	-	-	185	-	Raimundo et al, 2005
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	998	2541	-	-	32	-	

<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	6.76	2632.46	2220.65	-	-	23.66	22.55	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	9.49	1124.43	1901.48	-	-	92.02	17.58	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	1061.67	4349.80	-	-	41.91	15.35	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	1781.77	2606.59	-	-	44.05	11.94	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	6.75	1864.49	1828.83	-	-	125.42	5	Raimundo et al, 2004
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	860.78	858.79	-	-	109.58	2.09	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	944.42	1800.56	-	-	61.26	2.04	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	8.15	1133.50	701.65	-	-	146.40	2.02	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	731.64	538.08	-	-	111.97	1.63	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	636.52	651.13	-	-	181.26	0.31	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	4200	48050	-	-	555	-	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	1597.44	2805.92	-	-	23.92	7.18	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	1138.89	2873.45	-	-	29.95	5.74	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	1361.21	2125.64	-	-	15.49	5.20	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	1020.03	1455.98	-	-	234.51	4.61	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	1437.63	1552.92	-	-	9.79	4.50	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	1488.92	863.48	-	-	156.24	4.12	Raimundo et al, 2010 a
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	1405.84	2040.52	-	-	151.63	3.73	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	1415.66	1275.38	-	-	15.49	3.47	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	1121.92	741.45	-	-	11.97	3.04	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	859.00	410.37	-	-	56.77	2.37	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	648.39	491.36	-	-	143.73	1.57	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	640.75	849.74	-	-	251.51	1.55	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	5.87	0.53	14.45	5.01	422.50	2626.33	16.90	15.69	103.88	9.98	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	3.69	0.56	5.90	2.01	187.35	1288.04	25.96	9.09	43.86	9.02	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	4.95	0.98	8.48	4.15	218.23	1378.31	20.52	12.39	54.09	6.59	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	4.18	0.41	8.79	6.43	211.15	1369.59	23.69	10.93	37.87	6.43	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	3.68	0.33	10.36	3.49	377.61	1348.60	12.58	9.57	41.57	6.10	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	4.08	0.44	5.46	1.93	145.89	566.29	22.18	8.95	54.5	3.57	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	4.18	1.12	9.81	2.55	865.79	1344.43	17.56	28.15	326.05	1.81	Raimundo et al, 2010 b
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	2.71	1.96	4.26	1.89	134.73	537.17	23.95	13.06	156.81	1.62	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	3.48	1.38	4.77	2.08	217.77	677.93	23.97	14.256	140.30	1.34	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	3.83	1.32	5.41	3.92	123.52	744.46	21.48	22.88	143.77	1.24	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	6.97	0.54	16.92	1.83	930.95	1645.74	22.73	38.04	556.03	1.14	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	8.44	0.95	7.41	2.53	540.07	730.13	47.69	26.27	247.04	1.093	
<i>N.macromphalus</i>	Nautilida	Nautilidae	14.8	2.95	4.69	14	263	388	257	2.65	51.5	0.98	Pernice et al, 2009
<i>N.pompilius</i>	Nautilida	Nautilidae	8.42	1.37	3.85	9.77	311	470	210	2.35	74.3	0.62	
<i>N.macromphalus</i>	Nautilida	Nautilidae	8.8	4.4	7.8	16.3	106	672	166	-	4.45	0.46	Bustamante et al, 2000

**Table C2:** Trace elements concentration ( $\mu\text{g g}^{-1}$  dw) on cephalopods mantle from literature

Species	Order	Family	Cd	V	Cr	Co	Ni	Cu	Zn	As	Se	Pb	Ref
<i>Illex coindetti</i>	Oegopsida	Ommastrephidae	1.13	-	0.78	-	-	16.43	14.4	-	-	2.96	Duysak and Dural, 2015
<i>Illex coindetti</i>	Oegopsida	Ommastrephidae	1.69	-	0.66	-	-	16.48	17.85	-	-	1.86	
<i>Illex coindetti</i>	Oegopsida	Ommastrephidae	0.05	-	-	-	-	-	-	-	-	0.15	Storelli et al, 2008
<i>Illex argentinus</i>	Oegopsida	Ommastrephidae	0.08	-	-	-	-	2.13	15	-	-	0.14	Falandysz 1988
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	5.15	-	-	-	-	19.19	25.42	-	-	0.11	
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	1.48	-	-	-	-	7.21	20.19	-	-	0.06	
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	2.33	-	-	-	-	13.18	20.6	-	-	0.06	
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	18.14	-	-	-	-	29.04	24.19	-	-	0.04	
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	1.69	-	-	-	-	15.21	19.09	-	-	0.03	Panutrakul et al, 2007
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	2.92	-	-	-	-	6.93	17.84	-	-	0.03	
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	3.21	-	-	-	-	8.31	18.99	-	-	0.03	
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	3.73	-	-	-	-	9.7	18.56	-	-	0.02	
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	2.46	-	-	-	-	10.35	19.9	-	-	0.01	
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	5.96	-	2.64	-	-	13.13	45.85	0.06	-	-	Wu et al, 2016
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	1.8	-	-	-	-	-	-	-	-	-	Murthy et al, 2008
<i>Todarodes pacificus</i>	Oegopsida	Ommastrephidae	1.6	-	-	-	-	-	-	3.28	-	0.09	Nho et al, 2016
<i>Todarodes pacificus</i>	Oegopsida	Ommastrephidae	0.07	0.01	0.03	0.01	0.14	3.1	13	0.74	-	0.04	
<i>Todarodes pacificus</i>	Oegopsida	Ommastrephidae	0.04	0.01	0.03	0.02	0.1	3.4	16	2.9	-	0.04	Ichihashi et al, 2001
<i>Todarodes pacificus</i>	Oegopsida	Ommastrephidae	0.084	0.01	0.07	0.03	0.25	2.7	15	2.5	-	0.04	
<i>Todarodes pacificus</i>	Oegopsida	Ommastrephidae	-	-	-	0.01	-	1.5	15	-	-	-	Ueda et al, 1979
<i>Todaropsis eblanae</i>	Oegopsida	Ommastrephidae	1.55	-	-	-	-	-	-	-	-	-	Pierce et al, 2008
<i>Todarodes filippovae</i>	Oegopsida	Ommastrephidae	0.93	-	-	0.09	-	6.36	55.6	14.4	-	-	Kojadinovic et al, 2011
<i>Todarodes filippovae</i>	Oegopsida	Ommastrephidae	0.2	-	-	-	-	7.2	63.1	15.3	-	-	
<i>Todarodes sagittatus</i>	Oegopsida	Ommastrephidae	0.53	-	0.14	-	-	-	-	-	-	-	Georgantelis et al, 2001
<i>Todarodes sagittatus</i>	Oegopsida	Ommastrephidae	0.3	-	-	-	-	-	-	-	-	-	Pierce et al, 2008
<i>Ommastrephes bartramii</i>	Oegopsida	Ommastrephidae	-	-	-	0.01	-	3.25	17.5	-	-	-	Ueda et al, 1979
<i>Doryteuthis bleekeri</i>	Myopsida	Loliginidae	-	-	-	0.01	-	1.5	10	-	-	-	
<i>Sepioteuthis lessoniana</i>	Myopsida	Loliginidae	0.02	-	0.02	0.01	0.11	0.59	17.65	-	-	0.01	Jinadasa, 2014
<i>Alloteuthis sp.</i>	Myopsida	Loliginidae	0.8	-	-	-	-	-	-	-	-	-	Pierce et al, 2008
<i>Loligo forbesi</i>	Myopsida	Loliginidae	0.71	-	-	-	-	-	-	-	-	-	
<i>Loligo forbesi</i>	Myopsida	Loliginidae	0.13	-	-	-	1.38	5.13	21.25	-	-	0.75	Craig and Overnell, 2003
<i>Loligo opalescens</i>	Myopsida	Loliginidae	0.25	-	-	-	-	7.63	21.25	-	-	1.38	Falandysz, 1991
<i>Loligo patagonica</i>	Myopsida	Loliginidae	1	-	-	-	-	10	16.25	-	-	0.65	Falandysz, 1989
<i>Loligo vulgaris</i>	Myopsida	Loliginidae	0.05	-	-	-	0.03	1.88	15.75	-	-	0.13	Lourenço et al, 2009
<i>Loligo vulgaris</i>	Myopsida	Loliginidae	0.36	-	-	-	-	-	-	-	-	0.05	Storelli et al, 2008

<i>Loligo vulgaris</i>	Myopsida	Loliginidae	0.06	-	-	-	-	1.53	12.25	-	-	-	Rjeibi et al, 2015
<i>Loligo vulgaris</i>	Myopsida	Loliginidae	-	-	-	-	0.03	1.88	15.75	-	-	-	Lourenço et al, 2009
<i>Loligo duvauceli</i>	Myopsida	Loliginidae	0.03	-	0.18	0.02	0.01	20.44	26.53	-	-	0.02	Jinadasa, 2014
<i>Sepia officinalis</i>	Sepiida	Sepiidae	3.28	-	0.45	-	-	5.24	50.38	-	-	0.49	
<i>Sepia officinalis</i>	Sepiida	Sepiidae	2.46	-	0.5	-	-	2.71	24.44	-	-	0.4	
<i>Sepia officinalis</i>	Sepiida	Sepiidae	3.56	-	0.48	-	-	7.64	50.87	-	-	0.36	Ayas and Ozogul, 2011
<i>Sepia officinalis</i>	Sepiida	Sepiidae	2.79	-	0.45	-	-	4.06	26.74	-	-	0.29	
<i>Sepia officinalis</i>	Sepiida	Sepiidae	2.66	-	0.48	-	-	7.57	24.16	-	-	0.25	
<i>Sepia officinalis</i>	Sepiida	Sepiidae	1.1	0.5	-	-	-	70	156	-	-	0.2	Miramand et al, 2006
<i>Sepia officinalis</i>	Sepiida	Sepiidae	2.78	-	0.49	-	-	13.67	28	-	-	0.18	Ayas and Ozogul, 2011
<i>Sepia officinalis</i>	Sepiida	Sepiidae	0.39	-	-	-	0.06	5.63	22.13	-	-	0.05	Lourenço et al, 2009
<i>Sepia pharaonis</i>	Sepiida	Sepiidae	0.05	-	-	-	0.26	2.74	6.46	-	-	0.35	Farraj et al, 2011
<i>Sepia pharaonis</i>	Sepiida	Sepiidae	0.42	-	-	-	-	2.54	10.36	-	-	0.01	Thanonkaew et al, 2006
<i>Sepia latimanus</i>	Sepiida	Sepiidae	0.02	-	0.08	0.03	0.012	0.92	17.7	-	-	0.01	Jinadasa, 2014
<i>Sepia recurvirostra</i>	Sepiida	Sepiidae	0.05	-	-	-	-	7.13	24.53	-	0.03	-	Nurjanah et al, 2012
<i>Sepia sp</i>	Sepiida	Sepiidae	1.6	-	2.75	-	-	12.13	68.75	-	1.38	1.38	
<i>Octopus sp</i>	Octopoda	Octopodidae	-	2.13	3.36	-	-	14.63	86.25	-	2.25	1.63	Hoda et al, 2007
<i>Octopus vulgaris</i>	Octopoda	Octopodidae	0.06	-	-	-	2.98	17.63	90.87	-	-	2.78	
<i>Octopus vulgaris</i>	Octopoda	Octopodidae	0.45	-	-	-	2.27	29.92	87.61	-	-	0.87	
<i>Octopus vulgaris</i>	Octopoda	Octopodidae	0.45	-	-	-	-	32.22	85.94	-	-	0.73	
<i>Octopus vulgaris</i>	Octopoda	Octopodidae	0.65	-	-	-	3.1	25.04	85.78	-	-	0.72	
<i>Octopus vulgaris</i>	Octopoda	Octopodidae	0.92	-	-	-	-	16.63	104.35	-	-	0.7	
<i>Octopus vulgaris</i>	Octopoda	Octopodidae	0.2	-	-	-	2.08	23.01	82.25	-	-	0.66	
<i>Octopus vulgaris</i>	Octopoda	Octopodidae	1.81	-	-	-	-	33.24	86.88	-	-	0.61	Raimundo et al, 2004
<i>Octopus vulgaris</i>	Octopoda	Octopodidae	0.69	-	-	-	2.69	24.76	134.74	-	-	0.45	
<i>Octopus vulgaris</i>	Octopoda	Octopodidae	0.29	-	-	-	-	60.34	40.49	-	-	0.12	
<i>Octopus vulgaris</i>	Octopoda	Octopodidae	2.05	-	-	-	-	77.32	15.07	-	-	0.06	
<i>Octopus vulgaris</i>	Octopoda	Octopodidae	0.25	-	-	-	-	14.68	117.82	-	-	-	
<i>Octopus vulgaris</i>	Octopoda	Octopodidae	0.17	-	-	-	-	44.07	87.39	-	-	-	
<i>Octopus vulgaris</i>	Octopoda	Octopodidae	-	-	-	-	-	-	64.19	-	-	1.61	
<i>Octopus vulgaris</i>	Octopoda	Octopodidae	1.74	-	-	-	-	20.8	56.36	-	-	1.05	
<i>Octopus vulgaris</i>	Octopoda	Octopodidae	1.48	-	-	-	-	16.63	56.11	-	-	0.73	
<i>Octopus vulgaris</i>	Octopoda	Octopodidae	0.56	-	-	-	-	12.39	51.33	-	-	0.63	Raimundo and Vale, 2008
<i>Octopus vulgaris</i>	Octopoda	Octopodidae	0.32	-	-	-	-	12.3	51.31	-	-	0.52	
<i>Octopus vulgaris</i>	Octopoda	Octopodidae	0.25	-	-	-	-	8.32	47.32	-	-	0.3	
<i>Octopus vulgaris</i>	Octopoda	Octopodidae	0.48	-	-	-	0.03	4.75	22.13	-	0.023	-	Lourenço et al, 2009
<i>Eledone moschata</i>	Octopoda	Octopodidae	0.33	-	0.14	-	-	-	-	-	-	-	Georgantelis et al 2001

**Table C3:** Trace elements concentration ( $\mu\text{g g}^{-1}$  dw) on cephalopods tentacles from literature

Species	Order	Family	V	Cr	Co	Ni	Cu	Zn	As	Se	Cd	Pb	Ref
<i>Illex argentinus</i>	Oegopsida	Ommastrephidae	-	-	-	-	4.25	13.75	-	-	0.07	0.1875	Falandysz, 1988
<i>Illex coindetti</i>	Oegopsida	Ommastrephidae	-	0.78	-	-	8.44	11.41	-	-	0.85	2.35	Duysak and Dural, 2015
<i>Illex coindetti</i>	Oegopsida	Ommastrephidae	-	0.76	-	-	7.46	11.72	-	-	1.45	2.275	
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	-	-	-	-	-	-	-	-	1.85	-	Murthy et al, 2008
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	-	-	-	-	-	-	-	-	1.05	-	
<i>Sthenoteuthis oualaniensis</i>	Oegopsida	Ommastrephidae	-	0.51	-	-	47.85	71.29	0.07	-	-	-	Wu et al. 2016
<i>Loligo opalescens</i>	Myopsida	Loliginidae	-	-	-	-	5.375	31.25	-	-	0.18	1.075	Falandysz 1991
<i>Loligo patagonica</i>	Myopsida	Loliginidae	-	-	-	-	6.875	16.25	-	-	0.4	0.425	Falandysz 1989
<i>Loligo vulgaris</i>	Myopsida	Loliginidae	-	-	-	-	2.425	15.88	-	-	0.05	-	Rjeibi et al, 2015
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	30.64	72.43	-	-	0.24	1.11	Raimundo et al, 2004
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	22.66	69.23	-	-	0.32	0.53	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	0.56	14.12	79.28	-	-	0.03	0.34	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	7.98	67.92	-	-	0.14	0.4	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	8.15	12.41	83.01	-	-	0.41	0.11	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	27.28	133.8	-	-	0.32	-	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	10.94	79.19	-	-	0.28	0.17	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	9.86	71.51	-	-	3.5	0.11	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	28.04	106.3	-	-	0.77	0.08	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	0.89	13.36	76.95	-	-	0.12	-	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	1.58	13.1	73.11	-	-	0.17	0.80	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	0.65	21.07	84.94	-	-	0.04	0.52	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	12.49	49.60	-	-	0.25	0.51	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	12.39	49.38	-	-	0.18	0.51	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	12.38	46.09	-	-	0.16	0.4	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	8.23	44.28	-	-	0.14	0.3	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	8.22	43.48	-	-	0.11	0.29	
<i>Octopus vullgaris</i>	Octopoda	Octopodidae	-	-	-	-	16.43	65.61	-	-	0.262	-	
<i>Benthoctopus thielei</i>	Octopoda	Octopodidae	-	-	-	-	3	138	-	-	0.21	-	Bustamante et al, 1998
<i>Graeledone sp.</i>	Octopoda	Octopodidae	-	-	-	-	15	113	-	-	0.37	-	



## **Annex D**

The tables present in this Annex correspond to Dunn test results

### **List of tables**

Table D1: Dunn test p-value in the cranchiids digestive gland

Table D2: Dunn test p-value in the cranchiids mantle

Table D3: Dunn test p-value in the cranchiids tentacles

Table D4: Dunn test p-value between cranchiid tissues

Table D5: Dunn test p-value in the cephalopods digestive gland

Table D6: Dunn test p-value in the cephalopods mantle

Table D7: Dunn test p-value in the cephalopods tentacles

**Table D1:** Dunn test p-value in the cranchiids digestive gland

	<i>C.scabra</i>	<i>H.pfefferi</i>	<i>L.atlantica</i>	<i>Leachia</i> sp.	<i>L.reinhardtii</i>	
<i>Helicocranchia pfefferi</i>	0.25					<b>V</b>
<i>Leachia atlantica</i>	0.49	0.27				
<i>Leachia</i> sp.	0.24	0.45	0.25			
<i>Liocranchia reinhardtii</i>	0.29	0.16	0.37	0.16		
<i>Teuthowenia</i> sp.	0.09	0.06	0.13	0.07	0.13	
<i>Helicocranchia pfefferi</i>	0.25					<b>Cr</b>
<i>Leachia atlantica</i>	0.18	0.46				
<i>Leachia</i> sp.	0.21	0.12	0.09			
<i>Liocranchia reinhardtii</i>	0.25	0.38	0.29	0.12		
<i>Teuthowenia</i> sp.	0.01	0.07	0.06	0.01	0.01	
<i>Helicocranchia pfefferi</i>	0.14					<b>Co</b>
<i>Leachia atlantica</i>	0.41	0.20				
<i>Leachia</i> sp.	0.40	0.30	0.45			
<i>Liocranchia reinhardtii</i>	0.34	0.18	0.45	0.48		
<i>Teuthowenia</i> sp.	0.01	0.16	0.02	0.09	0.01	
<i>Helicocranchia pfefferi</i>	0.01					<b>Ni</b>
<i>Leachia atlantica</i>	-	-				
<i>Leachia</i> sp.	0.14	0.22	-			
<i>Liocranchia reinhardtii</i>	0.36	0.01	-	0.17		
<i>Teuthowenia</i> sp.	0.06	0.22	-	0.45	0.07	
<i>Helicocranchia pfefferi</i>	0.13					<b>Cu</b>
<i>Leachia atlantica</i>	0.45	0.18				
<i>Leachia</i> sp.	0.08	0.35	0.12			
<i>Liocranchia reinhardtii</i>	0.10	0.31	0.23	0.19		
<i>Teuthowenia</i> sp.	0.27	0.31	0.34	0.21	0.44	
<i>Helicocranchia pfefferi</i>	0.10					<b>Zn</b>
<i>Leachia atlantica</i>	0.30	0.06				
<i>Leachia</i> sp.	0.50	0.18	0.37			
<i>Liocranchia reinhardtii</i>	0.47	0.08	0.30	0.49		
<i>Teuthowenia</i> sp.	0.33	0.22	0.21	0.38	0.30	
<i>Helicocranchia pfefferi</i>	0.01					<b>As</b>
<i>Leachia atlantica</i>	0.01	0.44				
<i>Leachia</i> sp.	0.04	0.30	0.43			
<i>Liocranchia reinhardtii</i>	0.04	0.02	0.03	0.15		
<i>Teuthowenia</i> sp.	0.01	0.40	0.22	0.22	0.01	
<i>Helicocranchia pfefferi</i>	0.42					<b>Se</b>
<i>Leachia atlantica</i>	0.15	0.29				
<i>Leachia</i> sp.	0.50	0.45	0.26			
<i>Liocranchia reinhardtii</i>	0.50	0.41	0.13	0.50		
<i>Teuthowenia</i> sp.	0.06	0.17	0.32	0.16	0.04	
<i>Helicocranchia pfefferi</i>	0.46					<b>Cd</b>
<i>Leachia atlantica</i>	0.01	0.04				
<i>Leachia</i> sp.	0.05	0.1	0.46			
<i>Liocranchia reinhardtii</i>	0.02	0.16	0.08	0.22		
<i>Teuthowenia</i> sp.	0.02	0.07	0.42	0.48	0.16	
<i>Helicocranchia pfefferi</i>	0.36					<b>Pb</b>
<i>Leachia atlantica</i>	0.12	0.28				
<i>Leachia</i> sp.	0.30	0.39	0.46			
<i>Liocranchia reinhardtii</i>	0.21	0.46	0.24	0.4		
<i>Teuthowenia</i> sp.	0.5	0.38	0.19	0.31	0.31	

**Table D2:** Dunn test p-value in the cranchiids mantle

	<i>C.scabra</i>	<i>H.pfefferi</i>	<i>L.atlantica</i>	<i>Leachia sp.</i>	<i>L.reinhardtii</i>
<i>Helicocranchia pfefferi</i>	0.13				
<i>Leachia atlantica</i>	0.46	0.24			V
<i>Leachia sp.</i>	0.04	0.25	0.10		V
<i>Liocranchia reinhardtii</i>	0.34	0.17	0.46	0.05	
<i>Teuthowenia sp.</i>	0.42	0.20	0.49	0.07	0.46
<i>Helicocranchia pfefferi</i>	0.37				
<i>Leachia atlantica</i>	0.32	0.49			Cr
<i>Leachia sp.</i>	0.04	0.05	0.03		Cr
<i>Liocranchia reinhardtii</i>	0.01	0.07	0.02	0.24	
<i>Teuthowenia sp.</i>	0.41	0.44	0.42	0.04	0.02
<i>Helicocranchia pfefferi</i>	-				Co
<i>Liocranchia reinhardtii</i>	0.41	-			Co
<i>Teuthowenia sp.</i>	-	-			-
<i>Helicocranchia pfefferi</i>	0.31				
<i>Leachia atlantica</i>	0.35	0.24			Ni
<i>Leachia sp.</i>	-	-	-		Ni
<i>Liocranchia reinhardtii</i>	0.40	0.25	0.42	-	
<i>Teuthowenia sp.</i>	0.25	0.43	0.19	-	0.19
<i>Helicocranchia pfefferi</i>	0.10				
<i>Leachia atlantica</i>	0.34	0.10			Cu
<i>Leachia sp.</i>	0.37	0.11	0.48		Cu
<i>Liocranchia reinhardtii</i>	0.32	0.15	0.26	0.27	
<i>Teuthowenia sp.</i>	0.49	0.12	0.39	0.39	0.34
<i>Helicocranchia pfefferi</i>	0.24				
<i>Leachia atlantica</i>	0.18	0.45			Zn
<i>Leachia sp.</i>	0.34	0.44	0.40		Zn
<i>Liocranchia reinhardtii</i>	0.10	0.48	0.46	0.41	
<i>Teuthowenia sp.</i>	0.44	0.30	0.24	0.38	0.20
<i>Helicocranchia pfefferi</i>	0.30				
<i>Leachia atlantica</i>	0.01	0.07			As
<i>Leachia sp.</i>	0.04	0.13	0.45		As
<i>Liocranchia reinhardtii</i>	0.04	0.28	0.07	0.18	
<i>Teuthowenia sp.</i>	0.01	0.08	0.42	0.49	0.09
<i>Helicocranchia pfefferi</i>	0.12				
<i>Leachia atlantica</i>	0.43	0.12			Se
<i>Leachia sp.</i>	0.26	0.08	0.32		Se
<i>Liocranchia reinhardtii</i>	0.12	0.02	0.24	0.50	
<i>Teuthowenia sp.</i>	0.27	0.28	0.25	0.15	0.04
<i>Helicocranchia pfefferi</i>	0.30				
<i>Leachia atlantica</i>	0.29	0.19			Cd
<i>Leachia sp.</i>	-	-	-		Cd
<i>Liocranchia reinhardtii</i>	0.22	0.48	0.14	-	
<i>Teuthowenia sp.</i>	0.43	0.37	0.26	-	0.34
<i>Helicocranchia pfefferi</i>	0.06				
<i>Leachia atlantica</i>	-	-			Pb
<i>Leachia sp.</i>	-	-	-		Pb
<i>Liocranchia reinhardtii</i>	0.07	0.27	-	-	
<i>Teuthowenia sp.</i>	0.37	0.11	-	-	0.18

**Table D3:** Dunn test p-value in the cranchiids tentacles

	<i>C.scabra</i>	<i>H.pfefferi</i>	<i>L.atlantica</i>	<i>L.reinhardtii</i>
<i>Helicocranchia pfefferi</i>	0.5			V
<i>Leachia atlantica</i>	0.19	0.25		
<i>Liocranchia reinhardtii</i>	0.06	0.16	0.42	
<i>Teuthowenia sp.</i>	0.30	0.34	0.38	
<i>Leachia atlantica</i>	-			Cr
<i>Liocranchia reinhardtii</i>	-		-	
<i>Teuthowenia sp.</i>	-		-	
<i>Liocranchia reinhardtii</i>				Ni
<i>Leachia atlantica</i>	-			
<i>Liocranchia reinhardtii</i>	0.46		-	
<i>Helicocranchia pfefferi</i>	0.27			Cu
<i>Leachia atlantica</i>	-	-		
<i>Liocranchia reinhardtii</i>	0.04	0.23	-	
<i>Teuthowenia sp.</i>	0.43	0.35	-	
<i>Leachia atlantica</i>				Zn
<i>Liocranchia reinhardtii</i>			-	
<i>Helicocranchia pfefferi</i>	0.19			As
<i>Leachia atlantica</i>	0.37	0.32		
<i>Liocranchia reinhardtii</i>	0.21	0.35	0.41	
<i>Teuthowenia sp.</i>	0.31	0.12	0.24	
<i>Helicocranchia pfefferi</i>	0.26			Se
<i>Leachia atlantica</i>	0.22	0.11		
<i>Liocranchia reinhardtii</i>	0.34	0.33	0.12	
<i>Teuthowenia sp.</i>	0.23	0.12	0.48	
<i>Helicocranchia pfefferi</i>	-			Cd
<i>Leachia atlantica</i>	-	-		
<i>Liocranchia reinhardtii</i>	-	-	-	
<i>Teuthowenia sp.</i>	-	-	-	
<i>Liocranchia reinhardtii</i>				Pb

**Table D4:** Dunn test p-value between cranchiid tissues

	digestive gland	mantle	tentacles	
digestive gland				V
mantle	0			
tentacles	0.31	0		
digestive gland				Cr
mantle	0			
tentacles	0.01	0.17		
digestive gland				Co
mantle	0.16			
tentacles	0.47	0.33		
digestive gland				Ni
mantle	0			
tentacles	0.01	0.42		
digestive gland				Cu
mantle	0.01			
tentacles	0.01	0.19		
digestive gland				Zn
mantle	0.01			
tentacles	0.12	0.01		
digestive gland				As
mantle	0.15			
tentacles	0	0		
digestive gland				Se
mantle	0.12			
tentacles	0	0		
digestive gland				Cd
mantle	0			
tentacles	0	0.49		
digestive gland				Pb
mantle	0			
tentacles	0	0.49		

**Table D5:** Dunn test p-value in the cephalopods digestive gland

	Cranchiids	other Oegopsida	Myopsida	Sepioidea	Octopoda	
other Oegopsida	0.07					V
Sepioidea	0	0.01				
Octopoda	0	0.01		0.47		
Nautiloidea	0.01	0.01		0.23	0.17	
other Oegopsida	0.01					Cr
Sepioidea	-	-				
Octopoda	0.39	0.01		-		
Nautiloidea	0.01	0.01		-	0.01	
other Oegopsida	0.01					Co
Myopsida	-	-				
Sepioidea	0.01	0.2	-			
Octopoda	0	0.04	-	0.43		
Nautiloidea	0.01	0.22	-	0.44	0.34	
other Oegopsida	0.06					Ni
Myopsida	-	-				
Sepioidea	-	-	-			
Octopoda	0	0.06	-	-		
Nautiloidea	0.01	0.02	-	-	0.12	
other Oegopsida	0					Cu
Myopsida	0.01	0.4				
Sepioidea	0	0.06	0.07			
Octopoda	0	0.01	0.01	0.5		
Nautiloidea	0.01	0.31	0.28	0.24	0.19	
other Oegopsida	0					Zn
Myopsida	0.04	0.13				
Sepioidea	0	0.02	0.01			
Octopoda	0	0	0.01	0.42		
Nautiloidea	0.01	0.2	0.08	0.24	0.23	
other Oegopsida	0.4					As
Myopsida	-	-				
Octopoda	0	0.01	-			
Nautiloidea	0.01	0.01	-		0.31	
other Oegopsida	0.02					Se
Octopoda	0	0.34				
Nautiloidea	0.04	0.40			0.24	
other Oegopsida	0					Cd
Myopsida	0.46	0				
Sepioidea	0.02	0.29	0.03			
Octopoda	0	0.12	0	0.14		
Nautiloidea	0.11	0.17	0.11	0.36	0.08	
other Oegopsida	0.03					Pb
Myopsida	0.01	0.03				
Sepioidea	-	-	-			
Octopoda	0	0	0.34	-		
Nautiloidea	0.04	0.18	0.24	-	0.09	

**Table D6:** Dunn test p-value in the cephalopods mantle

	Cranchiids	other Oegopsida	Myopsida	Sepioidea
<b>other Oegopsida</b>	0.01			<b>V</b>
<b>Sepioidea</b>	-	-		
<b>Octopoda</b>	-	-		
<b>other Oegopsida</b>	0			<b>Cr</b>
<b>Myopsida</b>	0.01	0.33		
<b>Sepioidea</b>	0	0.04	0.289	
<b>Octopoda</b>	0.05	0.29	0.21	
<b>Cranchiids</b>				<b>Co</b>
<b>other Oegopsida</b>	0.01			
<b>Myopsida</b>	0.01	0.19		
<b>Sepioidea</b>	-	-	-	
<b>Cranchiids</b>				<b>Ni</b>
<b>other Oegopsida</b>	0.01			
<b>Myopsida</b>	0.01	0.44		
<b>Sepioidea</b>	0.01	0.41	0.46	
<b>Octopoda</b>	0.27	0.04	0.01	
<b>Cranchiids</b>				<b>Cu</b>
<b>other Oegopsida</b>	0.11			
<b>Myopsida</b>	0.15	0.04		
<b>Sepiida</b>	0.5	0.18	0.19	
<b>Octopoda</b>	0	0.01	0	
<b>Cranchiids</b>				<b>Zn</b>
<b>other Oegopsida</b>	0.06			
<b>Myopsida</b>	0.02	0.23		
<b>Sepioidea</b>	0.4	0.08	0.03	
<b>Octopoda</b>	0	0	0	
<b>Cranchiids</b>				<b>As</b>
<b>other Oegopsida</b>	0.39			
<b>Myopsida</b>	-	-		
<b>Cranchiids</b>				<b>Se</b>
<b>Sepiida</b>	0.08			
<b>Octopoda</b>	0.2			
<b>Cranchiids</b>				<b>Cd</b>
<b>other Oegopsida</b>	0.13			
<b>Myopsida</b>	0.02	0.01		
<b>Sepioidea</b>	0.19	0.49	0.01	
<b>Octopoda</b>	0.32	0.08	0.06	
<b>Cranchiids</b>				<b>Pb</b>
<b>other Oegopsida</b>	0.01			
<b>Myopsida</b>	0.22	0.09		
<b>Sepioidea</b>	0.18	0.06	0.49	
<b>Octopoda</b>	0.02	0	0.01	

**Table D7:** Dunn test p-value in the cephalopods tentacles

	Cranchiids	other Oegopsida	Myopsida
Cranchiids			
other Oegopsida			V
Myopsida			
Octopoda			
Cranchiids			Cr
other Oegopsida	0.04		
Cranchiids			Co
other Oegopsida			
Myopsida			
Octopoda			
Cranchiids			Ni
Octopoda	0.14		
Cranchiids			Cu
other Oegopsida	0.5		
Myopsida	0.06	0.1	
Octopoda	0.11	0.23	0.01
Cranchiids			Zn
other Oegopsida	0.34		
Myopsida	0.33	0.48	
Octopoda	0	0.01	0.01
Cranchiids			As
other Oegopsida	-		
Cranchiids			Se
other Oegopsida			
Myopsida			
Octopoda			
Cranchiids			Cd
other Oegopsida	0.24		
Myopsida	0.16	0.08	
Octopoda	0.11	0.05	0.38
Cranchiids			Pb
other Oegopsida	0.1		
Myopsida	0.15	0.46	
Octopoda	0.38	0.09	0.16