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Metal and metalloid concentrations in the giant squid *Architeuthis dux* from Iberian waters

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1 **Metal and metalloid concentrations in the giant squid *Architeuthis dux* from**
2 **Iberian waters**

3

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14 **Abstract:** This study investigated 14 trace elements (Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni,
15 Pb, Se, V and Zn) in the tissues of the giant squid *Architeuthis dux* from the Mediterranean
16 and Atlantic Spanish waters. As for other families of cephalopods, the digestive gland and
17 the branchial hearts of *Architeuthis* showed the highest concentrations of Ag, Cd, Co, Cu, Fe,
18 Ni, Se, V and Zn, highlighting their major role in the bioaccumulation and detoxification
19 processes. With the exception of Hg, the muscles showed relatively low trace element
20 concentrations. Nevertheless, this tissue contained the main proportion of the total As, Cr, Hg,
21 Mn, Ni, and Zn body burden because muscles represent the main proportion of the squid
22 mass. These findings suggest that the metal metabolism is overall the same as other
23 cephalopod families from neritic waters. In females, Zn concentrations increased in the
24 digestive gland with the squid's weight likely reflecting physiological changes during sexual
25 maturation. Comparing the trace element concentrations in the tissues of *Architeuthis*, higher
26 Ag, Cu, Hg and Zn concentrations in the squid from the Mediterranean reflected different
27 exposure conditions. In comparison to other meso-pelagic squids from the Bay of Biscay, Cd
28 concentrations recorded in the digestive gland suggest that *Architeuthis* might feed on more
29 contaminated prey or that it displays a longer life span than other cephalopods.

30

31 **Key words:** trace element; bioaccumulation; cephalopod; giant squid, *Architeuthis*

32 **1. Introduction**

33

34 Cephalopods play a key role in marine ecosystems both as predators and prey. They
35 constitute a class of marine molluscs which are found in a great variety of habitats from
36 coastal waters to very deep-ocean environments (Boyle and Rodhouse, 2005). Independently of
37 the species, the habitat or the life span they display, cephalopods share the ability to
38 accumulate inorganic and organic pollutants such as metals, PCBs or organochlorine
39 pesticides (e.g. Martin and Flegal, 1975; Tanabe et al., 1984; Miramand and Bentley, 1992;
40 Yamada et al., 1997; Bustamante et al., 2000, 2006a; Ueno et al., 2003; Storelli et al., 2006).
41 Consequently, they were reported to constitute a significant vector of contaminants to the
42 species feeding on them, in particular seabirds and marine mammals (e.g. Honda et al., 1983;
43 Muirhead and Furness 1988; Bustamante et al., 1998; Lahaye et al., 2005). However, most
44 studies focused on commercially targeted species which are 1) easy to sample and 2) of high
45 economic and health interest concerning human consumption. Many of these cephalopod
46 species are also consumed by top marine predators, but there is a gap in the information
47 concerning non-targeted species. This lack of data on bioaccumulation of contaminants is
48 particularly obvious for oceanic and deep-sea species, like the giant squid *Architeuthis*.

49

50 Overall, the biology, behaviour and life cycle of *Architeuthis* are still poorly known in many
51 aspects even if this squid has received considerable attention over the last decade (see
52 González et al., 2002; Guerra et al., 2004, 2006; Kubodera and Mori 2005). Most of the
53 information available on this squid comes from dead stranded animals and from predator
54 trophic ecology studies. Indeed, squid flesh and beaks are often recorded in the stomach of
55 sperm whales, but also of seabirds and sharks (Roper and Boss 1982; Clarke 1996; Santos et
56 al., 2002; Cherel and Hobson 2005). Giant squids are also increasingly captured by trawling

57 nets because of the development of deep-sea fisheries (Guerra et al., 2006). In deep water
58 conditions, the giant squid would have a particular diet and exposure conditions to trace
59 elements and metals. For example, Hg bioavailability seems to be enhanced in these deep
60 environments because the absence of solar radiation and the low oxygen concentrations in
61 the deep environment favors a high methylation rate by bacteria (Monteiro et al., 1996). Also,
62 Cd is enriched in mesopelagic waters while depleted in the surface ocean because of its
63 regeneration from sinking biological debris from epipelagic zone and its uptake by organisms
64 at the surface (Boyle et al., 1976).

65

66 In this framework, the objectives of this study were to provide baseline data on a wide range
67 of trace elements in the giant squid *Architeuthis dux* from the Spanish waters. To this end, the
68 concentrations and tissue distribution of 12 metals (Ag, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb,
69 V and Zn) and 2 metalloids (As and Se) were determined in the tissues and organs of fished
70 and stranded specimens. The recorded values were then compared to the data from the
71 current literature for other cephalopod species.

72

73 **2. Material and methods**

74

75 **2.1. Sampling and sample preparation**

76 Six specimens of giant squid were collected between 2001 and 2005. Table 1 shows the main
77 data of the specimens as well as the sampling site, date, and mode of capture. Five specimens
78 were caught in the Bay of Biscay (Asturias, North Spain) and one in the western
79 Mediterranean Sea. Two of them were mature males and four were immature or maturing
80 females.

81

82 Collected specimens were immediately frozen prior to dissection. In the laboratory, each
83 individual was weighed and measured (mantle length ML, total length TL), and the sex and
84 maturity stage determined. The digestive gland, gills, ink sack, branchial hearts and their
85 appendages, systemic heart and brain were totally removed. In addition, pieces of muscle,
86 skin, digestive and genital tissues (i.e. oviduct gland, ovary and testis), were sampled to
87 determine trace element concentrations. As it was not possible to separate the different
88 tissues and to weigh them, the total concentrations in the whole *Architeuthis* specimens were
89 estimated according to the measured concentrations in the different tissues and to their
90 relative weight in fishery targeted squids.

91

92 **2.2. Analytical procedure**

93 All samples were freeze-dried for several days then grounded. Aliquots of the samples
94 ranging from 50 to 300 mg were digested using a 3:1 v:v nitric-hydrochloric acid mixture
95 with 65% HNO₃ (Merck, suprapur quality) and 70% HCl (Merck, suprapur quality). Acidic
96 digestion was performed overnight under ambient temperature and then heated in a
97 microwave during 30 min with increasing temperature until 105°C, and 15 min at 105°C
98 (1200 W). After the mineralization process, each sample was diluted to 30 or 50 ml with
99 milli-Q quality water, according to the volume of acid added to the mineralization (3.0 ml or
100 4.5 ml).

101 Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni Pb, Se, V and Zn were analysed either by Inductively
102 Coupled Plasma-Optical Emission Spectrometry (Varian[®] Vista-Pro) or Inductively Coupled
103 Plasma-Mass Spectrometry (Varian[®] Ultra Mass 700). For Hg, two aliquots ranging from 10
104 to 50 mg of dried material were directly analysed in an Advanced Mercury Analyser
105 spectrophotometer (Altec[®] AMA 254).

106 Reference tissues (dogfish liver DOLT-3, NRCC, and lobster hepatopancreas TORT-2,
107 NRCC) were treated and analysed in the same way as the samples. Results were in good
108 agreement with the certified values, and the standard deviations were low, proving good
109 repeatability of the method (Table 2). The results for standard reference materials displayed
110 recoveries of the elements ranging from 88% to 116% (n=10).

111 The detection limits ($\mu\text{g}\cdot\text{g}^{-1}$ dry wt) for ICP-OES were 8.3 (As, Fe, Zn), 3.3 (Ag, Se), 1.67
112 (Pb, V), 0.83 (Cd, Co, Cr, Cu, Mn, Ni), and were 0.167 (Ni, V), 0.083 (Cd, Co, Cr, Cu, Mn,
113 Pb), 0.033 (Ag) for ICP-MS. Trace element concentrations are given relative to the dry
114 weight ($\mu\text{g}\cdot\text{g}^{-1}$ dry wt) while the distribution percentages are calculated for wet weight.

115

116 3. Results

117

118 3.1. Trace element concentrations in soft tissues

119 Trace element concentrations in the tissues and organs of *Architeuthis* are reported in Figure
120 1. Among the sampled tissues, the digestive gland was the major site of concentration for Ag,
121 Cd, Co, Cu, Fe and Se with $1.13\text{-}14.0 \mu\text{g Ag}\cdot\text{g}^{-1}$, $27.2\text{-}134 \mu\text{g Cd}\cdot\text{g}^{-1}$, $2.0\text{-}4.8 \mu\text{g Co}\cdot\text{g}^{-1}$, 64-
122 $1218 \mu\text{g Cu}\cdot\text{g}^{-1}$, $52\text{-}1862 \mu\text{g Fe}\cdot\text{g}^{-1}$ and $9.7\text{-}19.5 \mu\text{g Se}\cdot\text{g}^{-1}$ (Figure 1). The digestive gland also
123 concentrated Ni, V and Zn at concentrations closed to the highest concentrations recorded in
124 the other tissues with $0.11\text{-}1.42 \mu\text{g Ni}\cdot\text{g}^{-1}$, $0.60\text{-}4.34 \mu\text{g V}\cdot\text{g}^{-1}$, and $34\text{-}219 \mu\text{g Zn}\cdot\text{g}^{-1}$.
125 Interestingly, the digestive gland also exhibited the lowest concentrations of As, Cr and Hg
126 with $31\text{-}65 \mu\text{g As}\cdot\text{g}^{-1}$, $0.41\text{-}1.40 \mu\text{g Cr}\cdot\text{g}^{-1}$, and $0.32\text{-}1.56 \mu\text{g Hg}\cdot\text{g}^{-1}$ (Figure 1).

127 The concentrations of As, Co, Hg, Ni, Se and V were also remarkable in branchial hearts,
128 which play an important excretory role in cephalopods. In this tissue, the concentrations of
129 trace elements were the highest for Ni or very close to the highest for As, Co, Hg, Se and V.
130 Branchial hearts concentrated As from 86 to $111 \mu\text{g}\cdot\text{g}^{-1}$, Co from 3.15 to $3.22 \mu\text{g}\cdot\text{g}^{-1}$, Hg

131 from 0.71 to 4.37 $\mu\text{g}\cdot\text{g}^{-1}$, Ni from 0.74 to 2.60 $\mu\text{g}\cdot\text{g}^{-1}$, Se from 8.2 to 9.3 $\mu\text{g}\cdot\text{g}^{-1}$ and V from
132 1.50 to 2.46 $\mu\text{g}\cdot\text{g}^{-1}$ (Figure 1).

133 Muscles exhibited generally among the lowest concentrations for all trace elements except
134 for Hg which exhibited the highest values in this tissue (1.86-3.32 $\mu\text{g}\cdot\text{g}^{-1}$ dry weight;
135 Figure 1). Cr and Zn were highly concentrated in the ovary with concentrations ranging from
136 0.65 to 6.12 $\mu\text{g}\cdot\text{g}^{-1}$ and 131 to 149 $\mu\text{g}\cdot\text{g}^{-1}$, respectively. The oviduct gland also displayed high
137 concentrations of As and Mn as well as the digestive gland appendages had high
138 concentrations of Cr and Pb (Figure 1).

139

140 **3.2. Distribution of the trace elements in soft tissues**

141 The proportions of the whole body burden of the trace elements contained in each organ and
142 tissue are shown in Figure 2. With the exception of As, Cr, Hg and Mn which were mainly
143 found in the body muscular parts ($69 \pm 9\%$, $68 \pm 20\%$, $87 \pm 5\%$, and $55 \pm 22\%$, respectively),
144 the digestive gland contained the largest quantities of all trace elements : $98 \pm 1\%$ of Ag, 99
145 $\pm 1\%$ of Cd, $97 \pm 2\%$ of Co, $92 \pm 5\%$ of Cu, $85 \pm 16\%$ of Fe, $53 \pm 28\%$ of Ni, $69 \pm 22\%$ of
146 Pb, $77 \pm 5\%$ of Se, $71 \pm 19\%$ of V, and $53 \pm 17\%$ of Zn (Figure 2).

147 Although the concentrations of some trace elements were high in the branchial hearts or in
148 the gills, these tissues contained in fact low amounts of the considered elements because of
149 their small masses relative to the whole body weight (Figure 2).

150

151 **3.3. Influence of the size/weight and origin**

152 The size/weight only influenced Zn concentrations in the digestive gland ($R^2=0.934$, $p=0.020$)
153 and Cr and Ni concentrations in the gills ($R^2=0.968$ $p=0.007$ and $R^2=0.969$ $p=0.007$).

154 No statistical tests were performed to compare the influence of the origin on the accumulated
155 trace elements because our sampling only included one specimen from the Mediterranean.

156 However, this giant squid clearly displayed higher concentrations of Hg than any of those
157 from the Bay of Biscay with 1.56 vs $0.47 \pm 0.13 \mu\text{g.g}^{-1}$ in the digestive gland (see Table 3),
158 2.97 vs $1.38 \pm 0.34 \mu\text{g.g}^{-1}$ in the gills, and 3.32 vs $2.07 \pm 0.19 \mu\text{g.g}^{-1}$ in the mantle muscle
159 (data not shown). Important differences also appeared for Ag, Cu and Zn with 14.0 vs $1.90 \pm$
160 $0.47 \mu\text{g Ag.g}^{-1}$, 1218 vs $108 \pm 83 \mu\text{g Cu.g}^{-1}$, and 219 vs $103 \pm 51 \mu\text{g Zn.g}^{-1}$ in the digestive
161 gland (Table 3), and 4.80 vs $0.31 \pm 0.11 \mu\text{g Ag.g}^{-1}$, 206 vs $31 \pm 24 \mu\text{g Cu.g}^{-1}$ and 111 vs $60 \pm$
162 $35 \mu\text{g Zn.g}^{-1}$ in the gills (data not shown).

163

164 **4. Discussion**

165

166 Previous studies have demonstrated the ability of cephalopods to accumulate high
167 concentrations of trace elements in their tissues but very little data is available in the current
168 literature for non-targeted and/or deep-waters species such as the giant squid *Architeuthis*.
169 Although globally poorly documented, metal and metalloid concentrations in cephalopod
170 tissues have received increasing interest over the last decades, particularly in Europe and
171 Japan, as these molluscs play a major role both as predators and food items in marine
172 ecosystems (see the reviews by Clarke 1996; Croxall and Prince 1996; Klages 1996; Smale
173 1996; Boyle and Rodhouse, 2005). The central role of the digestive gland in trace element
174 bioaccumulation and detoxification has been highlighted many times, particularly for toxic
175 metals such as Ag and Cd (e.g. Martin and Flegal 1975; Miramand and Bentley 1992;
176 Bustamante et al., 2002, 2004; Ichiashi et al., 2001a; Miramand et al., 2006). Beside the
177 digestive gland, which also plays a major role in the energetic metabolism of cephalopods
178 (e.g. Rosa et al., 2005; Moltschaniwskyj and Johnston 2006), the branchial hearts and their
179 appendages are involved in trace element excretion processes, allowing the depuration and/or
180 the storage of various metals and radionuclides (e.g. Nardi and Steinberg 1974; Miramand

181 and Guary 1980; Guary et al., 1981; Miramand and Bentley 1992; González et al., 1998;
182 Bustamante et al., 2002, 2006b). As for coastal and/or oceanic targeted cephalopods, the
183 digestive gland and the branchial hearts of *Architeuthis* generally contained the highest
184 concentrations of most of the considered elements, i.e. Ag, Cd, Co, Cu, Fe, Ni, Se, V and Zn
185 (Figure 1). This finding strongly suggests that the metabolism of trace elements in
186 *Architeuthis* is very close to, or even the same as other families of cephalopods. This is
187 supported by the fact that trace element concentrations in the digestive gland of *Architeuthis*
188 closely fall within the same range than for other cephalopod species (Table 3), indicating that
189 the potential of *Architeuthis* for their bioaccumulation is relatively similar. Moreover,
190 according to the elevated proportions of the total element body burden, the digestive gland of
191 *Architeuthis* might play a central role in the detoxification and storage of most of the
192 analysed elements, i.e. Ag, Cd, Co, Cu, Fe, Ni, Pb, Se, V and Zn (Figure 2). It would be a
193 great interest to investigate the detoxification strategies in the digestive gland of *Architeuthis*
194 in comparison to that of other families of cephalopods.

195 Besides the digestive gland, muscles contained very high proportions of total body burdens
196 of As, Cr, Hg, Mn, Ni, and Zn (Figure 2). With the exception of Hg, trace element
197 concentrations in *Architeuthis* muscles were relatively low and these high proportions
198 resulted from the elevated muscular mass respective to the whole body weight. For Hg, the
199 concentrations recorded in the muscles were the highest among the different organs and
200 tissues (Figure 1). Previous studies have reported relatively similar Hg concentrations
201 between the different tissues of different squid species from the Northern Atlantic waters
202 (Bustamante et al., 2006a; Pierce et al., 2008). It is therefore noteworthy that muscular Hg
203 concentrations in *Architeuthis* were 2 to 4 times higher than in the digestive gland. In
204 comparison with Ag, Cd, Co, Cu, Fe, Ni, Pb, Se, V and Zn, the role of the digestive gland in
205 the storage of Hg appeared to be relatively limited in *Architeuthis*. This may be due to an

206 excretion function of Hg by the digestive gland, and/or a preferential redistribution of Hg to
207 muscular tissues where it binds to the sulphhydryl groups of proteins (Bloom, 1992;
208 Bustamante et al., 2006a). Such a redistribution might indicate that most of the Hg ingested
209 from the prey would be in the organic form such as fish in which Hg content is virtually
210 100% in the methylated form (Bloom, 1992). Little is known about the diet of *Architeuthis*,
211 it includes other cephalopods (Pérez-Gándaras and Guerra, 1978, 1989), crustaceans (e.g.
212 *Nephros norvergicus*) as well as a large proportion of fish of different families accordingly to
213 the available prey in the area (for instance *Trachurus trachurus*, *Maurollicus muelleri* and
214 *Micromesistius poutassou* in Ireland waters and equivalent species from Namibia and New
215 Zealand) (see Guerra et al., 2006 for a review). Further studies therefore should focus on
216 trace elements in the typical prey of *Architeuthis* to provide insights on this aspect.

217 As in other cephalopod species, trace element concentrations in *Architeuthis* may vary with
218 biological and environmental factors such as age (size/weight), sex, and geographical origin
219 (e.g. Monteiro et al., 1992; Bustamante et al., 1998; Raimundo et al., 2004; Pierce et al.,
220 2008). Our limited sampling did not allow making comparisons for all these factors.
221 However, considering the 5 specimens from the Bay of Biscay, it appears that size/weight
222 poorly influenced trace element concentrations in *Architeuthis* tissues. The increase of Zn
223 concentrations from 34 to 160 $\mu\text{g.g}^{-1}$ dwt in the digestive gland might be related to metal
224 physiological changes related to the sexual maturation as reported for other cephalopod
225 species like *Sepia officinalis* from the English Channel (Miramand et al., 2006). In females,
226 high concentrations of Zn in the ovary of *Architeuthis* ($120 \pm 38 \mu\text{g.g}^{-1}$ dwt) were close to
227 that in the genital tract of *Sepia officinalis* ($123 \pm 3 \mu\text{g.g}^{-1}$ dwt; Miramand and Bentley,
228 1992). Within the ovary, essential elements such as Zn are stored in metal-containing
229 enzymes and metalloproteins (Gerpe et al., 2000) and transferred to the yolk of the eggs
230 (Villanueva and Bustamante, 2006, Lacoue-Labarthe et al., 2008). Cr and Ni in the gills also

231 displayed a significant increase with size/weight. In the current literature, very little data is
232 available on the variation of Cr and Ni concentrations in cephalopod tissues. For example, in
233 the squid *Sthenoteuthis oualaniensis* Cr concentrations were higher in juveniles than in
234 adults, whereas juveniles displayed lower Ni concentrations than adults (Ichihashi et al.,
235 2001a). Such a difference for *S. oualaniensis* was explained by the evolution of food habits
236 between the juvenile and adult stages, juvenile feeding more on crustaceans while adults
237 primarily preyed on fish. According to the lack of significant variation in the digestive gland,
238 such a switch is not likely to occur in the size range of *Architeuthis* we analysed.
239 Furthermore, even if the diet could represent the main pathway for many elements - as
240 experimentally shown for Am, Cd, Co and Zn (Koyama et al., 2000; Bustamante et al., 2002,
241 2004, 2006b) - seawater could also be an important uptake pathway, as elements pass
242 through the skin and through the gills. For instance, seawater represents the main pathway for
243 Ag in *Sepia officinalis* (Bustamante et al., 2004). Therefore, Cr and Ni bioaccumulation in
244 gills might also result from a direct uptake from seawater all along the lifespan of
245 *Architeuthis*.

246 Trace element concentrations in cephalopods could also vary according the location where
247 individuals were captured (Bustamante et al., 1998; Seixas et al., 2005ab; Pierce et al., 2008).
248 In the case of *Architeuthis*, this is clearly exemplified by the much higher Hg concentrations
249 in the tissue of the specimen from the Mediterranean. Higher Hg concentrations in
250 Mediterranean organisms have been highlighted many times and are typically explained by
251 high temperatures and absence of solar radiation in the deep environment. These conditions
252 favor a high methylation rate of the metal, methyl-Hg being highly bioavailable for marine
253 biota, which consistently biomagnifies through the food chain. Moreover, natural sources of
254 Hg in the Mediterranean Sea may contribute to Hg enrichment through the benthic food webs,
255 as it constitutes the richest natural reserve of this element (Bacci, 1989). Higher Ag, Cu and

256 Zn concentrations in the digestive gland and in the gills also indicated different exposure
257 conditions of this specimen compared to those from the Bay of Biscay. In cephalopods, Ag
258 bioaccumulation in the digestive gland might reflect the global contamination of the
259 surrounding waters (e.g. Martin and Flegal, 1975; Miramand et al., 2006), seawater being the
260 main pathway of exposure and Ag having a fast turn-over in the tissues (Bustamante et al.,
261 2004). Interestingly, Ag, Cu, Hg and Zn are metals that bind to metallothionein proteins
262 (MTs), which play a role in the homeostasis of the essential metals (i.e. Cu and Zn) and in
263 the detoxification of non-essential metals (i.e. Ag, Cd and Hg) (Roesijadi, 1992, 1996;
264 Viarengo and Nott, 1993). The role of MTs in metal sequestration in cephalopods is not
265 completely clear (Bustamante et al., 2006c) and this issue clearly deserves further
266 investigation.

267 Even though trace elements are generally considered for their potential toxicity in
268 ecotoxicological studies and biomonitoring surveys, there is increasing interest in their use
269 for providing information on life history and trophic ecology of cephalopods (Jackson et al.,
270 2007). Thus, Cd is of particular interest because it is highly bioaccumulated by cephalopods.
271 Indeed, Cd is efficiently absorbed and strongly retained in the digestive gland (Bustamante et
272 al., 1998, 2002). Even if most of cephalopod species display short life spans i.e. typically less
273 than 2 years, they can accumulate very high Cd concentrations in their digestive gland
274 reaching up $1000 \mu\text{g g}^{-1}$ wet wt in the squid *Illex argentinus* (Dorneles et al., 2007). Because
275 of Cd incorporation by organisms in epipelagic waters and its regeneration from sinking
276 biological debris in the mesopelagic environment (Boyle et al., 1976), deep-water
277 cephalopods might show relatively high Cd concentrations. In the Bay of Biscay, Cd
278 concentrations reached 9.1 and $33.1 \mu\text{g.g}^{-1}$ dry wt in the digestive gland of the mesopelagic
279 squids *Histioteuthis reversa* and *Teuthowenia megalops*, respectively (unpublished data) and
280 would be due to the consumption of prey highly contaminated with Cd. Even elevated
281 compared to neritic squid species (Bustamante et al., 1998), these concentrations are lower

282 than those measured in the digestive gland of *Architeuthis*, suggesting that 1) the giant squid
283 feed on more contaminated prey than Histioteuthidae and Cranchidae, or 2) it displays a
284 much longer life span than other cephalopods. Age estimation and growth rates of giant
285 squids are still open questions. Indeed, isotopic analysis indicated that the age for the giant
286 squid *Architeuthis sanctipauli* from Tasmania was 14 years for specimens ranging from 191
287 to 240 cm ML (Landman et al., 2004). This completely disagrees with the age estimated from
288 growth increment counts in statoliths of *Architeuthis dux* and *Architeuthis* sp. from the
289 Atlantic and caught off New Zealand. Specimens ranging from 43 to 161 cm ML had
290 between 153 and 435 increments (Jackson et al., 1991; Gauldie et al., 1994; Lordan et al.,
291 1998; González et al., 2002). If the increments were daily deposited, as it occurs in other
292 cephalopods, the age of these animals will not exceed two years. This finding implies that
293 *Architeuthis* would have a very fast growth rate with intense food intakes that in turn would
294 lead to the bioaccumulation of relatively high Cd concentrations in its digestive gland.

295

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297

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546 Caption to figures

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549 Figure 1. Mean trace element concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ dry wt) in the tissues of *Architeuthis dux* from
550 Spanish waters. Scale bars represent 1 standard deviation (n=6). B: Brain; BH: branchial hearts;
551 BHA: branchial heart appendages; DG: digestive gland; DGA: digestive gland appendages; DT:
552 digestive tract; G: gills; IS: ink sack; M: muscles; O: ovary; OG: oviduct gland; S: skin; SH:
553 systemic heart; T: testis.

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556 Figure 2. Percentage distribution of trace elements in the tissues of *Architeuthis dux* from Spanish
557 waters. Scale bars represent 1 standard deviation. BH: branchial hearts; DG: digestive gland; DGA:
558 digestive gland appendages; DT: digestive tract; G: gills; IS: ink sack; M: muscles; O: ovary; S: skin;
559 SH: systemic heart.

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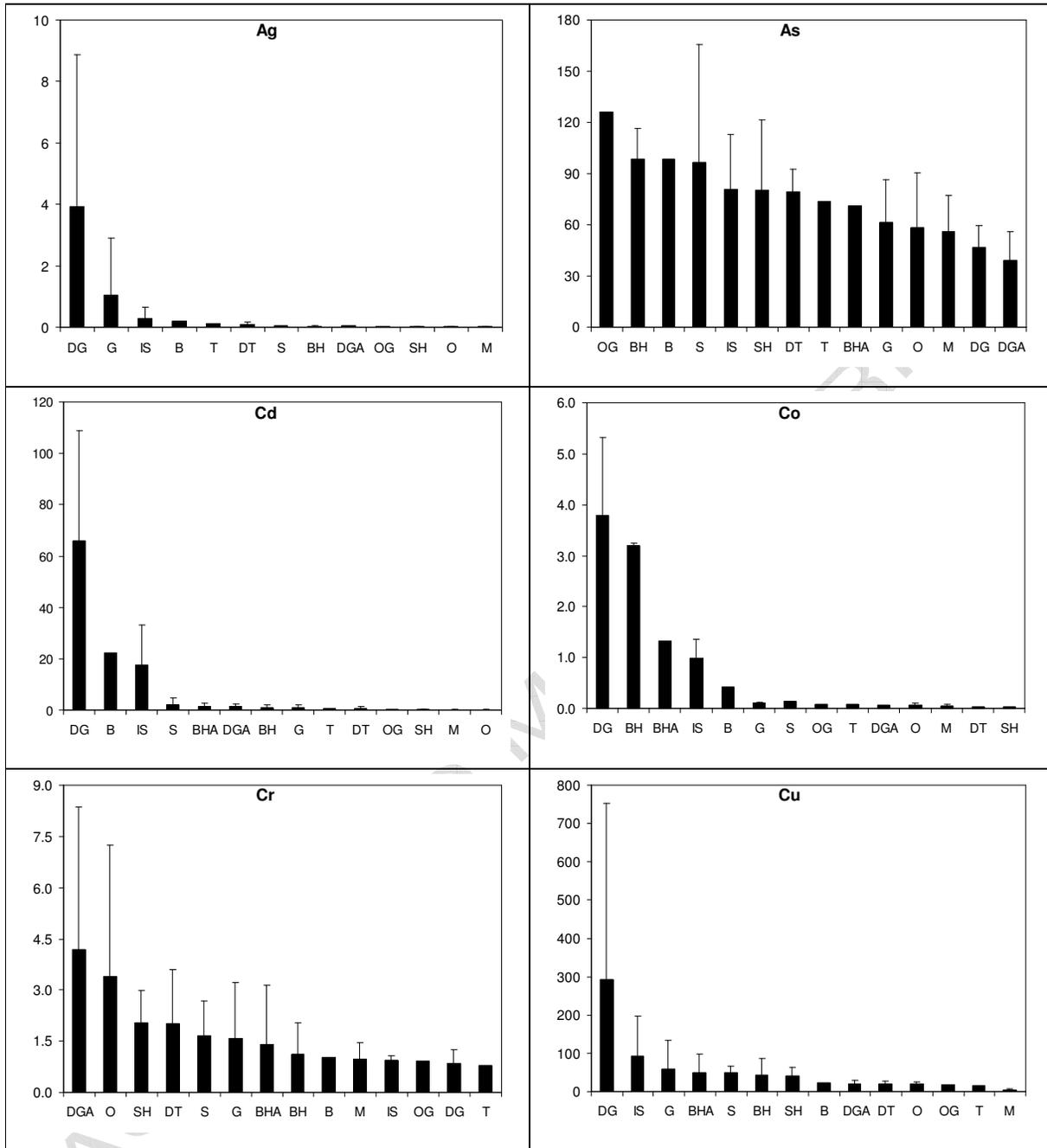
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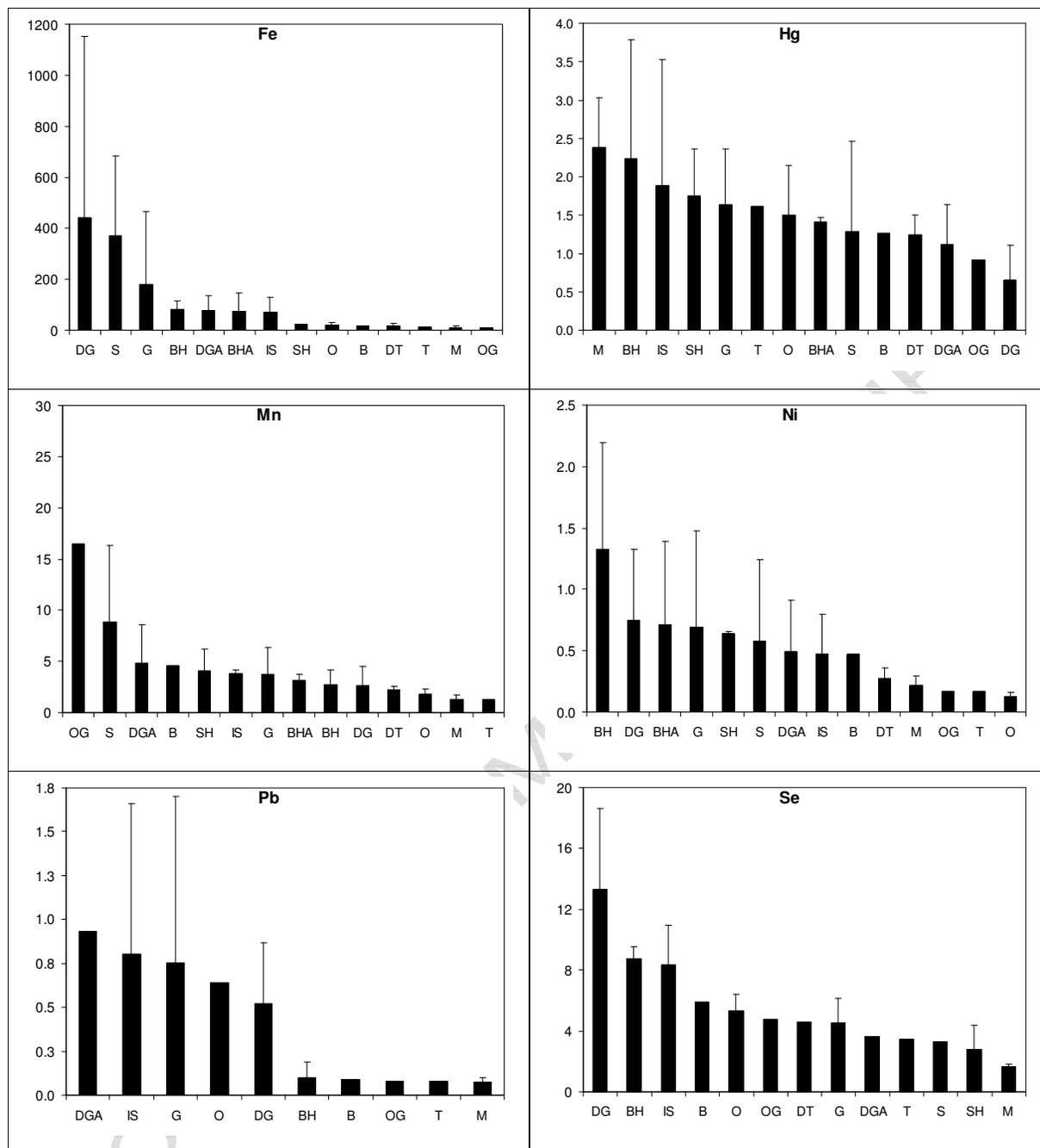
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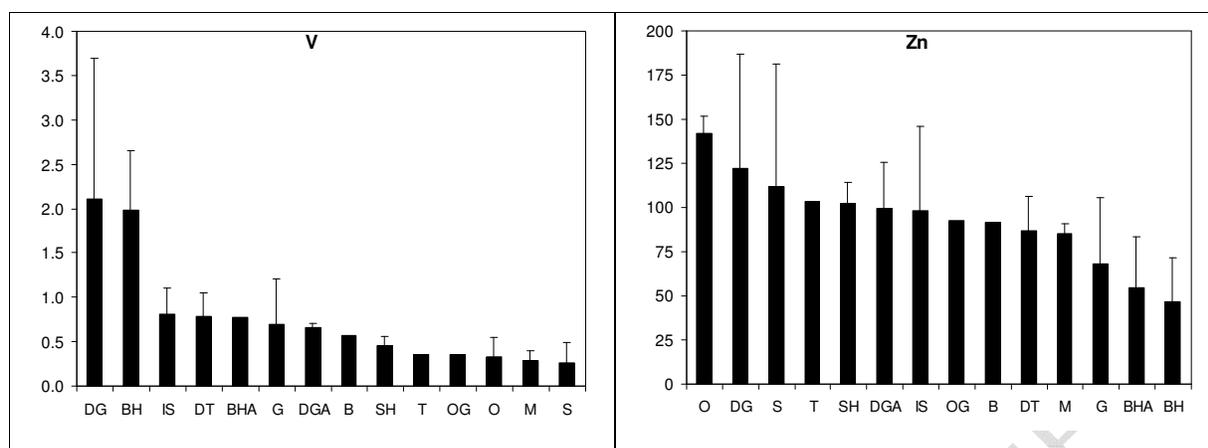
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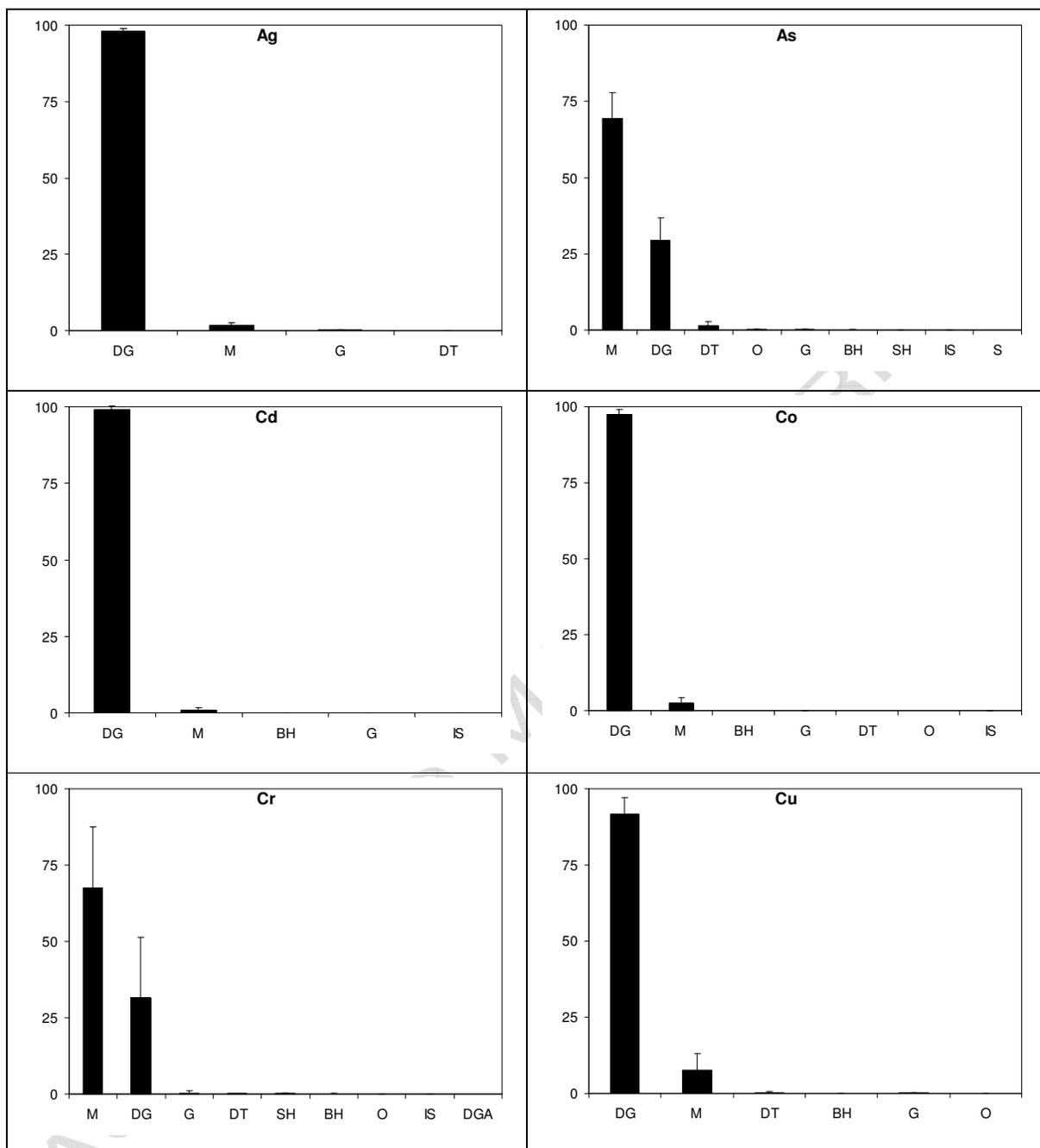
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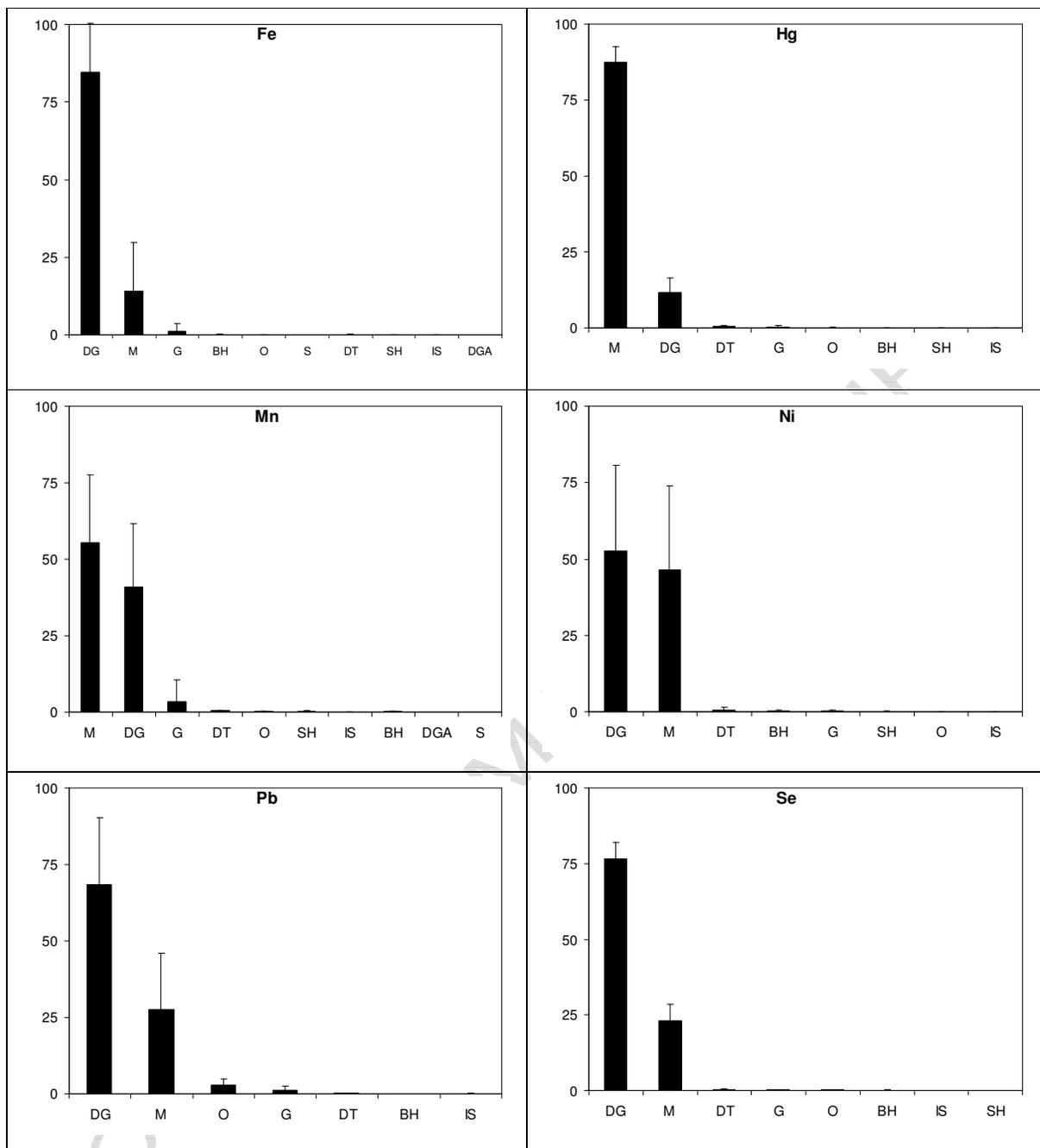
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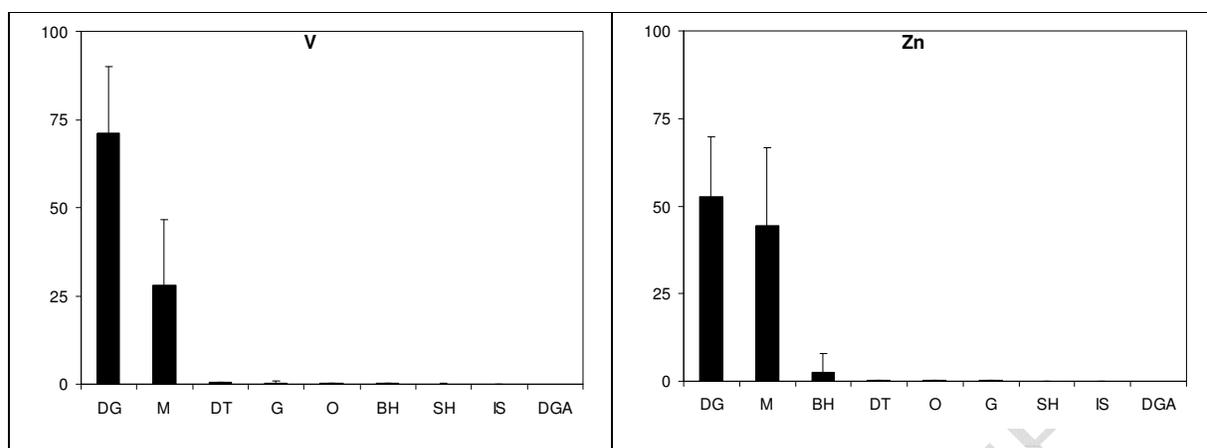
Figure 1.

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Figure 2.

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Table 1. Sampling information and squid biological characteristics. T: trawling; S: Stranded; F: Floating at the surface; ML: Dorsal mantle length; TL: Total length.

N°	Sampling site	Sampling date	Mode of capture	Weight (kg)	ML (cm)	TL (cm)	Sex / Maturation
1	Off Luarca (Asturias, North Spain)	12 September 2001	T	90	127	710	F / Immature
2	Ribadesella (Asturias, North Spain)	23 October 2001	S	104	150	800	F / Maturing
3	Colunga (Asturias, North Spain)	15 September 2003	S	80	152	1200	F / Immature
4	Off Gijón (Asturias, North Spain)	16 September 2003	F	66	122	620	M / Mature
5	Off Gandía (Valencia, Western Mediterranean)	19 July 2005	T	50	107	600	M / Mature
6	Off Gijón (Asturias, North Spain)	22 July 2005	T	139	146	820	F / Maturing

Table 2. Comparison of certified trace elements concentrations ($\mu\text{g g}^{-1}$ dry weight) in reference materials (n=10) with the values determined in the present study (nc: not certified value, nd: not determined). ICP-MS - Inductively Coupled Plasma Mass Spectrometry; ICP-OES - Inductively Coupled Plasma Optical Emission Spectrometry; AMA – Advanced Mercury Analyser.

Element	Method	TORT-2			DOLT-3						
		Measured		Certified	Recovery	Measured		Certified	Recovery		
		Mean	\pm SD	Mean		\pm SD	Mean	\pm SD		Mean	\pm SD
Ag	ICP-MS	6.21	\pm 1.69	nc	-	1.21	\pm 0.10	1.20	\pm 0.07	101	
As	ICP-OES	21.8	\pm 2.4	21.6	\pm 1.8	101	9.9	\pm 0.3	10.2	\pm 0.5	97
Cd	ICP-MS	26.4	\pm 2.2	26.7	\pm 0.6	99	19.3	\pm 0.7	19.4	\pm 0.6	99
Co	ICP-MS	0.45	\pm 0.09	0.51	\pm 0.09	88	0.29	\pm 0.05	nc	-	-
Cr	ICP-OES	0.69	\pm 0.18	0.77	\pm 0.15	90	4.02	\pm 0.93	nc	-	-
Cu	ICP-OES	95	\pm 15	106	\pm 10	90	31.9	\pm 0.7	31.2	\pm 1.0	99
Fe	ICP-OES	100	\pm 10	105	\pm 13	95	1349	\pm 76	1484	\pm 57	91
Hg	AMA	0.27	\pm 0.01	0.27	\pm 0.06	100	3.36	\pm 0.08	3.37	\pm 0.14	100
Mn	ICP-OES	13.5	\pm 2.0	13.6	\pm 1.2	99	9.73	\pm 0.14	nc	-	-
Ni	ICP-OES	2.44	\pm 0.56	2.50	\pm 0.19	98	2.46	\pm 0.45	2.72	\pm 0.35	90
Pb	ICP-MS	0.32	\pm 0.17	0.35	\pm 0.13	91	0.294	\pm 0.056	0.319	\pm 0.045	92
Se	ICP-MS	6.48	\pm 0.48	5.63	\pm 0.67	115	7.56	\pm 0.65	7.06	\pm 0.48	107
V	ICP-MS	1.55	\pm 0.24	1.64	\pm 0.19	95	nd	-	nc	-	-
Zn	ICP-OES	188	\pm 20	180	\pm 6	104	97.3	\pm 1.4	86.6	\pm 2.4	116

Table 3. Reported metal concentrations (Mean \pm SD, $\mu\text{g g}^{-1}$ dry weight) in the digestive gland of different cephalopod species.

Species	Ag	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	V	Zn	Reference
Architeuthidae														
<i>Architeuthis dux</i>	1.90 \pm 0.47	48 \pm 14	60.8 \pm 46.2	3.27 \pm 1.76	0.93 \pm 0.41	108 \pm 83	497 \pm 779	0.47 \pm 0.13	2.72 \pm 2.12	0.62 \pm 0.54	0.41 \pm 0.33	2.24 \pm 1.91	103 \pm 51	a
<i>A. dux</i>	14	44	90.7	4.8	0.49	1218	158	1.56	2.26	1.37	0.85	1.72	219	b
Nautilidae														
<i>Nautilus macromphalus</i>	4.43 \pm 1.95	185 \pm 64	45 \pm 13	5.9 \pm 3.6	4.2 \pm 0.8	106 \pm 46	554 \pm 238	-	8.9 \pm 2.0	11.9 \pm 7.8	-	8.0 \pm 2.3	672 \pm 208	c
Sepiidae														
<i>Sepia officinalis</i>	6.15 \pm 1.75	-	12.7 \pm 0.4	3.3 \pm 0.6	1.1 \pm 0.1	315 \pm 3	244 \pm 28	-	3.3 \pm 0.1	1.3 \pm 0.4	1.14 \pm 0.06	5.0 \pm 1.3	571 \pm 47	d
<i>S. officinalis</i>	13 \pm 2	-	25 \pm 5	10 \pm 2	-	600 \pm 10	390 \pm 10	-	-	-	2.2 \pm 0.5	3.3 \pm 0.1	1400 \pm 500	e
Loliginidae														
<i>Loligo opalescens</i>	25.1 \pm 12.6	-	85.0 \pm 51.6	-	-	5350 \pm 3210	111 \pm 73	-	-	-	-	-	247 \pm 131	f
<i>L. opalescens</i>	45.9 \pm 19.0	-	122 \pm 58	-	-	8370 \pm 3130	87 \pm 49	-	-	-	-	-	449 \pm 201	f
Ommastrephidae														
<i>Nototodarus gouldi</i>	-	-	33 \pm 30	-	-	363 \pm 238	-	-	-	-	-	-	830 \pm 355	g
<i>N. gouldi</i>	3.3 \pm 1.4	-	50 \pm 25	-	-	246 \pm 298	745 \pm 440	-	4.2 \pm 1.1	-	-	-	696 \pm 295	h
<i>Ommastrephes bartrami</i>	12.1 \pm 8.6	-	287 \pm 202	-	-	195 \pm 212	399 \pm 204	-	-	-	-	-	163 \pm 55	f
<i>Stenoteuthis oualaniensis</i>	24.1 \pm 10.9	-	782 \pm 255	-	-	1720 \pm 151	319 \pm 67	-	-	-	-	-	513 \pm 288	f
<i>S. oualaniensis</i> *	14.0	22.4	199	3.28	0.163	558	293	0.125	1.36	1.91	1.10	1.85	128	i
<i>Todarodes pacificus</i> *	3.5	7.5	60	0.78	0.375	27.5	325	0.133	3.5	7.0	0.60	13.8	195	j
Octopodidae														
<i>Eledone cirrhosa</i>	3.20 \pm 1.74	-	24.0 \pm 1.8	2.06 \pm 0.08	0.8 \pm 0.1	456 \pm 11	287 \pm 13	-	4.2 \pm 1.6	2.5 \pm 0.1	1.17 \pm 0.09	3.3 \pm 0.5	646 \pm 86	d
<i>Octopus vulgaris</i>	-	-	-	8.8	-	275	275	-	2.7	-	-	-	1300	k
<i>O. vulgaris</i>	-	-	50 \pm 10	-	-	2500 \pm 700	700 \pm 130	-	7.0 \pm 0.5	-	-	4.5 \pm 1.0	1450 \pm 400	l
<i>O. vulgaris</i>	-	-	-	-	-	-	-	0.58 \pm 0.08	-	-	4.9 \pm 1.9	7.2 \pm 6.9	-	m

a: Present study (Bay of Biscay); b: Present study (Mediterranean); c: Bustamante et al., (2000); d: Miramand and Bentley (1992); e: Miramand et al., (2006); f: Martin and Flegal (1975); g: Finger and Smith (1987); h: Smith et al., (1984); i: Ichihashi et al., (2001a); j: Ichihashi et al., (2001b); k: Ueda et al., (1979); l: Miramand and Guary (1980); m: Seixas and Pierce (2005ab) and Seixas et al., (2005a)

* converted to dwt using a factor of 2.5

in italics: median