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Original article

The effect of size, weight, body compartment, sex and reproductive status on the bioaccumulation of 19 trace elements in rope-grown *Mytilus galloprovincialis*

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ABSTRACT

Numerous trace elements (TEs) can be considered as potential pollutants of the environment, their mining productions and industrial uses increasing worldwide. Their monitoring can be achieved through the use of bioindicator species, such as the Mediterranean mussel *Mytilus galloprovincialis* (Lamarck, 1819). That species has been widely used to monitor the chemical pollution of coastal ecosystems by Cr, Ni, Cu, Zn, Cd, Pb, As, Ag and V. Conversely, environmental levels of Be, Al, Fe, Mn, Co, Se, Mo, Sn, Sb and Bi have been little or not monitored so far in mussel watch programs. Bioaccumulation processes of these 19 TEs in rope-grown *M. galloprovincialis* purchased from a salt pond with good chemical water quality were thus investigated in the present study.

Mussels efficiently accumulated the 19 studied TEs. Bioaccumulation processes were driven by numerous mutually dependent biological parameters such as the mussel size and flesh weight, the sex and the reproductive status and the body compartment considered. TE bioaccumulation was a power function of the mussel soft body dry weight; total contents linearly increased with the shell length. Small-size mussels overall concentrated more TEs, with a high inter-individual variability, consequently influencing the modelling of their bioaccumulation in the whole rope population. Although a large range of rope-grown *M. galloprovincialis* sizes can be used for monitoring purposes, one will thus take care not to use extreme size individuals. The influence of gametogenesis in determining female body higher TE concentrations prior to spawning could not be neglected and varied depending on the element. TEs were preferentially accumulated in the hepatopancreas, except for Zn, Se, Cd and Mo, more concentrated in gills. Gametogenesis did not influence TE distribution between body compartments, but likely diluted their concentrations as a direct consequence of massive reproductive tissue production.

So, results from the present study underline the potential use of *M. galloprovincialis* in the biomonitoring of numerous little studied TEs and give some insights into the decisive role played by some relevant biological parameters in bioaccumulation processes of the 19 investigated TEs in rope-grown mussels.

1. Introduction

In the ocean, trace element (TE) biogeochemical balance varies spatially and with depth depending on water masses physico-chemical parameters (salinity, pH, temperature etc.) and biological processes (Bruland and Lohan, 2003). Natural continental runoff and atmospheric deposition of TEs to the oceans (Nriagu, 1989, 1990; Bruland and Lohan, 2003) can be dramatically increased in coastal and estuarine waters due to anthropogenic activities such as urbanization, industry, agriculture or mining (Laubier, 2005; Fernandez et al., 2007; Benedicto et al., 2011). TEs are moreover considered as non-degradable pollutants (Navratil and Minarik, 2011; Pan and Wang, 2012); this persistent character can thus alter, sometimes quite strongly, their natural biogeochemical balance in contaminated environments (Doney, 2010).

Since the mid-70ies, when Goldberg (1975) proposed the mussel-watch concept to record the quality of marine waters, mussels from the genus *Mytilus* have been used worldwide in biomonitoring surveys (e.g. Andral et al., 2004; Kimbrough et al., 2008). To biomonitor the extend and the importance of the coastal pollution by TEs, some researchers resort to indigenous populations of wild or cultivated mussels (passive biomonitoring), while others rely upon transplanting individuals from a reference site (active biomonitoring). Indeed, the active biomonitoring using caged raft mussels allows to bypass the natural variability of internal biological factors (size, sex, sexual maturity, reproduction stages, seasonal growth cycles) governing TE uptake and depuration processes in mussels (Casas and Bacher, 2006; Casas et al., 2008), and to solve the scarcity of mussel stocks along certain coasts (Andral et al., 2004).

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Mussel watch programs have historically focussed on a limited number of metals such as Pb, Zn or Cu. Nowadays, the development of very sensitive equipment (GFAAS, ICP-MS, NAA etc.) allows scientists and environment managers to measure some TEs found at very low environmental levels (e.g. Be; Hsu et al., 2004). In parallel, recent technological developments lead to an increase of mining extractions and industrial refinements of TEs of previous little concern (e.g. Sb; Filella et al., 2002). Environmental quantification of certain less studied potential pollutants is henceforth now possible and relevant (Luy et al., 2012).

The first purpose of this paper was to measure concentrations of numerous TEs (Be, Al, Fe, Mn, Co, Se, Mo, Sn, Sb and Bi) that have been little or never studied in the Mediterranean mussel *Mytilus galloprovincialis* (Lamarck, 1819), in addition to TEs classically monitored with that species (Cr, Ni, Cu, Zn, Cd, Pb, As, Ag and V), and to evaluate the contamination status of the Corsican (France) shellfish pond they were purchased from. As the influence of the mussel size on soft tissue TE levels still appears to be ambiguous (Przytarska et al., 2010), the second objective of this study was to model the effect of the mussel shell length and dry flesh weight on concentrations and contents of the 19 investigated TEs. Knowing that the accumulation of reserves during the sexual dormancy and their subsequent mobilization during gonadal development influence TE levels and body compartmentalization in *Mytilus* spp. (Cossa, 1989), the last objective of this work was to study the importance of these physiological processes in *M. galloprovincialis*.

2. Materials and methods

Only materials in ceramic, plastic and glass were used for sample treatment and storage. All materials were previously decontaminated in HCl 5% of analytical grade for a minimum of 48 h. Powder-free nitril gloves were used, and dissection material and lab benches were systematically cleaned with HCl 2% of analytical grade.

2.1. Site and biological material

Shellfish farming activities occur in 2 Corsican salt ponds of its eastern coast, the Urbino pond and the Diane pond. The oyster *Crassostrea gigas* is rope-grown in both sites, while the Mediterranean mussel *M. galloprovincialis* is nowadays only rope-grown in the Diane pond (Orsoni et al., 2001; Ramsar, 2008; Bouchoucha et al., 2012). The Diane pond is the third largest (surface area: 570 ha) and the deepest (maximal depth: 11 m; mean depth: 6 m) Corsican salt pond. Freshwater inputs are from the Arena stream (and the two smaller Pietroni and Ronsignese streams) and from the water runoff from part of the 69 km^2 catchment basin of the Bravona river. The pond is connected to the open sea through a small "grau", a channel silted up part of the year. The low water renewing of the pond takes several months (total volume: 30.2 million m^3), which makes it sensitive to anthropisation (Longere et al., 1972; Orsoni et al., 2001; Orsoni and Laugier, 2004). Although the pond receives some effluents (mainly agricultural) from the catchment basin, the general chemical diagnostic of its water quality remains good (Orsoni and Laugier, 2004; Bouchoucha et al., 2012). The high phytoplankton productivity of this relatively stable salt pond, mainly sustained by the recycling of nutrients stored in sediments, is a real advantage for the shellfish farming industry (Orsoni and Laugier, 2004).

The 3rd March 2010 (after mussel spawning) and the 11th February 2011 (before mussel spawning), rope-grown *M. galloprovincialis* were purchased from the shellfish farm *SARL Etang de Diane* (42°07′45.00″N, 9°31′01.00″E). Mussels were carefully detached from ropes with a ceramic scalpel. 74 mussels sampled in February 2011 were used to study the bioaccumulation behaviour of the 10 TEs little or never studied in that species, and to biomonitor the chemical contamination of the Diane pond by the 19 investigated TEs. They were also used to model relationships between the mussel flesh dry weight (from 0.17 to 3.36 g) or shell length (from 43.40 to 86.41 mm) and TE levels. In *Mytilus* spp., it is possible to tell the sex of individuals before they spawn by the colour of their gonadal follicles developed in the mantle (pink to orange for females and creamy-white to yellow for males, Mikhailov et al., 1995). On the basis of this colour pattern, the 74 mussels were segregated according to their sex to study differences between male and female TE accumulation prior to spawning. 40 supplementary large-size (70–80 mm shell length) mussels purchased in March 2010 ($n = 20$) and February 2011 ($n = 20$) were used for body compartmentalization analysis after and before spawning, respectively. Collected mussels were stored at −28 °C, without previous defecation of their digestive tract.

2.2. Sample preparation

In the laboratory, mussels were measured with an electronic calliper (0.01 mm). Soft tissues (byssus excluded) were carefully removed from shells with a ceramic scalpel. For the body compartmentalization study, mussels were dissected and tissues were sorted as follows: gills, hepatopancreas, mantle and remaining soft tissues. All samples (body compartments or whole individuals) were freeze dried (BenchTop 3L, VirTis Company Inc.) and weighed. Samples weighing more than 300 mg were further ground with liquid nitrogen in an agate mortar and then re-lyophilized to eliminate condensed ambient water vapour. Dried powders and unground samples were mineralized in Teflon bombs in a closed microwave digestion labstation (Ethos D, Milestone Inc.). The digestion procedure performed was a nitric acid-hydrogen peroxide mineralization (HNO_3/H_2O_2; suprapure grade, Merck). Digestates were diluted to an appropriate volume of 50 ml prior to being analysed. Mussel shells were oven dried (48 h at 60 °C) and weighed to calculate individual condition indices (Andral et al., 2004).

2.3. TE analysis

TE levels were determined by inductively coupled plasma mass spectrometry (ICP-MS) using dynamic reaction cell (DRC) technology (ICP-MS ELAN DRC II, PerkinElmer Inc.). Analytical accuracy was checked by analysing Certified Reference Materials: BCR 278 (mussel tissue; $n = 3$) from the Belgian Institute for Reference Materials and Measurements, and NIST 1566b (oyster tissue; $n = 10$) and NIST 2976 (mussel tissue; $n = 10$) from the American National Institute of Standards and Technology. Mean TE recoveries, all 3 CRMs together, ranged from 86% for Sb to 108 ± 9% for Se; only Cr mean recovery was lower (mean recovery: 72 ± 3%). The global mean recovery, all elements together, was 95 ± 9% (no certified or indicative value for Be and Bi). For each TE, detection decision (L_C), detection limit (L_D) and quantification limit (L_Q) were calculated according to Currie (1999) or Grinzaid et al. (1977), depending on their specific blank distribution (normal or not). Data were analysed as TE concentrations or total contents on a dry weight basis and are expressed in $\mu g\, g^{-1}{}_{DW}$ or in μg, respectively.

2.4. Mathematical and statistical analysis

Statistical analyses were performed with STATISTICA 10 (StatSoft Inc.) and GraphPad Prism 5 (GraphPad Software Inc.) softwares.

2.4.1. Pairwise and multiple comparison tests of means

TEs measured in the 4 body compartments of each mussel were added to calculate individual TE total contents, and weighted by their respective dry weight to calculate individual TE concentrations. Significant differences between mean TE levels (concentrations and contents) measured in the 4 body

compartments and in whole mussels (for each reproductive status, respectively) were highlighted through one-way analysis of variance (one-way ANOVA) followed by Tukey HSD pairwise comparison test of means with equal n's ($p < 0.05$), after testing for normality or homogeneity of variances (Levene test) on raw or log-transformed data. Non-parametric analysis of variance (Kruskal–Wallis test) was performed when assumptions prior to ANOVAs (normality and/or homoscedasticity) were not achieved, followed by Dunn pairwise comparison test of means ($p < 0.05$). An equivalent statistical procedure (for unequal n's) was performed to compare TE concentrations between mussels separated into 4 equivalent size-classes of about 10 mm (43–54, 55–64, 65–74 and 75–87 mm; Saavedra et al., 2004).

TE levels (concentrations and contents) in each body compartment and in whole mussels were then compared between reproductive statuses, using Student pairwise comparison test of means with equal n's ($p < 0.05$), after testing for normality and homogeneity of variances (Levene test) on raw or log-transformed data. Non-parametric pairwise comparison test of means (test U of Mann–Whitney, $p < 0.05$) was performed when assumptions prior to Student tests (normality and/or homoscedasticity) were not achieved. An equivalent statistical procedure (for unequal n's) was performed to compare TE concentrations between females and males prior to spawning.

2.4.2. Condition index calculation

Mussel condition index (CI = the ratio of dry flesh weight to dry shell weight) was calculated according to Andral et al. (2004), to verify that this physiological index did not statistically ($p > 0.05$) evolve with the size of mussels sampled prior to spawning. There was no significant linear relationship ($p = 0.7818$) between the CI and the shell length. The mussel gamete maturation coupled together with the important food availability in late winter–early spring planktonic bloom resulted in the relatively high mean CI (0.226 ± 0.053) calculated (Casas, 2005). As mussels showed the same physiological status independently of their size, they could be all used for modelling the effect of the mussel weight and size on TE levels (concentrations and contents).

2.4.3. Principal component analysis and cluster analysis

Data matrices, where rows are the objects (mussel samples) and columns are the variables (Cr, Ni, Cu, Zn, Cd, Pb, As, Ag, V, Be, Al, Fe, Mn, Co, Se, Mo, Sn, Sb and Bi concentrations), were built. The data pre-treatment used in this study was the half-range central value transformation (Moreda-Pineiro et al., 2001). Unsupervised pattern recognition techniques used were principal component analysis (PCA) and cluster analysis (CA). Samples were clustered using the Ward's method (Euclidean distance between objects as measure of similarity). PCA and CA were carried out on a first data set composed of mussels sampled prior to spawning ($n = 74$), with sizes ranging from 43.40 to 86.41 mm. PCA and CA were also carried out on a 2nd data set composed of body compartments (gills, hepatopancreas, mantle and remaining soft tissues) and whole mussels ($n = 200$) sampled at both reproductive statuses, i.e. before and after spawning.

2.4.4. Data modelling

The effect of the mussel weight and size on measured TE levels in the 74 individuals sampled prior to spawning were modelled using a power function, according to previous studies (Lobel et al., 1992; Saavedra et al., 2004; Mubiana et al., 2006): TE concentration (M, in $\mu g\,g^{-1}{}_{DW}$) is a power function of the mussel soft tissue dry weight (W), and total TE content (M, in μg) is a power function of the mussel shell length (L):

$$M = aW^b \quad (1)$$

$$M = aL^b \quad (2)$$

Relationships of Eqs. (1) and (2) were double logarithmically transformed to yield 2 linear functions:

$$\log_{10} M = \log_{10} a + b \log_{10} W \quad (3)$$

$$\log_{10} M = \log_{10} a + b \log_{10} L \quad (4)$$

The effect of the mussel weight and size on TE levels was also modelled using a linear regression model, TE concentration (M, in $\mu g\,g^{-1}{}_{DW}$) and total TE content (M, in μg) being a linear function of the mussel soft tissue dry weight (W) or shell length (L), respectively:

$$M = bW + a \quad (5)$$

$$M = bL + a \quad (6)$$

b is the slope of linear functions (3)–(6); $\log_{10} a$ and a are the Y-intercepts. To select the most adequate model (power function or linear regression) that the best described the effect of the mussel weight and size on TE levels (concentrations and contents), an Akaike information criterion (AIC) analysis was performed. In particular, the second order information criterion, often called AICc, which takes into account sample size was used (Burnham and Anderson, 2002). An equivalent modelling procedure was performed for mussels larger than 55 mm only ($n = 55$). The linear regression model was further used to model relationships between the mussel shell length and TE concentrations, all mussel sizes together ($n = 74$) or limited to mussels larger than 55 mm ($n = 55$), segregated or not according to their sex.

3. Results and discussion

3.1. Chemical status of the Diane pond

TE concentrations discussed in following Sections 3.1.1–3.1.4 are all expressed in $\mu g\,g^{-1}{}_{DW}$ (Tables 1 and 2).

3.1.1. Cr, Ni, Cu, Zn, Cd, As and Pb

Cr, Ni, Cu, Zn, Cd, As and Pb concentrations in rope-grown mussels from the Diane pond were within the range of values measured in the Mediterranean, notably by French mussel watch programs (RINBIO, 2001; Andral et al., 2004; RNO, 2006; Benedicto et al., 2011). For example, Cd (0.374 ± 0.131), Pb (0.336 ± 0.192) and Ni (1.41 ± 0.54) concentrations were very low to moderate when compared to 123 sites in the western Mediterranean basin (mean levels for Cd = 1.33, Pb = 1.40, Ni = 1.10) surveyed with caged mussels between years 2004 and 2006 (Benedicto et al., 2011). Cr (0.554 ± 0.320), Cd and Pb were in the low range of concentrations when compared to 97 mussel cages immersed in year 2000 along the French Mediterranean coasts (mean levels for Cr = 1, Cd = 0.9, Pb = 1 and As = 20, Ni = 2, Cu = 4.1) and in the intermediate range for As (31.2 ± 6.1), Ni (1.41 ± 0.54) and Cu (4.82 ± 1.50); Zn (72.6 ± 33.6) concentrations were half the mean stable value measured in this area (Zn = 148.3; RINBIO, 2001; Andral et al., 2004). Since Cr, Ni, Cu, Zn, Cd, As and Pb concentrations measured in the present study ranged from very low to medium when compared to previous large spatial scale monitoring surveys in the western Mediterranean (RINBIO, 2001; Andral et al., 2004; RNO, 2006; Benedicto et al., 2011), the chemical status of the Diane pond for these 7 elements can thus be considered as good; Cr, Ni, Cu, Zn, Cd, As and Pb concentrations were moreover similar to concentrations previously reported in mussels from the Diane pond (RINBIO, 2001; Andral et al., 2004; RNO, 2006). Finally, Cd and Pb concentrations were under the maximal value of 1 $\mu g\,g^{-1}$ of bivalve mollusc fresh weight fixed by the European Legislation EC No. 466-2001 (EC, 2001).

Table 1
Trace element mean concentrations, ranges of mean concentrations or ranges of individual (asterisks, *) concentrations ($\mu g\, g^{-1}_{DW}$) in *Mytilus galloprovincialis* and other mollusc species. Numbers between brackets represent the number of sampled sites in each study, respectively (not available for ref. 18).

	Reference	Studied area	Species	Al	V	Fe	Cr	Mn
1.	Benedicto et al. (2011)	Western Mediterranean bassin (123)	*M. galloprovincialis*					
2.	RINBIO (2001) and Andral et al. (2004)	French Mediterranean coasts (93)	*M. galloprovincialis*				1	
3.	Bartolomé et al. (2010)	Atlantic coast of northwestern Spain (10)	*M. galloprovincialis*		1.7–7.1		2.6–6.1	5.6–55.3
4.	Desideri et al. (2009)	Central Adriatic Sea (7)	*M. galloprovincialis*			45–755		73–83
5.	Çevik et al. (2008)	Eastern Black Sea (5)	*M. galloprovincialis*			1150–4030	1–3	41–59
6.	Favretto et al. (1997)	Muggia Bay, north Adriatic Sea (1)	*M. galloprovincialis* *			15.5–64.8		0.70–2.42
7.	This study	Diane pond, east Corsica (1)	*M. galloprovincialis* *	39–877	2.3–12.5	66–656	0.19–2.17	3.4-20.6
8.	RNO (2006)	French Med. and English Channel-Atlantic coasts (83)	*Mytilus* spp.		0.43–15.4		0.12–9.21	
9.	Giltrap et al. (2013)	Irish coasts (4)	*M. edulis*	40–201	0.95–1.59	30–261	0.71–1.51	4.6–13.4
10.	Gobert et al. (1992)	Belgian coast (4)	*M. edulis*			166–247	0.9–1.2	
11.	Brooks and Rumsby (1965)	Tasman Bay, New Zealand (1)	*M. edulis aoteanus* *		2–8	960–2640	9–24	12–38
12.	Watling and Watling (1976)	Saldanha Bay, South Africa (3)	*C. meridionalis*					9–11
13.	Giltrap et al. (2013)	Irish coasts (4)	*C. gigas*	81–148	0.88–1.29	175–254	0.79–1.70	21.3–47.1
14.	Hsu et al. (2004)	Chigu Lagoon, southwestern Taiwan (1)	*C. gigas*					
15.	Watling and Watling (1976)	Langebaan Lagoon, South Africa (1)	*C. gigas*					12
16.	Brooks and Rumsby (1965)	Tasman Bay, New Zealand (1)	*O. sinuata* *		2–4	630–750	2–6	1–11
17.	Brooks and Rumsby (1965)	Tasman Bay, New Zealand (1)	*P. novae-zelandiae* *		5–14	1140–6900	3–23	12–306
18.	Papadopoulu (1973) in Eisler (2010)	Greek coasts	*A. noae*					

	Co	Ni	Cu	Zn	Se	Ag	Cd	Sn	Sb	As	Mo	Be	Pb	Bi
1.		0.1–3.4					0.46–2.89						0.5–8.3	
2.		2	2.9–9.2	116–203			0.1–5.9			20			0.5–5.4	
3.	0.4–69.3	0.8–15.4	6.9–59.9	192–301	5.4–9.3		0.4–2.3	0.1–1.4		14–32			1.1–13.3	
4.		1.3–7.6	18–156	61–190			0.6–1.0	0.6–3.9					2.0–9.0	
5.		1–6	90–260	180–630			2–4						5–21	
6.	0.06–0.53	0.41–2.30	0.77–2.23	6.9–29.6			0.12–0.38						0.48–1.79	
7.	0.37–1.37	0.71–3.39	2.8–7.4	35–224	1.5–4.3	0.005–0.033	0.21–1.03	0.015–0.089	0.007–0.028	17–46	4.8–33.1	0.005–0.037	0.14–1.10	0.005–0.021
8.		0.45–8.41	3.8–67.0	36–409		0.01–7.75	0.17–10.00						0.1–27.7	
9.	0.54–0.88	0.88–1.56	5.8–14.1	118–251			0.67–1.77		0.25–0.39	6.7–17.4	1.00–2.64		1.01–4.24	
10.			5.6–6.2	107–142			0.5–1.2						0.7–2.3	
11.		1–17	5–11	50–180		0.1–0.3	<10				0.1–1.0		3–25	
12.	2–3	2–3	7–14	73–113			1–8						2–5	5–6
13.	0.46–0.52	0.81–1.58	21–282	673–1466			1.14–1.49		0.27–0.28	9.5–20.7	0.83–1.85		0.74–1.65	
14.												0.0247		
15.	1	1	33	424			9						1	4
16.		1–3	21–53	850–1500		4.5–7.3	10–43				0.1–0.4		6–14	
17.		2–17	2–14	195–368		0.2–2.3	210–299				0.1–2.3		10–23	
18.											88.0			

Table 2
Trace element (TE) concentrations in rope-grown *Mytilus galloprovincialis* from the Diane pond (east Corsica), sampled in February 2011 before they spawned. Concentrations are given for all mussels together, independently of their sex (left side of the table; mean ± SD and median, in $\mu g\,g^{-1}{}_{DW}$; $n = 74$), or distinctly for females ($n = 29$) and males ($n = 45$; right side of the table; mean ± SD, in $\mu g\,g^{-1}{}_{DW}$). Asterisks (*) represent significant differences ($p < 0.05$) between sexes.

	TE concentrations for all mussels together		Mean TE concentrations by sex	
	Means	Medians	Females	Males
Al	200 ± 150	152	204 ± 143	197 ± 156
V	5.35 ± 2.02	5.07	*6.55 ± 2.40	*4.57 ± 1.24
Fe	177 ± 97	149	186 ± 89	172 ± 102
Cr	0.554 ± 0.320	0.459	0.581 ± 0.288	0.537 ± 0.341
Mn	9.86 ± 3.87	9.19	*12.18 ± 3.48	*8.36 ± 3.37
Co	0.634 ± 0.205	0.589	*0.707 ± 0.215	*0.587 ± 0.185
Ni	1.41 ± 0.54	1.28	*1.68 ± 0.59	*1.24 ± 0.43
Cu	4.82 ± 1.50	4.03	*6.50 ± 0.67	*3.74 ± 0.61
Zn	72.6 ± 33.6	64.3	*86.3 ± 36.4	*63.7 ± 28.8
Se	2.70 ± 0.78	2.45	*3.48 ± 0.34	*2.21 ± 0.54
Ag	0.0123 ± 0.0054	0.0111	*0.0151 ± 0.0064	*0.0104 ± 0.0038
Cd	0.374 ± 0.131	0.336	*0.397 ± 0.106	*0.358 ± 0.144
Sn	0.0318 ± 0.0167	0.0259	0.0323 ± 0.0160	0.0314 ± 0. 0174
Sb	0.0126 ± 0.0042	0.0115	*0.0140 ± 0.0048	*0.0118 ± 0.0036
As	31.2 ± 6.1	31.3	*36.3 ± 4.3	*27.8 ± 4.6
Mo	17.1 ± 5.8	17.1	*20.7 ± 5.8	*14.8 ± 4.5
Be	0.0135 ± 0.0056	0.0122	0.0127 ± 0.0056	0.0140 ± 0.0057
Pb	0.336 ± 0.192	0.279	0.378 ± 0.211	0.309 ± 0.175
Bi	0.0087 ± 0.0032	0.0078	*0.0097 ± 0.0034	*0.0080 ± 0.0028

3.1.2. Ag and V

The Ag (0.0123 ± 0.0054) mean concentration was similar to mussel (*Mytilus* spp.) Ag concentrations from the cleanest stations of 83 sites sampled along the English Channel-Atlantic and Mediterranean French coasts (range of Ag concentrations = 0.01–7.75; RNO, 2006). Unlike Ag, the V mean concentration (5.35 ± 2.02) was high compared to the range of available data for the Diane pond (0.92–3.62; www.ifremer.fr/envlit/) and to median values for the English Channel-Atlantic (1.62) and Mediterranean (1.40) French coasts in general (RNO, 2006). It can result from the combined effect of the pond confinement, the important aquaculture activity with regard to the pond size, inputs from the water runoff from part of the catchment basin of Bravona and from the 3 small streams feeding the pond (important precipitations in eastern Corsica in late January 2011; www.meteofrance.com) and releases from sediments (Orsoni et al., 2001; Amiard et al., 2008; Santos-Echeandia et al., 2009; Abi-Ghanem et al., 2013).

3.1.3. Fe, Mn, Co, Se and Sn

Available data from the literature for Fe, Mn, Co, Se and Sn in mollusc bivalves are more scarce. Bartolomé et al. (2010) studied seasonal and pluriannual variations of Co, Mn, Se and Sn concentrations in wild *M. galloprovincialis* from the Atlantic coast of northwestern Spain (median concentrations for Co = 0.8, Mn = 21.6, Se = 8.1 and Sn = 0.4). Concentrations measured in the Diane pond (Co = 0.634 ± 0.205, Mn = 9.86 ± 3.87, Se = 2.70 ± 0.78 and Sn = 0.0318 ± 0.0167) were similar or lower than concentrations reported by these authors. Moreover, mean Sn (1.3) and Mn (77.5) concentrations measured in wild and raft *M. galloprovincialis* from the central Adriatic Sea were about 40 and 9 times higher than in the Diane pond, respectively (Desideri et al., 2009). Conversely, mean levels of Mn (1.29) and Co (0.27) measured in raft *M. galloprovincialis* from the Muggia Bay, north Adriatic Sea, were lower than in the Diane pond (Favretto et al., 1997). Fe concentrations (177 ± 97) were similar to mean concentrations recorded in *Mytilus* spp. from the Belgian coast (217; Gobert et al., 1992) and the central Adriatic Sea (199; Desideri et al., 2009), but lower than in the contaminated eastern Black Sea (2462; Çevik et al., 2008).

3.1.4. Al, Sb, Mo, Be and Bi

For Al, Sb, Mo, Be and Bi, little data have been published; furthermore, at least 3 of these TEs (Sb, Mo and Bi) were identified as coastal pollutants (Luy et al., 2012). Giltrap et al. (2013) recently monitored Al and Sb in Irish coastal waters with caged mussels *Mytilus edulis* (mean levels for Al = 118, Sb = 0.28) and oysters *Crassostrea gigas* (mean levels for Al = 117, Sb = 0.32). Concentrations reported by these authors were lower than the mean Al concentration (200 ± 150) measured in the Diane pond; this terrigenous TE was more concentrated and bioavailable in this semi-enclosed salt pond catching part of streaming waters from the catchment basin of Bravona. Conversely, Sb concentrations (0.0126 ± 0.0042) were very below concentrations measured by these authors. Be concentrations in marine organisms are poorly documented mainly because they are often below detection limits of instrumental techniques employed. Nonetheless, Be concentrations in rope-grown mussels from the Diane pond (0.0135 ± 0.0056) were similar to concentrations measured by Hsu et al. (2004) in oysters *C. gigas* (0.0247) harvested from the Chigu Lagoon (southwestern Taiwan). The Mo mean concentration (17.1 ± 5.8) was in-between low concentrations measured by Brooks and Rumsby (1965) in three bivalves (mean Mo concentrations in the mussel *M. edulis aoteanus* = 0.6, in the oyster *Ostrea sinuata* = 0.3, in the scallop *Pecten novae-zelandiae* = 0.9) collected in the Tasman Bay (New Zealand) and the higher concentration reported by Papadopoulu (1973, in Eisler, 2010) for the bivalve *Arca noae* (88.0) from Greek coastal waters. Finally, the only available Bi concentrations in bivalves reported by Watling and Watling (1976) for the mussel *Choromytilus meridionalis* (5–6) and the oyster *C. gigas* (4) were high when compared to Bi concentrations in mussels from the Diane pond (0.0087 ± 0.0032).

3.2. TE bioaccumulation in rope-grown mussels prior to spawning

3.2.1. TE concentrations as a function of the mussel weight vs. TE contents as a function of the mussel shell length

The present section investigates which of the power function or the linear regression better models relationships between the mussel weight and TE concentrations or the mussel shell length and TE contents.

Tissue concentrations of the 19 studied TEs decreased when the mussel flesh dry weight increased; their tissue contents increased when the mussel shell length increased (slope *b* in Table 3A). This is consistent with previous studies modelling the effect of the mussel weight and size on TE bioaccumulation (e.g. Lobel et al., 1991; Martincic et al., 1992). Significant regressions were found for all

Table 3
Comparison of linear regressions and double log transformed power functions modelling relationships between trace element (TE) concentrations in rope-grown *Mytilus galloprovincialis* from the Diane pond (east Corsica), sampled in February 2011 before they spawned, and their flesh dry weight and relationships between total TE contents and the mussel shell length. Relationships between the mussel dry weight and their shell length are also modelled. (A) All mussels together ($n = 74$). (B) Mussels larger than 55 mm ($n = 55$). b is the slope of linear functions; a and $\log_{10}a$ are the Y-intercepts. Fitting parameters are also indicated (r^2; p-levels; deviation (dev.) from the model: s. = significant, n.s. = non-significant; AICc).

A.	Relationships between TE concentrations and the mussel flesh dry weight—all mussels												Relationships between TE contents and the mussel shell length—all mussels											
	Linear regressions						Double log transformed power functions						Linear regressions						Double log transformed power functions					
	b	a	r^2	p	dev.	AICc	b	$\log_{10}a$	r^2	p	dev.	AICc	b	a	r^2	p	dev.	AICc	b	$\log_{10}a$	r^2	p	dev.	AICc
Length	13.02	41.74	0.781	<0.001	n.s.	93.72%	0.289	1.752	0.764	<0.001	n.s.	6.28%												
Weight													0.0600	−2.134	0.781	<0.001	n.s.	92.16%	2.278	−3.903	0.766	<0.001	n.s.	7.84%
Al	−92.47	355.3	0.263	<0.001	n.s.	<0.01%	−0.672	2.349	0.475	<0.001	n.s.	>99.99%	6.7880	−158.9	0.273	<0.001	n.s.	37.89%	1.734	−0.703	0.282	<0.001	n.s.	62.11%
V	−1.029	7.076	0.180	<0.001	n.s.	94.85%	−0.172	0.752	0.113	<0.01	n.s.	5.15%	0.2320	−6.475	0.560	<0.001	n.s.	88.76%	1.669	−2.099	0.534	<0.001	n.s.	11.24%
Fe	−64.00	285.1	0.300	<0.001	n.s.	<0.01%	−0.531	2.298	0.507	<0.001	n.s.	>99.99%	6.9590	−188.0	0.520	<0.001	n.s.	40.93%	1.812	−0.874	0.525	<0.001	n.s.	59.07%
Cr	−0.205	0.898	0.283	<0.001	n.s.	<0.01%	−0.565	−0.208	0.514	<0.001	n.s.	>99.99%	0.0224	−0.632	0.520	<0.001	n.s.	34.62%	1.900	−3.541	0.528	<0.001	n.s.	65.38%
Mn	−3.055	14.99	0.430	<0.001	n.s.	72.89%	−0.336	1.035	0.414	<0.001	n.s.	27.11%	0.3317	−6.611	0.425	<0.001	n.s.	67.03%	1.385	−1.340	0.413	<0.001	n.s.	32.97%
Co	−0.112	0.822	0.206	<0.001	n.s.	13.12%	−0.230	−0.168	0.245	<0.001	n.s.	86.88%	0.0340	−1.174	0.820	<0.001	s.	97.18%	2.176	−3.946	0.801	<0.001	s.	2.82%
Ni	−0.429	2.136	0.436	<0.001	n.s.	0.16%	−0.375	0.194	0.527	<0.001	n.s.	99.84%	0.0591	−1.675	0.752	<0.001	n.s.	87.57%	1.788	−2.914	0.739	<0.001	n.s.	12.43%
Cu	−0.815	6.195	0.205	<0.001	n.s.	96.69%	−0.154	0.705	0.129	<0.01	s.	3.31%	0.1974	−5.002	0.443	<0.001	n.s.	76.62%	1.557	−1.934	0.425	<0.001	n.s.	23.38%
Zn	−12.60	93.76	0.097	<0.01	n.s.	22.23%	−0.240	1.892	0.127	<0.01	n.s.	77.77%	3.9500	−137.9	0.578	<0.001	n.s.	65.17%	2.246	−2.016	0.571	<0.001	n.s.	34.83%
Se	−0.559	3.645	0.356	<0.001	n.s.	90.27%	−0.224	0.462	0.316	<0.001	n.s.	9.73%	0.1145	−3.116	0.548	<0.001	n.s.	79.45%	1.685	−2.427	0.531	<0.001	n.s.	20.55%
Ag	−0.004	0.019	0.348	<0.001	n.s.	76.21%	−0.331	−1.870	0.327	<0.001	n.s.	23.79%	0.0004	−0.008	0.376	<0.001	n.s.	66.52%	1.362	−4.204	0.365	<0.001	n.s.	33.48%
Cd	−0.059	0.472	0.138	<0.01	n.s.	2.75%	−0.246	−0.397	0.217	<0.001	n.s.	97.25%	0.0194	−0.648	0.751	<0.001	n.s.	99.12%	2.004	−3.856	0.718	<0.001	s.	0.88%
Sn	−0.009	0.047	0.191	<0.001	n.s.	0.31%	−0.411	−1.454	0.308	<0.001	n.s.	99.69%	0.0014	−0.040	0.374	<0.001	n.s.	60.03%	1.836	−4.646	0.367	<0.001	n.s.	39.97%
Sb	−0.003	0.018	0.368	<0.001	n.s.	2.73%	−0.302	−1.861	0.426	<0.001	n.s.	97.27%	0.0006	−0.018	0.772	<0.001	n.s.	92.35%	1.923	−5.199	0.756	<0.001	s.	7.65%
As	−2.950	36.13	0.162	<0.001	n.s.	98.48%	−0.069	1.504	0.062	<0.05	s.	1.52%	1.5280	−46.84	0.697	<0.001	n.s.	96.25%	1.844	−1.633	0.669	<0.001	n.s.	3.75%
Mo	−2.006	20.49	0.083	<0.05	s.	93.09%	−0.059	1.242	0.016	n.s.	s.	6.91%	0.7283	−18.92	0.466	<0.001	s.	88.66%	1.500	−1.270	0.435	<0.001	s.	11.34%
Be	−0.003	0.018	0.191	<0.001	n.s.	0.03%	−0.360	−1.830	0.350	<0.001	n.s.	99.97%	0.0007	−0.023	0.646	<0.001	n.s.	51.21%	2.181	−5.638	0.646	<0.001	n.s.	48.79%
Pb	−0.077	0.465	0.111	<0.01	n.s.	12.10%	−0.322	−0.435	0.157	<0.001	n.s.	87.90%	0.0197	−0.741	0.521	<0.001	n.s.	6.00%	2.809	−5.398	0.556	<0.001	n.s.	94.00%
Bi	−0.002	0.012	0.294	<0.001	n.s.	3.41%	−0.306	−2.024	0.355	<0.001	n.s.	96.59%	0.0004	−0.011	0.672	<0.001	s.	93.65%	1.764	−5.069	0.648	<0.001	s.	6.35%
Mean			0.244			35.76%			0.294			64.24%			0.564			69.95%			0.553			30.05%
SD			0.109			42.51%			0.161			42.51%			0.153			26.03%			0.148			26.03%
Median			0.206			12.10%			0.316			87.90%			0.548			76.62%			0.534			23.38%

B.	Relationships between TE concentrations and the mussel flesh dry weight—mussels > 55 mm												Relationships between TE contents and the mussel shell lenght—mussels > 55 mm											
	Linear regressions						Double log transformed power functions						Linear regressions						Double log transformed power functions					
	b	a	r^2	p	dev.	AICc	b	$\log_{10}a$	r^2	p	dev.	AICc	b	a	r^2	p	dev.	AICc	b	$\log_{10}a$	r^2	p	dev.	AICc
Length	9.389	50.32	0.529	<0.001	n.s.	95.67%	0.255	1.767	0.473	<0.001	n.s.	4.33%												
Weight													0.0563	−1.874	0.529	<0.001	n.s.	40.04%	1.927	−3.245	0.536	<0.001	n.s.	59.96%
Al	−38.36	235.4	0.088	<0.05	n.s.	42.49%	−0.465	2.325	0.098	<0.05	n.s.	57.51%	9.1460	−329.6	0.211	<0.001	n.s.	43.54%	2.146	−1.476	0.218	<0.001	n.s.	56.46%
V	−1.807	8.875	0.383	<0.001	n.s.	0.05%	−0.743	0.915	0.531	<0.001	n.s.	99.95%	0.1358	0.454	0.154	<0.01	n.s.	49.92%	0.949	−0.753	0.155	<0.01	n.s.	50.08%
Fe	−36.16	224.3	0.181	<0.01	n.s.	28.08%	−0.468	2.307	0.208	<0.001	n.s.	71.92%	7.6150	−235.8	0.336	<0.001	n.s.	44.10%	1.829	−0.906	0.342	<0.001	n.s.	55.90%
Cr	−0.111	0.696	0.165	<0.01	n.s.	23.32%	−0.479	−0.197	0.200	<0.001	n.s.	76.68%	0.0258	−0.880	0.359	<0.001	n.s.	42.88%	1.988	−3.707	0.365	<0.001	n.s.	57.12%
Mn	−3.028	14.99	0.340	<0.001	n.s.	39.82%	−0.606	1.111	0.350	<0.001	n.s.	60.18%	0.1805	4.275	0.078	<0.05	n.s.	50.13%	0.751	−0.156	0.078	<0.05	n.s.	49.87%
Co	−0.163	0.948	0.308	<0.001	n.s.	1.66%	−0.550	−0.060	0.403	<0.001	n.s.	98.34%	0.0321	−1.036	0.597	<0.001	n.s.	49.64%	1.842	−3.319	0.597	<0.001	n.s.	50.36%
Ni	−0.423	2.139	0.406	<0.001	n.s.	0.16%	−0.678	0.290	0.530	<0.001	n.s.	99.84%	0.0537	−1.286	0.494	<0.001	n.s.	51.40%	1.507	−2.390	0.493	<0.001	n.s.	48.60%
Cu	−1.099	6.818	0.222	<0.001	s.	45.66%	−0.433	0.781	0.227	<0.001	n.s.	54.34%	0.1121	1.123	0.093	<0.05	n.s.	49.87%	0.871	−0.655	0.093	<0.05	n.s.	50.13%
Zn	−17.46	105.7	0.115	<0.05	n.s.	26.28%	−0.511	1.986	0.148	<0.01	n.s.	73.72%	3.9500	−138.1	0.317	<0.001	n.s.	47.38%	2.002	−1.559	0.320	<0.001	n.s.	52.62%
Se	−0.636	3.818	0.291	<0.001	s.	23.47%	−0.475	0.533	0.321	<0.001	n.s.	76.53%	0.0850	−1.007	0.208	<0.001	n.s.	49.76%	1.207	−1.534	0.208	<0.001	n.s.	50.24%
Ag	−0.004	0.019	0.355	<0.001	n.s.	2.37%	−0.728	−1.760	0.436	<0.001	n.s.	97.63%	0.0002	0.009	0.042	n.s.	n.s.	50.11%	0.599	−2.780	0.042	n.s.	n.s.	49.89%
Cd	−0.094	0.560	0.356	<0.001	n.s.	4.89%	−0.506	−0.295	0.422	<0.001	n.s.	95.11%	0.0145	−0.290	0.388	<0.001	n.s.	50.22%	1.394	−2.715	0.388	<0.001	n.s.	49.78%
Sn	−0.006	0.041	0.082	<0.05	n.s.	33.90%	−0.442	−1.424	0.104	<0.05	n.s.	66.10%	0.0013	−0.034	0.150	<0.01	n.s.	51.19%	1.557	−4.123	0.148	<0.01	n.s.	48.81%
Sb	−0.003	0.019	0.359	<0.001	n.s.	2.59%	−0.566	−1.775	0.438	<0.001	n.s.	97.41%	0.0005	−0.014	0.517	<0.001	n.s.	51.82%	1.604	−4.601	0.516	<0.001	n.s.	48.18%
As	−5.407	41.68	0.339	<0.001	n.s.	29.66%	−0.331	1.579	0.359	<0.001	n.s.	70.34%	1.0950	−15.57	0.322	<0.001	n.s.	50.05%	1.252	−0.525	0.322	<0.001	n.s.	49.95%
Mo	−6.253	30.17	0.458	<0.001	n.s.	7.82%	−0.661	1.421	0.504	<0.001	n.s.	92.18%	0.1684	21.42	0.023	n.s.	n.s.	48.73%	0.363	0.852	0.025	n.s.	n.s.	51.27%
Be	−0.002	0.016	0.072	<0.05	n.s.	50.10%	−0.238	−1.842	0.072	<0.05	n.s.	49.90%	0.0007	−0.027	0.432	<0.001	n.s.	51.34%	2.059	−5.409	0.430	<0.001	n.s.	48.66%
Pb	−0.078	0.473	0.089	<0.05	n.s.	24.45%	−0.539	−0.355	0.126	<0.01	n.s.	75.55%	0.0264	−1.221	0.437	<0.001	n.s.	35.13%	3.003	−5.764	0.449	<0.001	n.s.	64.87%
Bi	−0.003	0.014	0.394	<0.001	n.s.	0.55%	−0.676	−1.901	0.498	<0.001	n.s.	99.45%	0.0003	−0.003	0.294	<0.001	n.s.	49.92%	1.179	−3.976	0.294	<0.001	n.s.	50.08%
Mean			0.263			20.39%			0.315			79.61%			0.287			48.27%			0.289			51.73%
SD			0.129			17.31%			0.160			17.31%			0.169			4.14%			0.170			4.14%
Median			0.308			23.47%			0.350			76.53%			0.317			49.92%			0.320			50.08%

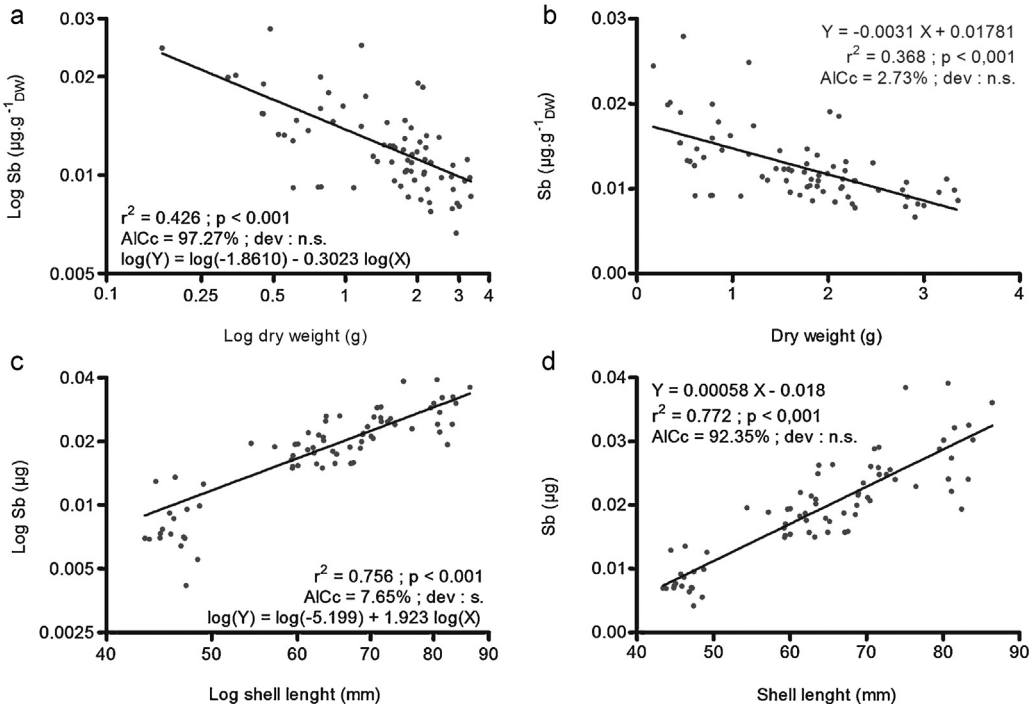

Fig. 1. Double log transformed power functions and linear regressions modelling relationships between (a and b) the soft tissue dry weight and Sb concentrations ($\mu g\,g^{-1}{}_{DW}$) and between (c and d) the shell length and Sb total contents (µg) of rope-grown *Mytilus galloprovincialis* ($n = 74$) from the Diane pond (east Corsica), sampled in February 2011 before they spawned. Linear equations and their corresponding fitting parameters (r^2; p-levels; deviation (dev.) from the model: s. = significant, n.s. = non-significant; AICc) are reported on graphs.

TEs ($p < 0.05$), except for the relationship between the weight and Mo concentrations in the double log transformed power function model. Mean goodness of fit was higher for relationships between the mussel shell length and TE contents (mean $r^2 = 0.564$ or 0.553 for the linear regression or the power function, respectively); linear equations deviated less often from the trend of data when modelling the effect of the mussel dry weight on TE concentrations (4 significant deviations – $p < 0.05$ – for Mo, Cu and As, both models together). The AICc showed that power functions better described relationships between the mussel dry weight and TE concentrations (mean AICc = 64.24%), while linear regressions better described relationships between the mussel shell length and TE contents (mean AICc = 69.95%). The example given in Fig. 1 for Sb properly illustrates statistical considerations of Table 3A.

Results of regression fitting parameters were inconsistent with observations made by Saavedra et al. (2004) who elected the power function to model the effect of raft *M. galloprovincialis* size, ranging from 52 to 87 mm, on TE contents. In order to work with similar data sets, both models were run again for mussels larger than 55 mm only (Table 3B). Mean calculated AICc were then equal to 48.27% and 51.73% for linear and power relationships between TE contents and the mussel shell length, respectively. As the difference in likelihood was very small (i.e. similar mean AICc), one may have concluded that both models were correct, as did Saavedra et al. (2004). Moreover, the 7 relationships between V, Mn, Cu, Se, Ag, As and Mo concentrations and the mussel dry weight, previously better modelled by linear regressions (AICc of Table 3A), were then better modelled by power functions (AICc of Table 3B), in agreement with Lobel et al. (1989, 1991) and Mubiana et al. (2006). Overall fitting parameters of power functions were also improved: the mean AICc increased from 64.24% to 79.61%, and the mean r^2 from 0.294 to 0.315. The example given in Fig. 2 for Bi properly illustrates statistical considerations of Table 3B.

It can be concluded that small-size rope-grown *M. galloprovincialis* had an antagonist effect on the modelling of relationships between the mussel size and weight and TE levels: they drove to elect linear regressions to model relationships between the mussel shell length and TE contents, but diminished the significance of power functions modelling relationships between the mussel dry weight and some TE concentrations. Graphical representations of power functions modelling relationships between the mussel dry weight and TE concentrations (e.g. Figs. 1a and 2a) or linear regressions modelling relationships between the mussel shell length and TE contents (e.g. Figs. 1d and 2d) are given in Annex A for the 19 studied TEs. For comparison purposes, in Annex A, paired graphs model relationships (power functions or linear regressions) for all mussels or for individuals larger than 55 mm, respectively.

3.2.2. *TE concentrations as a function of the mussel shell length*

The present section investigates if there is a relationship between TE concentrations and the mussel shell length, or if the size has no significant ($p > 0.05$) importance on TE concentrations bioaccumulated in rope-grown *M. galloprovincialis* and can consequently be overcome in biomonitoring surveys.

Results showed that concentrations of 14 out of the 19 studied TEs (Al, V, Fe, Cr, Mn, Ni, Cu, Se, Ag, Sn, Sb, As, Be and Bi) were correlated with the shell length ($p < 0.05$; Table 4). This is partially inconsistent with results obtained by Saavedra et al. (2004). These authors modelled relationships between TE concentrations and the shell length for raft *M. galloprovincialis* measuring from 52 to 87 mm, and separated into 4 size-classes; they observed no difference between Cd, Pb, Cr, Ni, As, Cu and Zn concentrations at different shell lengths, as shown by the lack of significance ($p > 0.05$) of corresponding linear regressions.

When the linear regression model was run again for mussels larger than 55 mm (i.e. excluding small-size individuals), only 7 TEs (V, Mn, Cu, Se, Ag, As and Mo) were still correlated ($p < 0.05$) with the shell length instead of the previous 14 (Table 4). Mean TE concentrations calculated for mussels separated into the 4 size-classes (43–54, 55–64, 65–74 and 75–87 mm) were moreover

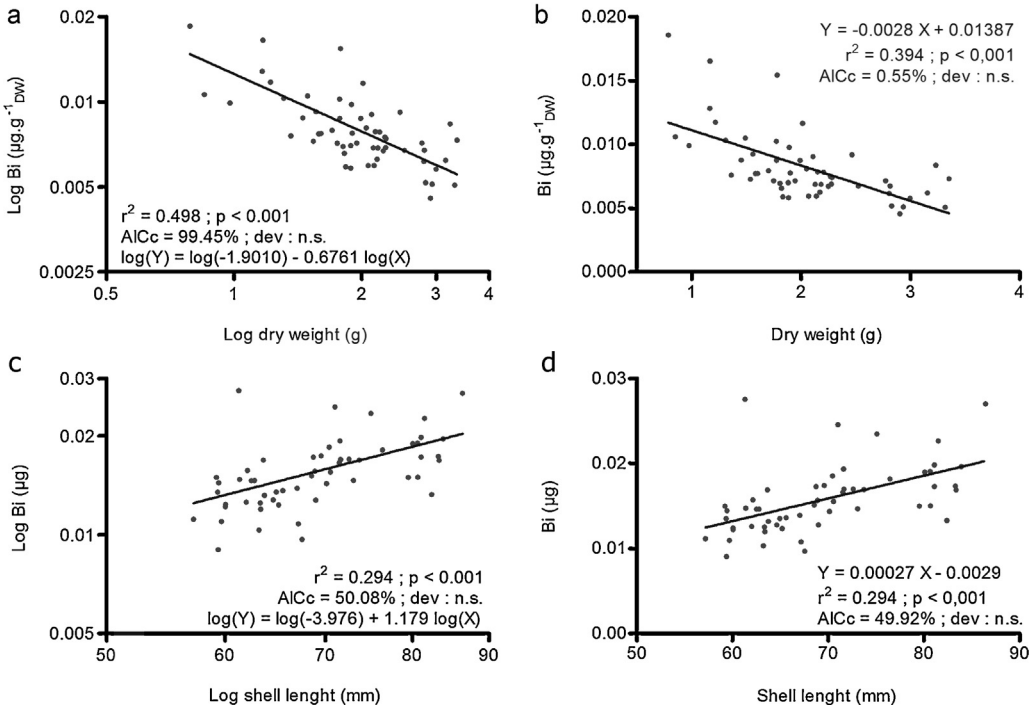

Fig. 2. Double log transformed power functions and linear regressions modelling relationships between (a and b) the soft tissue dry weight and Bi concentrations ($\mu g\,g^{-1}{}_{DW}$) and between (c and d) the shell length and Bi total contents (µg) of rope-grown *Mytilus galloprovincialis* from the Diane pond (east Corsica) larger than 55 mm ($n = 55$), sampled in February 2011 before they spawned. Linear equations and their corresponding fitting parameters (r^2; p-levels; deviation (dev.) from the model: s. = significant, n.s. = non-significant; AICc) are reported on graphs.

always higher for small-size mussels (43–54 mm), except for Mo, As and Cd; standard deviations were also generally larger (Table 4). Henceforth, if small-size individuals accumulated proportionally more TEs, they also presented an important inter-individual variability. The example given in Fig. 3 for Cr properly illustrates these results, as the significant linear relationship ($p = 0.0007$; Fig. 3a) existing between the mussel shell length and Cr concentrations disappeared ($p = 0.9116$; Fig. 3b) when mussels smaller than 55 mm were not taken into account. Small-size mussels were found inside the raft, close to the fixing rope, mixed with broken shells, covered by bigger individuals. Their shell also presented sometimes deformities due to the confinement. These unfavourable conditions, slowing down their growth, led to higher TE concentrations and to bigger variabilities observed within this size-class. Graphical representations of linear regressions modelling relationships between the mussel shell length and TE concentrations (e.g. Fig. 3) are given in Annex B for the 19 studied TEs. For comparison purposes, in Annex B, paired graphs model relationships for all mussels or for individuals larger than 55 mm, respectively.

Table 4
Left part of the table: p-values of linear regressions modelling relationships between trace element (TE) concentrations and the shell length of rope-grown *Mytilus galloprovincialis* from the Diane pond (east Corsica), sampled in February 2011 before they spawned, all mussel sizes together ($n = 74$) or limited to mussels larger than 55 mm ($n = 55$). Significant p-values < 0.05 are in bold. Rest of the Table: TE concentrations (mean ± SD, in $\mu g\,g^{-1}{}_{DW}$) in mussels separated into 4 equivalent size-classes of about 10 mm ($n = 19$, 21, 20 and 14 for size-classes cl. 1, 2, 3 and 4, respectively). Letters represent significant differences ($p < 0.05$) between size-classes.

	p-values of linear regressions		Mean concentrations by size-class			
	All mussels	>55 mm	cl. 1: 43-54 mm	cl. 2: 55–64 mm	cl. 3: 65–74 mm	cl. 4: 75–87 mm
Al	**0.0003**	0.9637	323 ± 223 a	162 ± 63 ab	150 ± 95 b	160 ± 88 ab
V	**0.0310**	**0.0229**	5.80 ± 2.50 ab	5.67 ± 1.84 a	5.43 ± 1.67 ab	4.11 ± 1.68 b
Fe	**0.0003**	0.6747	255 ± 146 a	156 ± 43 ab	148 ± 63 b	146 ± 55 b
Cr	**0.0007**	0.9116	0.803 ± 0.489	0.477 ± 0.146	0.462 ± 0.197	0.465 ± 0.181
Mn	**<0.0001**	**0.0081**	12.89 ± 4.03 a	9.84 ± 2.84 b	9.07 ± 3.62 b	6.88 ± 2.51 b
Co	0.2192	0.8275	0.688 ± 0.255	0.605 ± 0.135	0.626 ± 0.235	0.616 ± 0.173
Ni	**0.0002**	0.3083	1.81 ± 0.66 a	1.30 ± 0.37 b	1.34 ± 0.50 b	1.16 ± 0.34 b
Cu	**0.0004**	**0.0063**	5.55 ± 1.40 a	4.86 ± 1.47 a	4.98 ± 1.55 a	3.56 ± 0.69 b
Zn	0.4092	0.9809	79.7 ± 37.8	66.2 ± 22.2	75.7 ± 43.8	67.9 ± 25.2
Se	**<0.0001**	**0.0307**	3.24 ± 0.66 a	2.64 ± 0.76 ab	2.64 ± 0.81 b	2.17 ± 0.48 b
Ag	**<0.0001**	**0.0062**	0.0157 ± 0.0068 a	0.0124 ± 0.0040 a	0.0116 ± 0.0048 ab	0.0083 ± 0.0027 b
Cd	0.2599	0.1196	0.390 ± 0.200	0.389 ± 0.100	0.357 ± 0.089	0.352 ± 0.111
Sn	**0.0048**	0.4958	0.0413 ± 0.0222	0.0282 ± 0.0117	0.0312 ± 0.0170	0.0248 ± 0.0073
Sb	**0.0026**	0.4752	0.0152 ± 0.0052	0.0119 ± 0.0028	0.0119 ± 0.0040	0.0113 ± 0.0038
As	**0.0096**	**0.0048**	32.7 ± 6.8 a	31.7 ± 5.5 ab	32.3 ± 5.9 ab	26.9 ± 4.5 b
Mo	0.1741	**0.0002**	16.3 ± 6.0 ab	19.5 ± 4.9 a	18.8 ± 5.6 a	12.3 ± 4.2 b
Be	**0.0173**	0.5205	0.0169 ± 0.0085	0.0122 ± 0.0030	0.0121 ± 0.0042	0.0128 ± 0.0040
Pb	0.4056	0.1768	0.400 ± 0.253	0.268 ± 0.138	0.324 ± 0.185	0.369 ± 0.154
Bi	**0.0064**	0.0673	0.0100 ± 0.0039	0.0089 ± 0.0032	0.0082 ± 0.0026	0.0073 ± 0.0021

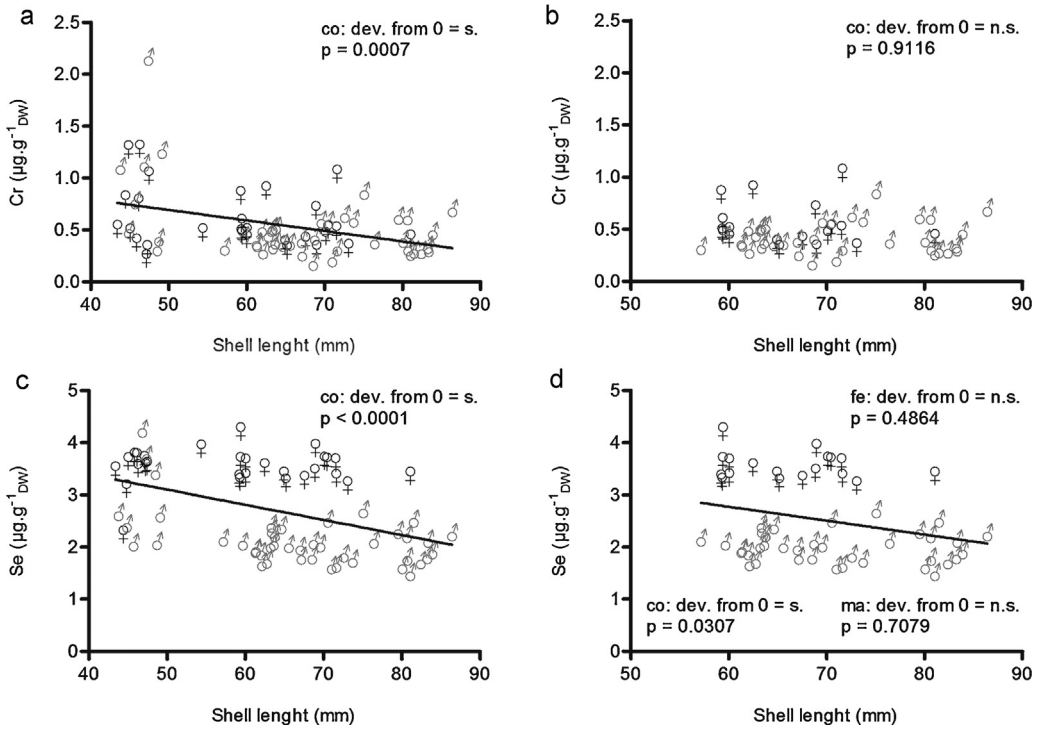

Fig. 3. Linear regressions modelling relationships between the shell length and Cr or Se concentrations ($\mu g\,g^{-1}{}_{DW}$), all mussels together (a and c; $n = 74$) or limited to mussels larger than 55 mm (b and d; $n = 55$), of rope-grown *Mytilus galloprovincialis* from the Diane pond (east Corsica), sampled in February 2011 before they spawned. ♀ and ♂ symbolize females and males, respectively. The common (co.) regression to ♀ and ♂ for Cr, only significant ($p < 0.05$) when considering all mussels (a), underlines the determining effect played by small-size mussels. The common regression (co.) to ♀ and ♂ for Se, significant ($p < 0.05$) when considering all individuals (c) or only mussels larger than 55 mm (d), underlines the difference in Se accumulation linked to sex prior to spawning, superimposed on the size effect; regressions specific to each sex (fe., ma.) are moreover not significant ($p > 0.05$). Probabilities of linear slopes to deviate (dev.) from 0 (s. = significant, n.s. = non-significant) are given on graphs.

Results further showed that for mid- to large-size *M. galloprovincialis* grown on ropes, the size did not influence body concentrations of most TEs (Table 4; little significant differences – $p < 0.05$ – between mean TE concentrations of size-classes cl. 2–4). As their culture starts synchronically and accordingly, all mussels on a rope have the same age (Saavedra et al., 2004), but may differ in size. Then, when sorting mussels for monitoring purposes, care will be taken not to use small- (restrained growth) but also large-size (rapid growth) mussels, these individuals being not representative of the rope population. This consideration has to be minimized as small (<50 mm) and large (>80 mm) mussels only represent around 5% of total individuals on a 10 kg rope.

3.2.3. *Pattern recognitions among mussels sampled prior to spawning using PCA and CA*

78% of the total variance of the data set built with the 74 mussels sampled prior to spawning was explained by the three first principal components (PCs; eigenvalues higher than 1) resulting from the PCA. More precisely, concentrations of 11 out of the 19 studied TEs (Al, Fe, Cr, Mn, Co, Ni, Cd, Sn, Sb, Be and Bi) were the dominating features in the first PC (PC1) and explained together 51% of the total variance of the data set. Cu, As and Mo concentrations showed the highest weights in the second PC (PC2), explaining 19% of the total variance. In the third PC (PC3), only Zn weight was close to but lower than 0.700, followed by V and Se; PC3 explained 8% of the total variance. Table 5 lists the loading variables along the first three PCs.

The dendrogram resulting from the CA on the data set built with the 74 mussels sampled prior to spawning showed 4 clusters at a linkage distance of 10 (Fig. 4a). The smallest central 1st cluster was formed by 6 mussels with significantly ($p < 0.05$) higher mean Mn, Ni, Cd and/or Al, Fe, Cr, Co, Sn, Sb, Be and Bi concentrations than clusters 3 (left side of the dendrogram) and 4 (right side of the dendrogram); the 2nd cluster was formed by 14 mussels with significantly ($p < 0.05$) higher mean Mn, Ni, Sb, Bi and/or Al, Fe, Cr, Sn and Be than clusters 3 and 4, respectively (results – not shown – of supplementary multiple comparison tests of means between mean transformed concentrations of the 4 clusters), although these differences were proportionally less important than for cluster 1. Examining the three-dimensional (3D) scores plot of the 74 mussels sampled prior to spawning (Fig. 4b), mussels of cluster 1 (dark grey patches) and half the mussels of cluster 2 (light grey patches), plotted according to PC1, distinctly emerged from the main scatter plot. Of the 20 mussels forming these 2 clusters, 9 were small-size individuals (<55 mm) of both sexes and 6 were large males (>69 mm). PCA and CA grouping methods thus gave results in agreement with the linear modelling of relationships between TE concentrations and the mussel shell length (Fig. 3; Table 4) and confirmed the significant ($p < 0.05$) effect of extreme sizes on TE bioaccumulation in rope-grown *M. galloprovincialis*.

Of the remaining 2 clusters at a linkage distance of 10 (Fig. 4a), the 3rd cluster (left side of the dendrogram) was formed by 32 of the 45 male mussels larger than 55 mm (except individuals M1.2 and M1.7; see Fig. 4 legend for label meaning) and the 4th cluster (right side of the dendrogram) was formed by 21 of the 29 female mussels (and male M1.6). The 3D scatter plot clearly separated individuals of both sexes according to PC2 (with dominating features Cu, As and Mo) and PC3 (with dominating features Zn, V and Se). This supplementary effect of the sex on the bioaccumulation of TEs in rope-grown *M. galloprovincialis* prior to spawning, superimposed on the size effect, is modelled and discussed in the next Section 3.2.4.

Table 5
Factor loadings for the first three principal components (PC1, PC2, PC3) resulting from principal component analysis when using trace element concentrations of rope-grown *Mytilus galloprovincialis* from the Diane pond (east Corsica). The first data set ($n = 74$; left part of the table) analysed is composed of whole mussels sampled in February 2011 before they spawned, with sizes ranging from 43.40 to 86.41 mm. The second data set ($n = 200$; right part of the table) analysed is composed of body compartments (gills, hepatopancreas, mantle and remaining soft tissues) and whole mussels sampled in February 2011 before they spawned and in March 2010 after they spawned. Factor loadings equal or greater than 0.700 in absolute terms are in bold.

	Factor loadings					
	Data set 1			Data set 2		
	PC1	PC2	PC3	PC1	PC2	PC3
Al	**−0.835**	−0.459	−0.092	**−0.880**	−0.417	−0.116
V	−0.499	0.653	−0.447	−0.636	0.581	−0.230
Fe	**−0.886**	−0.390	−0.099	**−0.905**	−0.366	−0.104
Cr	**−0.889**	−0.402	−0.062	**−0.895**	−0.383	−0.084
Mn	**−0.872**	0.105	−0.016	**−0.794**	−0.526	0.017
Co	**−0.821**	0.107	0.195	**−0.927**	0.109	0.143
Ni	**−0.888**	0.174	−0.172	**−0.960**	−0.043	0.024
Cu	−0.365	**0.702**	0.322	**−0.946**	−0.002	−0.007
Zn	−0.573	0.073	0.645	−0.409	−0.029	0.645
Se	−0.579	0.559	0.418	−0.373	0.436	0.622
Ag	−0.618	0.396	−0.183	−0.555	0.668	−0.142
Cd	**−0.721**	−0.106	0.288	−0.478	0.337	0.622
Sn	**−0.770**	−0.362	−0.105	**−0.920**	−0.241	−0.132
Sb	**−0.922**	0.025	−0.126	**−0.917**	0.297	−0.154
As	−0.196	**0.863**	−0.076	−0.617	0.637	−0.165
Mo	−0.278	**0.745**	−0.296	−0.335	−0.317	**0.716**
Be	**−0.780**	−0.445	−0.270	**−0.924**	−0.195	−0.119
Pb	−0.612	−0.192	0.415	**−0.775**	0.340	−0.130
Bi	**−0.857**	−0.022	−0.144	**−0.940**	−0.076	−0.131

3.2.4. TE bioaccumulation according to the sex

Even when considering only mussels with a size larger than 55 mm, sampled in February 2011 before their spawned, V, Mn, Cu, Se, Ag, As and Mo concentrations were still correlated ($0.05 < p < 0.0001$) with the shell length (Table 4). This observation resulted from the unequal accumulation of certain TEs between females and males during gametogenesis, as raised from PCA and CA results (Fig. 4) and shown for Se in Fig. 3: the significant relationship between Se concentrations and the mussel shell length (Fig. 3c) partly relied on small-size mussels (as for Cr; Fig. 3a), but this effect was overlapped by differences between female and male Se accumulation prior to spawning (Fig. 3d). This supplementary effect of the sex on the modelling of linear relationships between the mussel shell length and TE concentrations (e.g. Figs. 3b and d), for mussels larger than 55 mm, is graphically represented for the 19 studied TEs in Annex B.

Mean TE concentrations in female and male *M. galloprovincialis* dry flesh (Table 2) differed significantly for most of the 19 studied TEs ($p < 0.05$) and were higher in females (from 3% for Al up to 74% for Cu), except for Be. This is consistent with observations

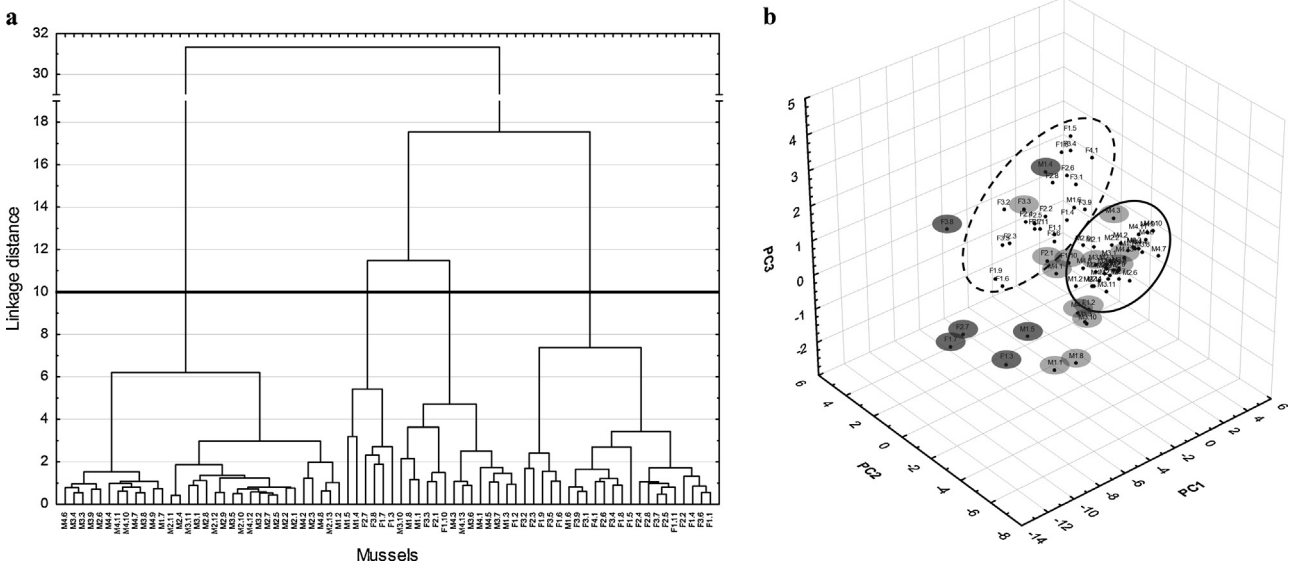

Fig. 4. (a) Dendrographic classification after cluster analysis (Euclidean distance as measure of similarity) using trace element (TE) concentrations and (b) three-dimensional (3D) scores plot after principal component analysis according to TE concentrations of rope-grown *Mytilus galloprovincialis* ($n = 74$) from the Diane pond (east Corsica), sampled in February 2011 before they spawned, with sizes ranging from 43.40 to 86.41 mm. To identify samples on the dendrogram and on the 3D scores plot, mussels are labelled as follows: (i) to each individual is given the capital letter of its gender, i.e. "M" for males or "F" for females; (ii) the 1st number is related to the size-class the mussel belongs to, i.e "1" for the size-class 43–54 mm, "2" for the size-class 55–64 mm, "3" for the size-class 65–74 mm and "4" for the size-class 75-87 mm; (iii) the 2nd number is related to the place of each individual within its size-class in ascending order. The dendrogram shows 4 clusters at a linkage distance of 10 (thick black line). On the 3D scores plot, male mussels forming the extreme left cluster are grouped within the full ellipse, female mussels forming the extreme right cluster are grouped within the dotted ellipse, and the 6 and 14 mussels forming the two smaller central clusters are highlighted with dark or light grey patches, respectively.

Table 6
Dry weights of the 4 body compartments and whole mussels (mean ± SD, in g), and corresponding TE concentrations (mean ± SD, in $\mu g\,g^{-1}{}_{DW}$), of rope-grown *Mytilus galloprovincialis* from the Diane pond (east Corsica), sampled (A) in February 2011 before they spawned ($n = 20$) and (B) in March 2010 after they spawned ($n = 20$). Letters represent significant differences ($p < 0.05$) between the 4 body compartments and whole mussels of a same gametogenic status (i.e. multiple comparison tests of means); asterisks (*) represent significant differences ($p < 0.05$) for a same body compartment or between mussels before or after spawning (i.e. pairwise comparison tests of means), respectively.

A

Before spawning	Gills	Hepatopancreas	Mantle	Remaining soft tissues	Whole mussel
Dry weight	0.110 ± 0.031	0.259 ± 0.136	0.178 ± 0.066	0.435 ± 0.135	0.981 ± 0.348
Al	8.8 ± 7.2 a*	40.3 ± 27.7 b*	3.7 ± 2.4 c*	8.5 ± 6.8 a*	14.9 ± 7.3 d*
V	1.8 ± 0.3 ad*	11.6 ± 5.3 b*	0.8 ± 0.3 c	1.0 ± 0.3 ac*	3.7 ± 1.3 bd*
Fe	50 ± 11 a*	125 ± 46 b*	33 ± 7 c*	30 ± 8 c*	57 ± 15 ab*
Cr	0.239 ± 0.060 a*	0.420 ± 0.140 b*	0.131 ± 0.071 c*	0.158 ± 0.056 ac*	0.227 ± 0.060 a*
Mn	2.68 ± 1.41*	2.54 ± 0.75 *	2.18 ± 1.04*	2.39 ± 1.07 *	2.45 ± 0.98 *
Co	1.01 ± 0.26 ab	1.44 ± 0.63 b*	0.37 ± 0.17 c*	0.46 ± 0.13 c*	0.75 ± 0.22 a*
Ni	1.19 ± 0.45 a*	2.64 ± 1.38 a*	0.46 ± 0.28 b*	0.55 ± 0.23 b*	1.12 ± 0.45 a*
Cu	3.52 ± 0.43 a*	5.79 ± 1.43 b*	3.02 ± 1.03 ac*	2.48 ± 0.54 c*	3.51 ± 0.65 a*
Zn	113 ± 42 a	79 ± 25 ab*	61 ± 13 b*	65 ± 18 b	74 ± 20 ab
Se	4.62 ± 0.61 a	3.71 ± 0.72 b	3.15 ± 0.78 bc	2.96 ± 0.46 c	3.33 ± 0.48 bc
Ag	0.0471 ± 0.0142 ab*	0.0672 ± 0.0202 b*	0.0286 ± 0.0137 c*	0.0295 ± 0.0113 c*	0.0405 ± 0.0119 ac*
Cd	1.09 ± 0.24 a	0.91 ± 0.26 a	0.49 ± 0.17 b*	0.61 ± 0.13 bc	0.72 ± 0.16 c
Sn	0.0193 ± 0.0035 ac*	0.0618 ± 0.0237 b*	0.0132 ± 0.0054 cd*	0.0127 ± 0.0034 d*	0.0252 ± 0.0057 ab*
Sb	0.0213 ± 0.0024 a*	0.0551 ± 0.0156 b	0.0123 ± 0.0049 c*	0.0102 ± 0.0022 c	0.0229 ± 0.0043 a
As	20.5 ± 2.6 a*	32.9 ± 4.5 b*	18.2 ± 2.0 c*	15.2 ± 1.4 d*	20.7 ± 2.0 a*
Mo	13.8 ± 5.0 a*	10.7 ± 4.1 a*	4.5 ± 1.5 b*	4.2 ± 0.9 b*	7.1 ± 2.2 c*
Be	0.0145 ± 0.0078 ab	0.0250 ± 0.0093 b*	0.0041 ± 0.0021 c*	0.0082 ± 0.0035 ac*	0.0125 ± 0.0042 a*
Pb	1.74 ± 0.39 ab*	2.38 ± 1.16 b	0.74 ± 0.24 c*	0.98 ± 0.28 cd*	1.32 ± 0.34 ad
Bi	0.0168 ± 0.0037 a	0.0419 ± 0.0126 b*	0.0064 ± 0.0032 c*	0.0076 ± 0.0024 c*	0.0170 ± 0.0038 a*

B

After spawning	Gills	Hepatopancreas	Mantle	Remaining soft tissues	Whole mussel
Dry weight	0.102 ± 0.025	0.176 ± 0.054	0.136 ± 0.052	0.326 ± 0.091	0.740 ± 0.182
Al	136.9 ± 68.4 a*	1493.2 ± 774.4 b*	39.2 ± 21.8 c*	187.6 ± 110.3 a*	468.4 ± 190.2 d*
V	1.2 ± 0.2 a*	6.6 ± 2.5 b*	0.6 ± 0.2 c	0.9 ± 0.3 c*	2.2 ± 0.6 a*
Fe	153 ± 35 ac*	1061 ± 563 b*	89 ± 23 c*	180 ± 66 a*	361 ± 130 b*
Cr	0.599 ± 0.119 a*	3.421 ± 1.851 b*	0.324 ± 0.117 c*	0.742 ± 0.325 ad*	1.254 ± 0.449 bd*
Mn	7.41 ± 1.75 a*	23.44 ± 8.97 b*	6.45 ± 1.83 a*	8.46 ± 3.10 ac*	11.38 ± 3.80 c*
Co	1.06 ± 0.28 a	2.53 ± 0.99 b*	0.62 ± 0.18 c*	0.57 ± 0.16 c*	1.11 ± 0.35 a*
Ni	1.70 ± 0.64 ad*	5.91 ± 2.51 b*	0.89 ± 0.45 c*	1.09 ± 0.42 ac*	2.26 ± 0.86 d*
Cu	4.27 ± 0.63 a*	9.89 ± 2.45 b*	4.04 ± 0.96 ac*	3.19 ± 0.57 c*	5.07 ± 0.95 a*
Zn	133 ± 59 a	112 ± 47 ab*	98 ± 34 b*	84 ± 30 b	93 ± 38 ab
Se	4.23 ± 0.64 a	3.56 ± 0.70 b	3.08 ± 0.50 bc	2.71 ± 0.38 c	3.19 ± 0.45 b
Ag	0.0212 ± 0.0063 ac*	0.0509 ± 0.0210 b*	0.0172 ± 0.0073 c*	0.0165 ± 0.0049 c*	0.0252 ± 0.0079 a*
Cd	1.08 ± 0.18 a	1.03 ± 0.29 a	0.66 ± 0.23 b*	0.60 ± 0.12 b	0.75 ± 0.15 b
Sn	0.0503 ± 0.0208 ad*	0.2346 ± 0.1015 b*	0.0197 ± 0.0041 c*	0.0373 ± 0.0153 d*	0.0763 ± 0.0222 ab*
Sb	0.0200 ± 0.0065 ad*	0.0659 ± 0.0207 b	0.0092 ± 0.0024 c*	0.0112 ± 00021 cd	0.0258 ± 0.0069 ab
As	18.4 ± 3.2 a*	27.2 ± 2.7 b*	16.1 ± 1.5 ac*	13.1 ± 1.7 c*	17.7 ± 1.4 a*
Mo	93.8 ± 29.2 a*	43.0 ± 14.1 b*	29.5 ± 8.7 c*	21.5 ± 7.2 c*	38.6 ± 12.4 b*
Be	0.0155 ± 0.0054 a	0.0835 ± 0.0311 b*	0.0075 ± 0.0043 a*	0.0150 ± 0.0053 a*	0.0295 ± 0.0088 b*
Pb	0.96 ± 0.24 ad*	3.01 ± 0.95 b	0.52 ± 0.17 c*	0.76 ± 0.18 ac*	1.27 ± 0.34 bd
Bi	0.0207 ± 0.0049 ad	0.1107 ± 0.0310 b*	0.0128 ± 0.0048 c*	0.0157 ± 0.0037 ac*	0.0381 ± 0.0115 bd*

made by Lobel et al. (1991) for Mn, Cu, As and Se measured in wild *M. edulis* from Newfoundland sampled in summer. Watling and Watling (1976) measured higher concentrations in wild female *Choromytilus meridionalis* from South Africa for Zn, Cu, Mn and Fe, similar in both sexes for Fe, Cd, Ag, Cr, Co and Ni, and slightly higher in males for Pb, and Bi. After the main breeding period, no difference was found between sexes (Orren et al., 1980), suggesting a reproduction-related metal accumulation. In wild *Perna perna* from the Gulf of Aden collected in summer, Cd, Cu, Mn, Pb and Zn were more concentrated in females than in males, and inversely for Fe (Sokolowski et al., 2004). Hellou et al. (2003) collected male and female *M. edulis* in Nova Scotia, prior and during the spawning period (between April and July), and measured systematically higher concentrations of Cd, Pb, Hg, Ag, As, Cr, Cu, Sn and Zn in female soft tissues. The influence of gametogenesis in determining body TE concentrations can thus not be neglected, even if its role is not clear yet and needs further studies. We can reasonably suggest that the closest mussels are from spawning (additional mussels from the present study effectively spawned 2–4 weeks later; Richir et al., unpubl. data), the most differences in TE concentrations rise. Sokolowski et al. (2004), from observations made by Lobel and Wright (1982) and Pieters et al. (1980) on *M. edulis*, suggested that the formation of reserves during the prespawning period, more pronounced in females, could favour the faster accumulation of TEs in this sex. However, Suárez et al. (2005) showed that the contribution of reproductive tissues to the total body weight of raft *M. galloprovincialis* was systematically higher in males all year round, this difference being even more pronounced in winter and spring, during gametogenesis. The hypothesis suggested by Sokolowski et al. (2004) must then be partially rejected, at least in the case of rope-grown *M. galloprovincialis*, since the contribution of female reproductive tissues to their total body weight does not exceed that in males (data not shown). Some evidences suggest that differences between female and male TE concentrations prior to spawning could rely on a functional role played by metallothioneins (MTs), as already suggested by Latouche and Mix (1981) in the early 80ies. Akberali et al. (1985, in Earnshaw et al., 1986) experimentally showed that Cu and Zn uptakes were higher into sperm than into eggs of *M. edulis*. Fitzpatrick et al. (2008) observed no influence of increasing Cu concentrations on *M. trossulus* egg

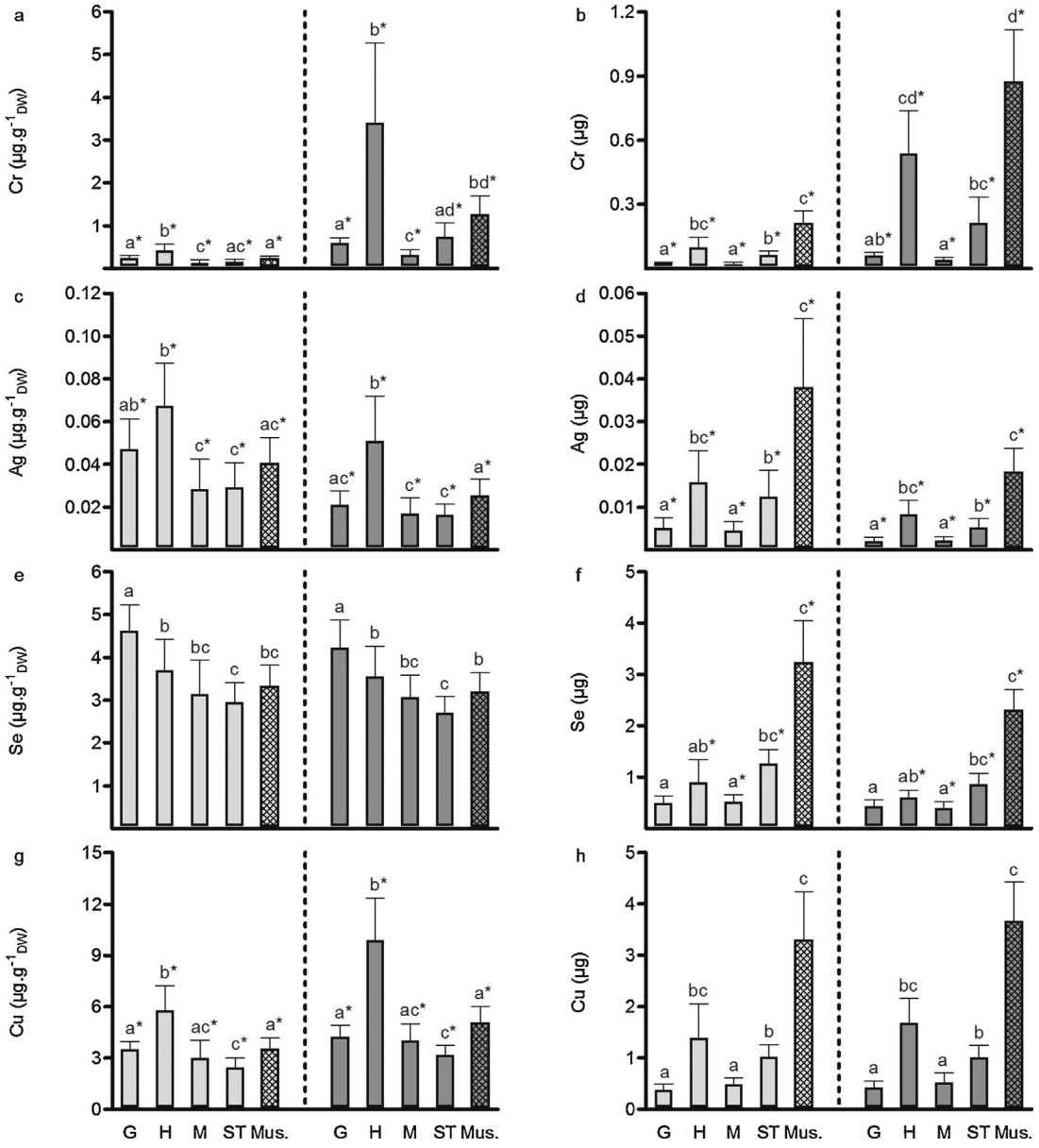

Fig. 5. (a and b) Cr, (c and d) Ag, (e and f) Se and (g and h) Cu levels in gills "G", hepatopancreas "H", mantle "M", remaining soft tissues "ST" and whole mussels "Mus." of rope-grown *Mytilus galloprovincialis* from the Diane pond (east Corsica), sampled in February 2011 before they spawned (light gray; $n = 20$) and in March 2010 after they spawned (dark grey; $n = 20$). Levels are expressed as concentrations ($\mu g\,g^{-1}{}_{DW}$–4 left graphs) or total contents (µg—4 right graphs). Letters represent significant differences ($p < 0.05$) between the 4 body compartments and whole mussels of a same gametogenic status (i.e. multiple comparison tests of means); asterisks (*) represent significant differences ($p < 0.05$) for a same body compartment or between mussels before or after spawning (i.e. pairwise comparison tests of means), respectively.

viability and fertilization rates, while sperm motility and fertilization rates decreased, and Meistertzheim et al. (2009) recently measured a more important increase of MT concentrations in female gonads of *C. gigas* than in males during gametogenesis. This hypothetical role played by MTs in *M. galloprovincialis* during gametogenesis therefore needs further investigations.

3.3. TE accumulation and tissue compartmentalization according to the reproductive status

All 40 mussels analysed for tissue compartmentalization measured between 70 and 80 mm. Mean body and gonad (i.e. follicles developed in the mantle) dry weights of mussels sampled in February 2011 (before spawning) were 0.981 and 0.178 g, respectively, and mean body and gonad dry weights of mussels sampled in March 2010 (after spawning) were 0.740 and 0.136 g, respectively. Gonad weights differed by 31%; this was similar to the 33% whole body weight difference between both reproductive statuses. In the genus *Mytilus*, up to 40% of soft tissue weight can be lost during spawning, which shows the importance of gametogenesis in their physiological cycle (Cossa, 1989).

TEs could be divided into 4 groups, depending on their concentrations and contents before and after spawning (Table 6). Fig. 5 gives one graphical example for each group; histograms of the tissue compartmentalization of the 19 studied TEs are given in Annex C. The first group was made up of Al, Fe, Cr (Figs. 5a and b), Mn, Ni, Sn, Mo, Be and Bi: they were less concentrated and less abundant prior to spawning, due to the combined effect of a tissue dilution during the gametogenesis body weight increase (lower concentrations) and an environmental diminution of available TEs between the two sampled years (lower contents). Inversely, a second group was made up of V and Ag (Figs. 5c and d), more concentrated and

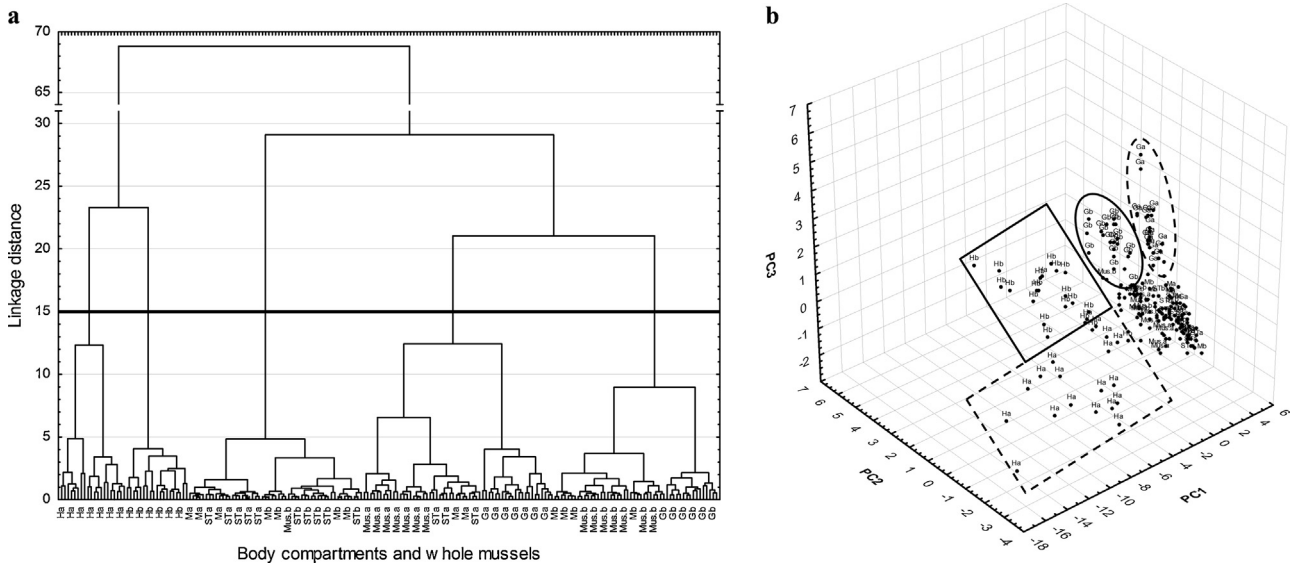

Fig. 6. (a) Dendrographic classification after cluster analysis (Euclidean distance as measure of similarity) using trace element (TE) concentrations and (b) three-dimensional (3D) scores plot after principal component analysis according to TE concentrations of body compartments and whole rope-grown *Mytilus galloprovincialis* ($n = 200$) from the Diane pond (east Corsica), sampled in February 2011 before they spawned and in March 2010 after they spawned. To identify samples on the dendrogram and on the 3D scores plot, body compartments and whole mussels are labelled as follows: (i) gills "G", remaining soft tissues "ST", hepatopancreas "H", mantle "M" and whole mussels "Mus.", (ii) with the letter "b" or "a" referring to the reproductive status, i.e. before or after spawning, respectively. The dendrogram shows 5 clusters at a linkage distance of 15 (thick black line). On the 3D scores plot, hepatopancreas samples are grouped within rectangles and gill samples are grouped within ellipses, drawn either with full or dotted lines for mussels sampled before or after spawning, respectively. For clarity purpose, one in three sample label is reported on the dendrogram, as well as one in three M, ST and Mus. label on the 3D scores plot.

more abundant in 2011 prior to spawning; these 2 TEs were sufficiently abundant that year to mask the dilution effect due to the gametogenesis.

The Diane pond is a small-size salt pond, highly influenced by the runoff of freshwater from part of the catchment basin of Bravona, bringing terrigenous TEs such as Al or Fe, and by the discontinuous connectivity with the open sea through a small "grau", a channel silted up part of the year (Longere et al., 1972). These particular physical characteristics influence the physicochemical properties of that water body, and so the bioavailability of TEs. Data compiled from 2 mussel caging campaigns in spring 2010 and 2011 in the Calvi Bay, northwestern Corsica, with mussels from the same ropes that the ones used in this study confirmed this confinement hypothesis: TE concentrations were similar between the 2 monitored years in the Calvi Bay (Richir et al., unpubl. data), even for TE showing differences in this study as important as for Al or Fe (Table 6; Annex C).

Se (Figs. 5e and f), Cd, Sb, As and Pb formed a third group, showing similar tissue concentrations at both reproductive statuses but with contents a little higher in 2011 prior to spawning. These 5 TEs, more accumulated in mussel tissues in 2011 prior to spawning (Fig. 5f), displayed similar concentrations with mussels having spawned (Fig. 5e) as they were diluted during gametogenesis. Finally, the fourth group was made up of Co, Cu (Figs. 5g and h) and Zn: these 3 essentials TEs displayed lower concentrations prior to spawning and similar contents at both physiological statuses. The evident role of MTs in Zn and Cu regulation probably accounts for stable contents observed between years (Bebianno and Serafim, 2003; Strogyloudi et al., 2012).

85% of the total variance of the data set composed of body compartments and whole mussels ($n = 200$) sampled before and after spawning was explained by the three first PCs (eigenvalues higher than 1) resulting from the PCA. More precisely, concentrations of 12 out of the 19 studied TEs (Al, Fe, Cr, Mn, Co, Ni, Cu, Sn, Sb, Be, Pb and Bi) were the dominating features in PC1, which explained 60% of the total variance of the data set. In PC2, only Ag, As and V weights were close to but lower than 0.700; PC2 explained 14% of the total variance. Mo concentrations showed the highest weight in PC3 (followed by Zn, Se and Cd with weights close to but lower than 0.700), which explained 10% of the total variance. Table 5 lists the loading variables along the first three PCs.

The dendrogram resulting from the CA on the data set composed of body compartments and whole mussels ($n = 200$) sampled at both reproductive statuses showed 5 clusters at a linkage distance of 15 (Fig. 6a). Hepatopancreas samples formed 2 distinct clusters on the left side of the dendrogram. The 2 clusters on the right side of the dendrogram were formed by whole mussels sampled before or after spawning, their associated gill samples and most mantle samples. The left central 5th cluster was formed by soft tissues and remaining mantle samples of indistinctly both reproductive statuses. Examining the 3D scatter plot of samples in the space defined by the three first PCs, 5 main groups could be observed (Fig. 6b), in agreement with the dendrographic classification. Hepatopancreas samples, plotted according to PC1 (dominating features of PC1: Al, Fe, Cr, Mn, Co, Ni, Cu, Sn, Sb, Be, Pb and Bi), distinctly emerged from the main scatter plot composed of mantle, remaining soft tissue and whole mussel samples. Environmental Ag and V, sufficiently abundant in 2011 (before spawning) to mask the dilution effect due to the gametogenesis (e.g. Ag in Fig. 5c), separated samples plotted according to PC2 (although that separation between both reproductive statuses was less obvious than on the dendrogram). Gill samples, plotted according to PC3 (dominating features of PC3: Mo, followed by Zn, Se and Cd) formed two more distinct groups opposite to hepatopancreas groups.

PCA and CA gave complementary information in agreement with results of pairwise and multiple comparison tests of means between sampling years (i.e. before or after spawning) and between body compartments and whole mussels for a given reproductive status (Table 6; Fig. 5; Annex C). So, most TEs were preferentially concentrated in the hepatopancreas, except for Zn, Se, Cd and Mo, more concentrated in gills, and their concentrations significantly ($p < 0.05$) differed between sampling years (i.e. before and after spawning). When expressed as total contents in each body compartment, the 19 investigated TEs were systematically more

accumulated in the hepatopancreas (Fig. 5; Annex C). Since the hepatopancreas preferentially concentrated most of the 19 studied TEs, this particular organ could be privileged in monitoring surveys, as previously suggested by many authors for classically investigated trace metals (e.g. Adami et al., 2002; Gupta and Singh, 2011). TE distribution between tissues further displayed the same pattern, independently of the sampling year or the reproductive status. During the prespawning period, mussels accumulate reserves (Pieters et al., 1980): glycogen is largely accumulated and stored in the mantle, and proteins and lipids in non-mantle tissues (Gabbot and Bayne, 1973, in Gabbott, 1975). TE accumulation is mainly limited to the hepatopancreas, kidneys and gills (Lobel and Wright, 1982; Cossa, 1989; this study); if the mantle can also take up soluble TEs, it has to be considered as a transitional tissue, TEs being rapidly transported to nongonadal tissues (Lobel and Wright, 1982). Energy for vitellogenesis, the final stage of gametogenesis, is supplied from glycogen reserves, from lipid reserves stored in adipogranular cells in the mantle, and from freshly ingested food materials (Newell, 1989). Following the conversion of the stored glycogen to the lipid reserve of developing eggs (Latouche and Mix, 1981), a movement of nutrients from the digestive gland to the mantle occurs (Gabbott, 1975), leading to a consecutive redistribution of TEs within mussel tissues. So, the similar pattern of TE distribution observed between tissues before and after spawning was closely related to the mussel reproductive cycle.

4. Conclusion

Rope-grown *M. galloprovincialis* from the Diane pond efficiently accumulated the 10 little studied TEs (Be, Al, Fe, Mn, Co, Se, Mo, Sn, Sb, Bi) in addition to the 9 TEs classically investigated (Cr, Ni, Cu, Zn, Cd, Pb, As, Ag and V) in that species. However, the important variability of TE concentrations sometimes recorded at regional or even local scale requires a coordination of monitoring programs between neighbouring countries, in order to define sensible reference conditions to properly monitor the chemical pollution of the coastal environment. Low concentrations measured in the Diane pond could serve as baseline levels for comparison with ulterior surveys, at least for the northwestern Mediterranean.

Relationships between TE concentrations and the body dry weight or between TE contents and the mussel shell length were better modelled by power functions or linear regressions, respectively; conversely, relationships between TE contents and the mussel shell length were properly modelled by both linear regressions and power functions when only considering individuals larger than 55 mm. Small-size *M. galloprovincialis* (<55 mm) had an antagonist effect: they drove to elect the linear regression to model relationships between the mussel size and TE contents, but diminished the significance of the power function modelling relationships between the mussel weight and some TE concentrations. Small-size mussels furthermore concentrated more TEs, and showed an important inter-individual variability of their concentrations. Although a large range of rope-grown *M. galloprovincialis* sizes can be used for monitoring purposes, one will thus take care not to use very small or large mussels.

Many differences were observed between sexes prior to spawning. Although the influence of gametogenesis in determining female body higher TE concentrations is not clear yet and needs further studies, the role played by MTs could be fundamental. The hepatopancreas preferentially accumulated TEs, except for Zn, Se, Cd and Mo, more concentrated in gills. Body compartmentalization was not influenced by the reproductive status, but gametogenesis diluted TEs due to the important tissue production prior to spawning. Based on these observations, it appears judicious to monitor the 19 studied TEs during mussel sexual dormancy. However, temporal variations of environmental TE concentrations within the confined Diane pond mostly hid this gametogenic dilution effect.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2013.06.021.

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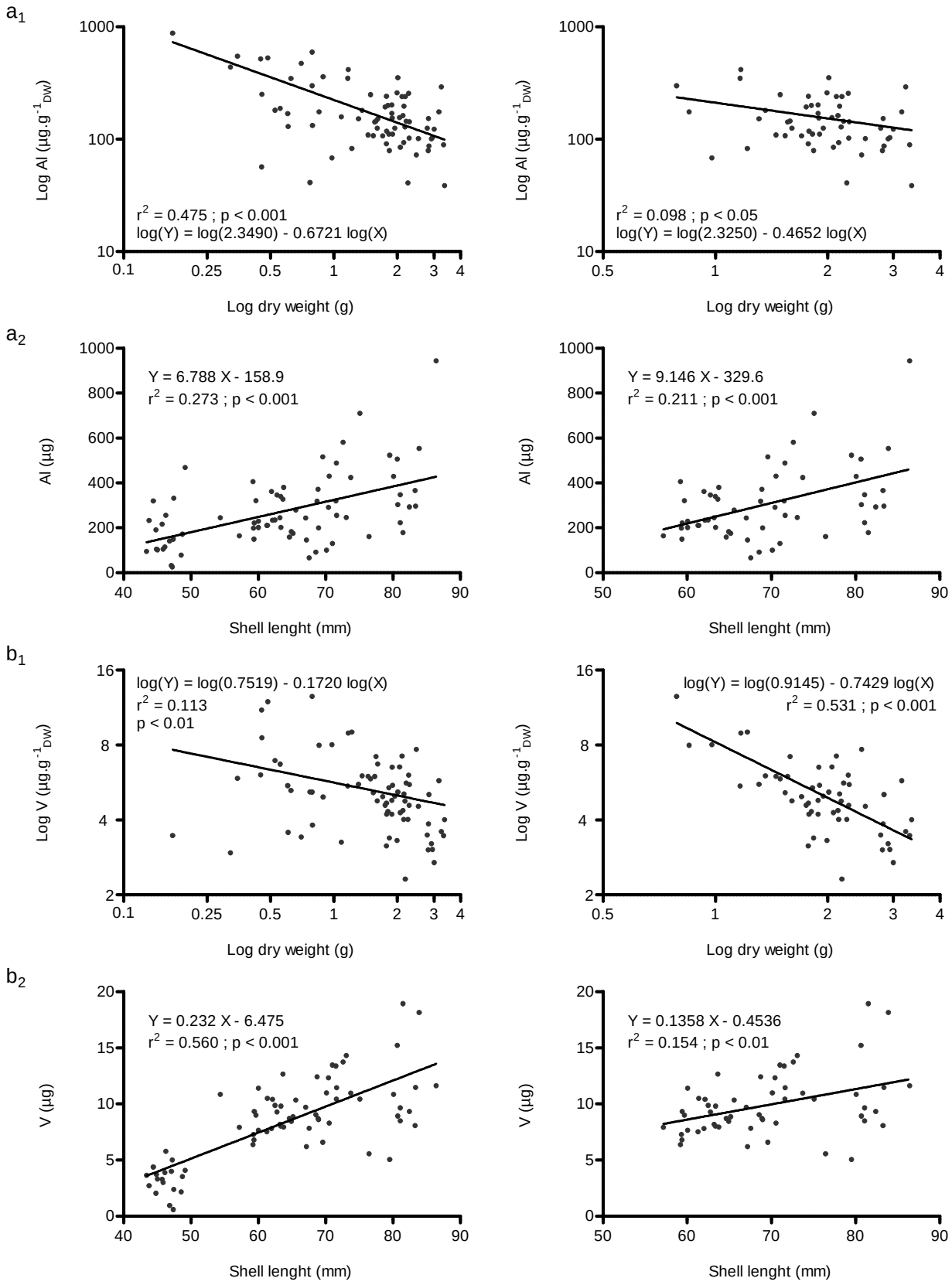
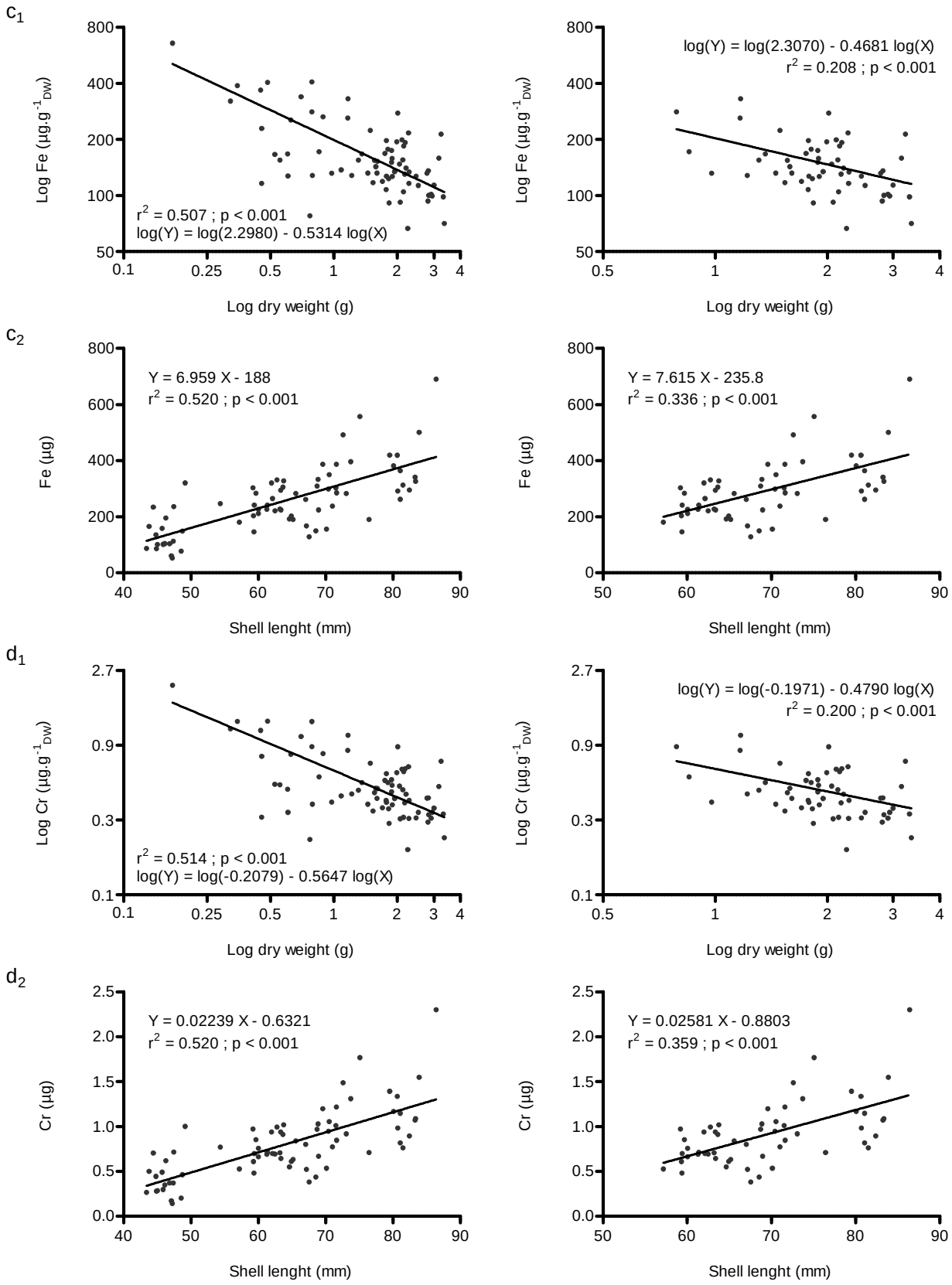
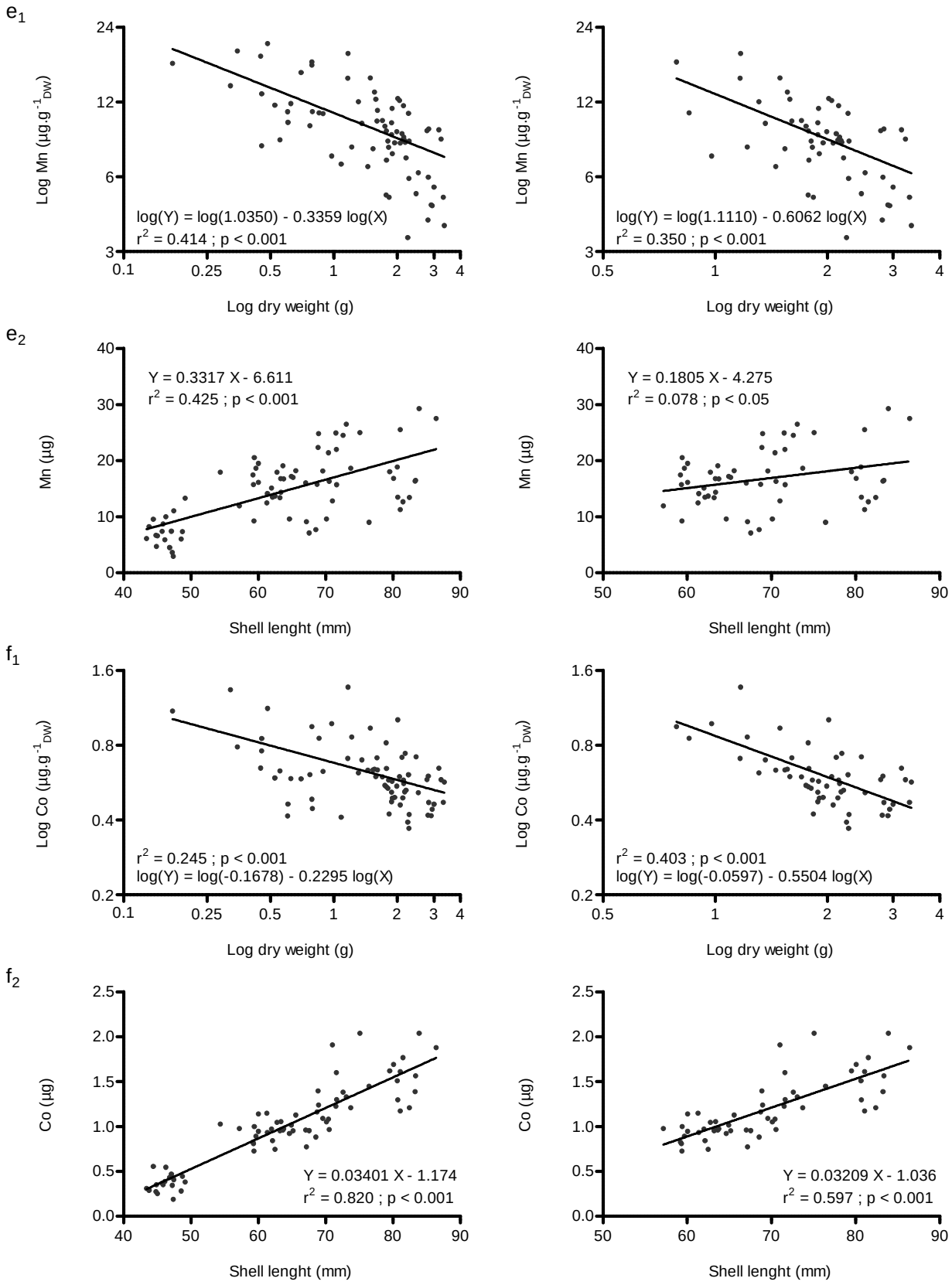


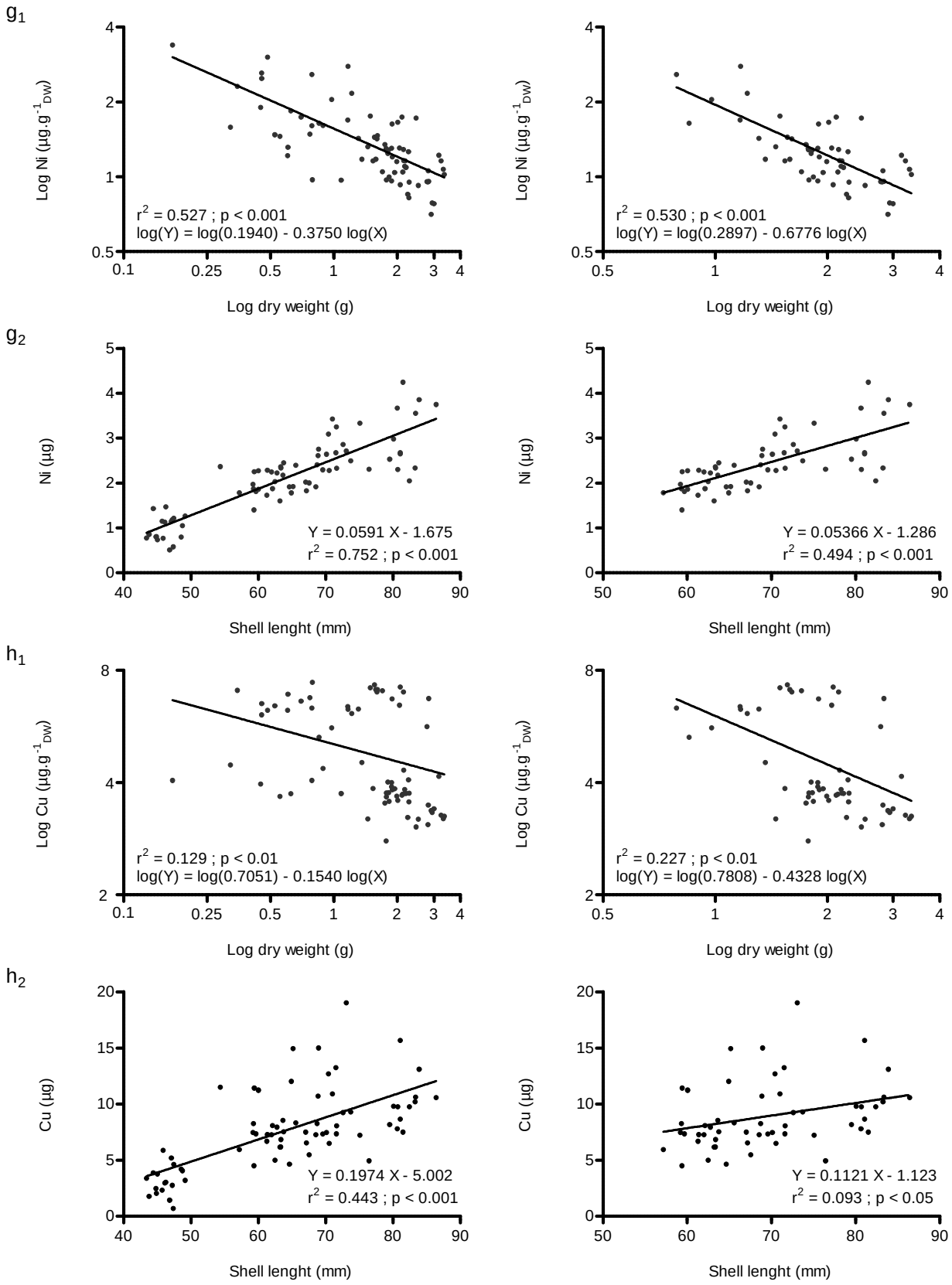
Annex A. Double log transformed power functions modelling relationships between a_1-b_1) the mussel soft tissue dry weight (g) and Al or V concentrations ($\mu g.g^{-1}_{DW}$) and linear regressions modelling relationships between a_2-b_2) the mussel shell length (mm) and Al or V total contents (µg), all individuals together (4 left graphs) or limited to mussels larger than 55 mm (4 right graphs). Equations and their corresponding fitting parameters (r^2 and p-levels) are reported on graphs.



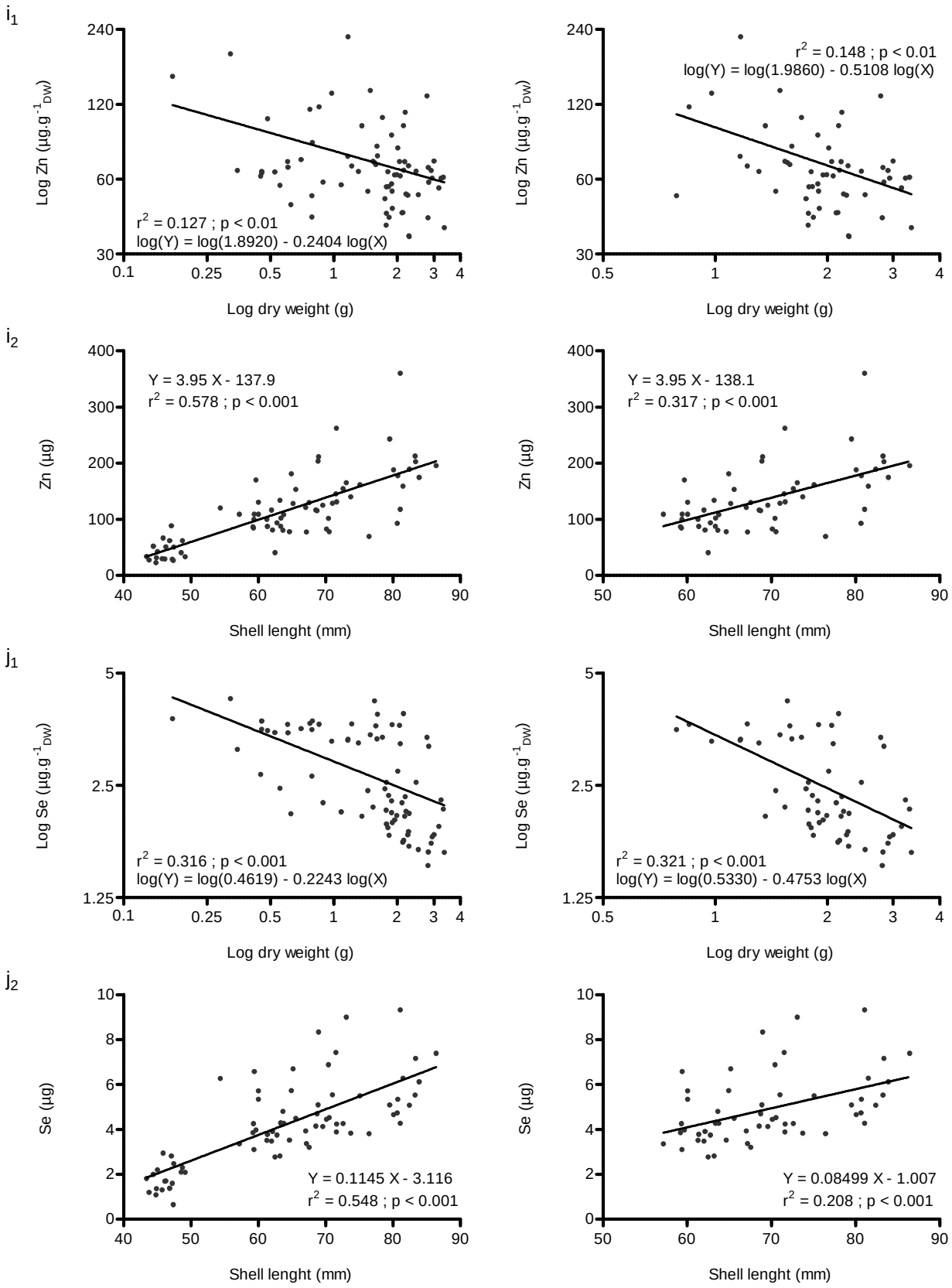
Annex A (*Continued*). Double log transformed power functions modelling relationships between c_1-d_1) the mussel soft tissue dry weight (g) and Fe or Cr concentrations ($\mu g.g^{-1}_{DW}$) and linear regressions modelling relationships between c_2-d_2) the mussel shell length (mm) and Fe or Cr total contents (µg), all individuals together (4 left graphs) or limited to mussels larger than 55 mm (4 right graphs). Equations and their corresponding fitting parameters (r^2 and p-levels) are reported on graphs.



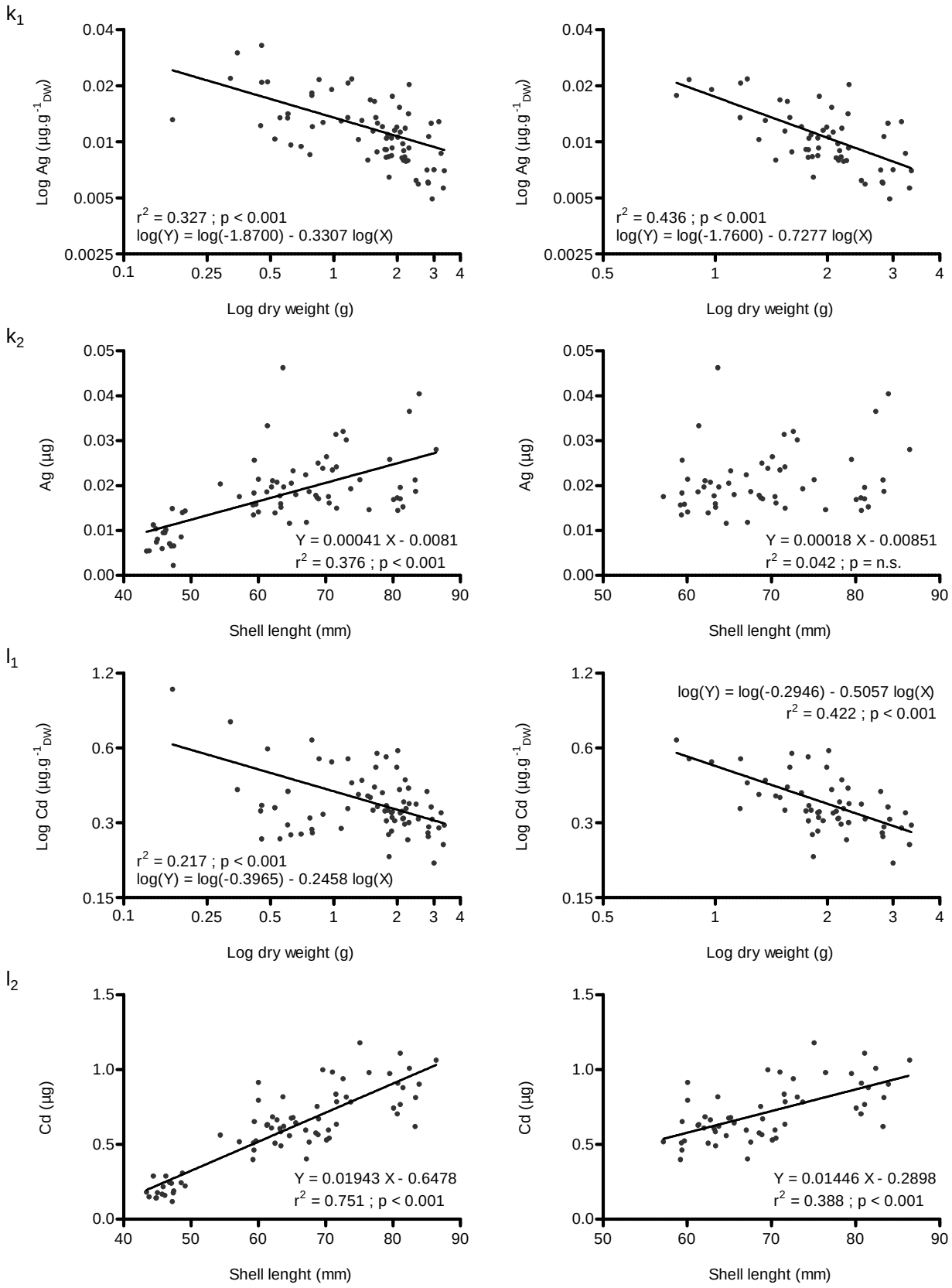
Annex A (*Continued*). Double log transformed power functions modelling relationships between e_1-f_1) the mussel soft tissue dry weight (g) and Mn or Co concentrations ($\mu g.g^{-1}_{DW}$) and linear regressions modelling relationships between e_2-f_2) the mussel shell length (mm) and Mn or Co total contents (µg), all individuals together (4 left graphs) or limited to mussels larger than 55 mm (4 right graphs). Equations and their corresponding fitting parameters (r^2 and p-levels) are reported on graphs.



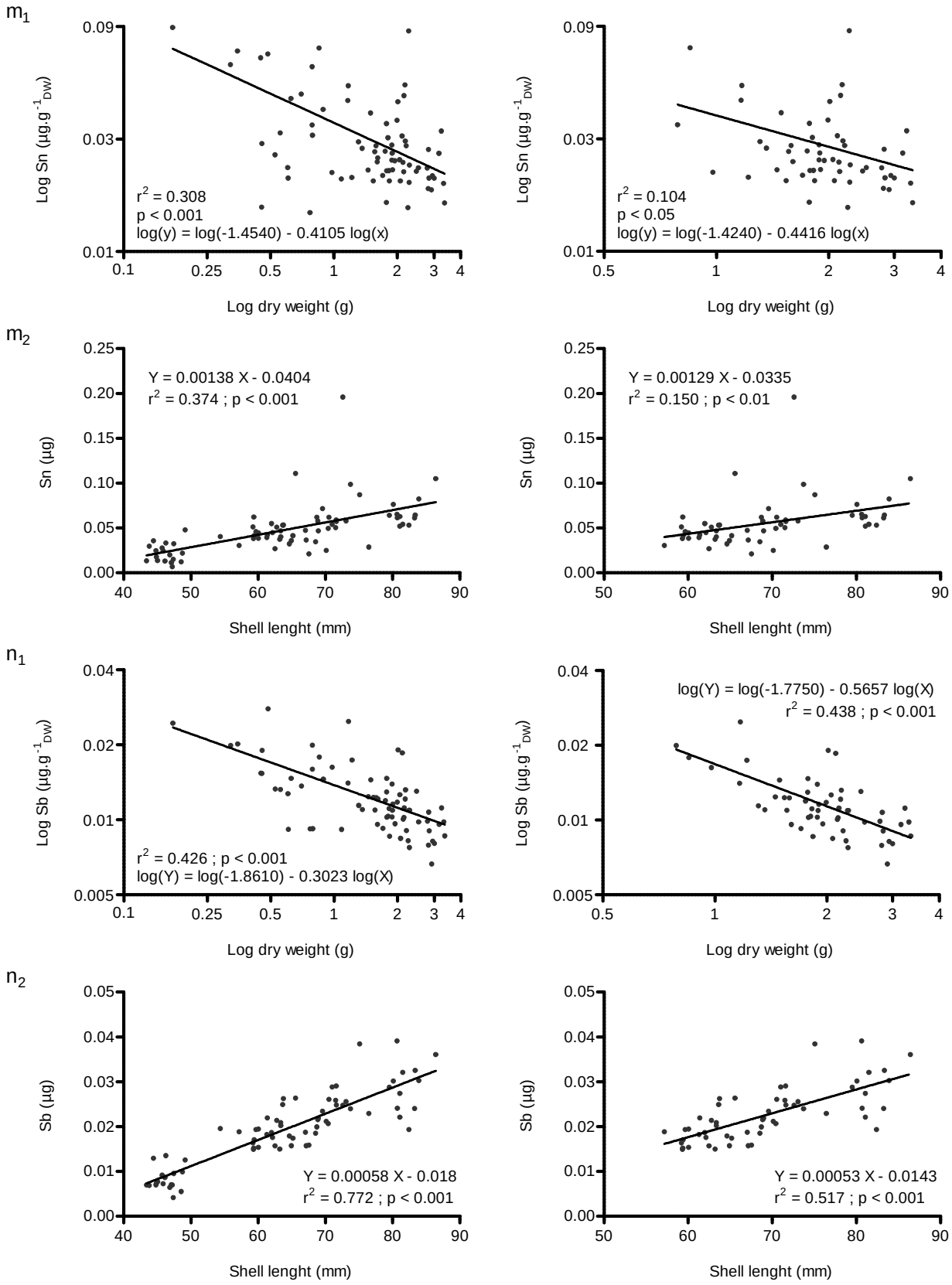
Annex A (*Continued*). Double log transformed power functions modelling relationships between g_1-h_1) the mussel soft tissue dry weight (g) and Ni or Cu concentrations ($\mu g.g^{-1}_{DW}$) and linear regressions modelling relationships between g_2-h_2) the mussel shell length (mm) and Ni or Cu total contents (µg), all individuals together (4 left graphs) or limited to mussels larger than 55 mm (4 right graphs). Equations and their corresponding fitting parameters (r^2 and p-levels) are reported on graphs.



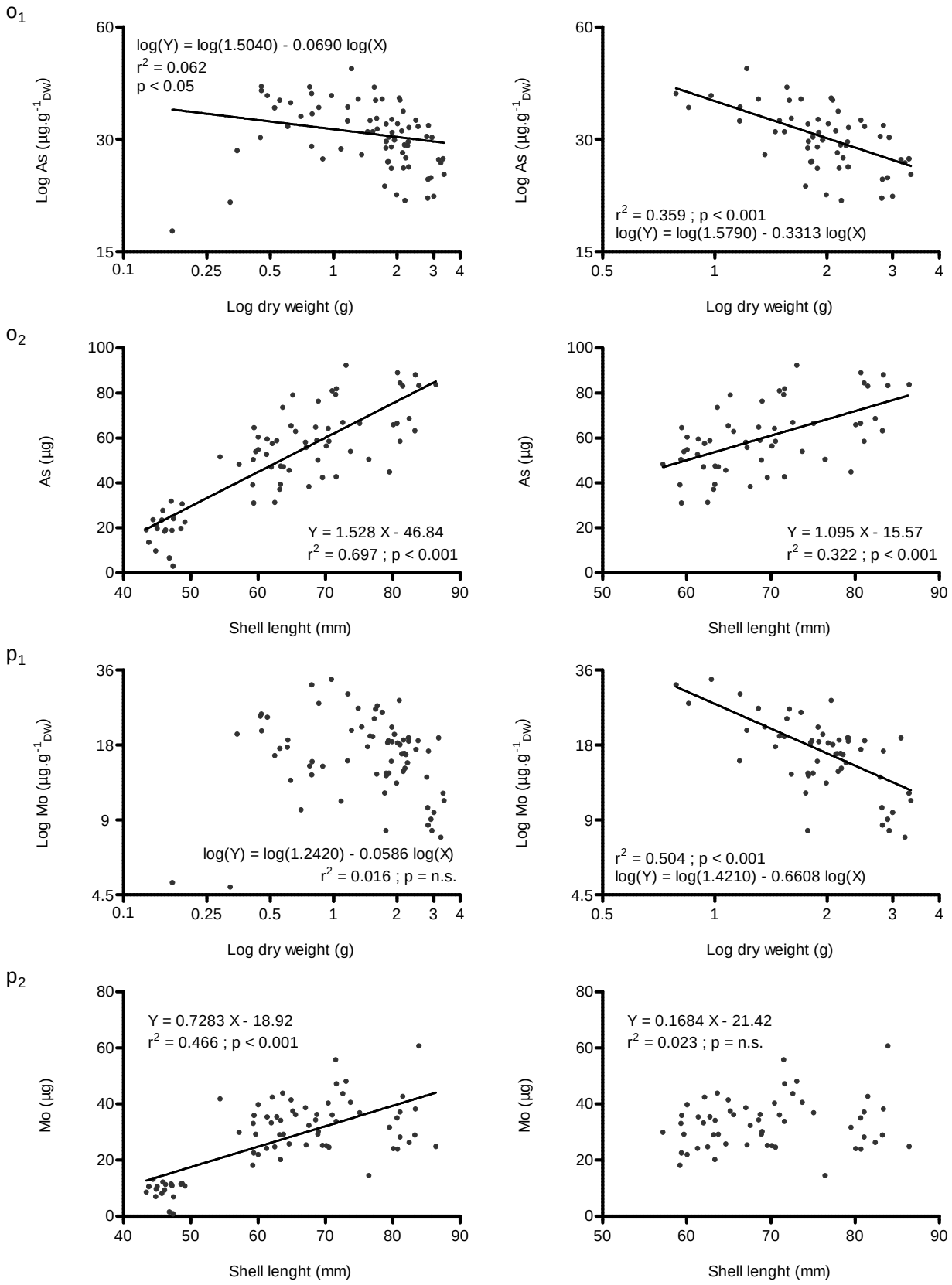
Annex A (*Continued*). Double log transformed power functions modelling relationships between i_1-j_1) the mussel soft tissue dry weight (g) and Zn or Se concentrations ($\mu g.g^{-1}_{DW}$) and linear regressions modelling relationships between i_2-j_2) the mussel shell length (mm) and Zn or Se total contents (µg), all individuals together (4 left graphs) or limited to mussels larger than 55 mm (4 right graphs). Equations and their corresponding fitting parameters (r^2 and p-levels) are reported on graphs.



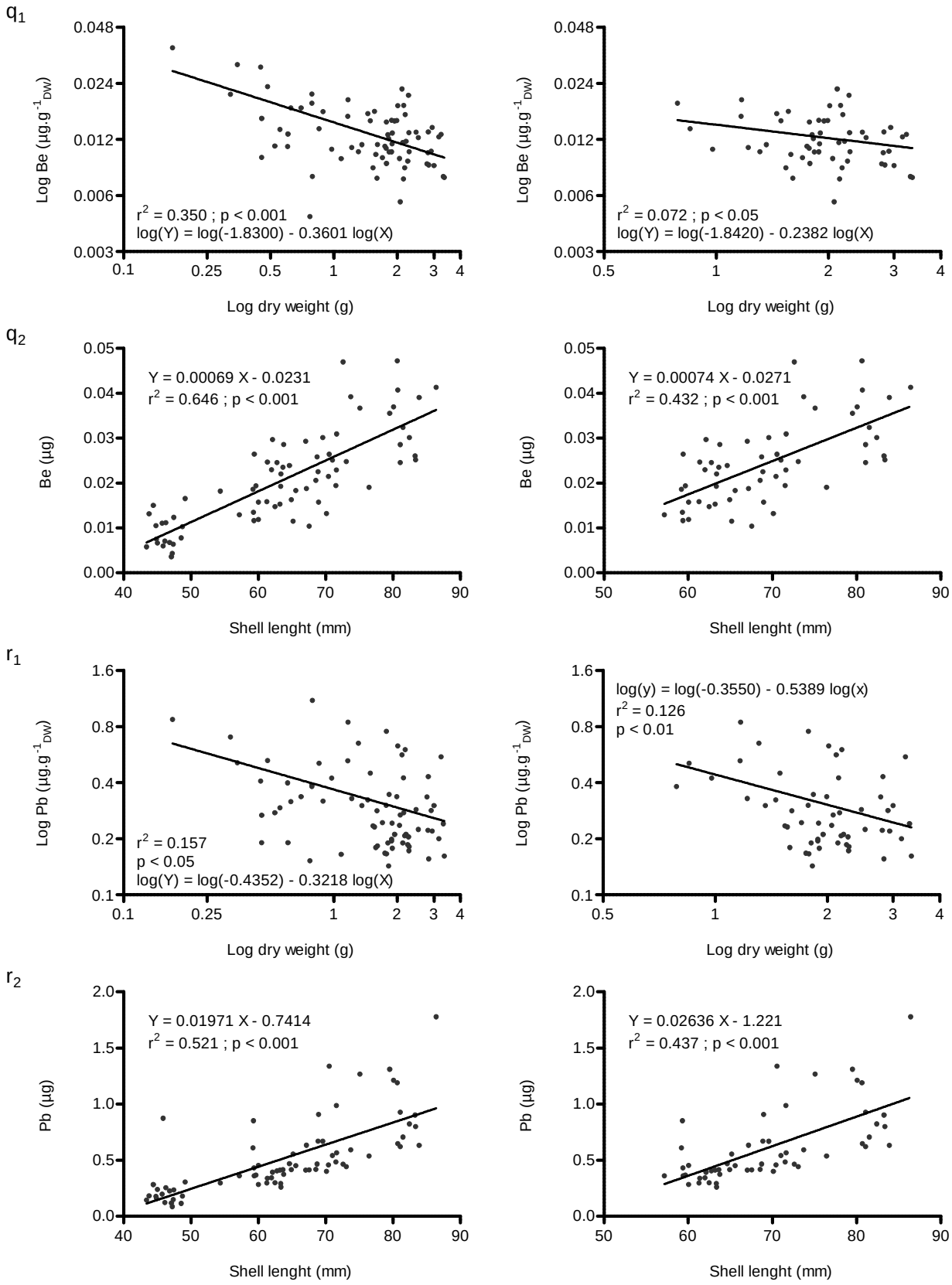
Annex A (*Continued*). Double log transformed power functions modelling relationships between k_1-l_1) the mussel soft tissue dry weight (g) and Ag or Cd concentrations ($\mu g.g^{-1}_{DW}$) and linear regressions modelling relationships between k_2-l_2) the mussel shell length (mm) and Ag or Cd total contents (µg), all individuals together (4 left graphs) or limited to mussels larger than 55 mm (4 right graphs). Equations and their corresponding fitting parameters (r^2 and p-levels) are reported on graphs.



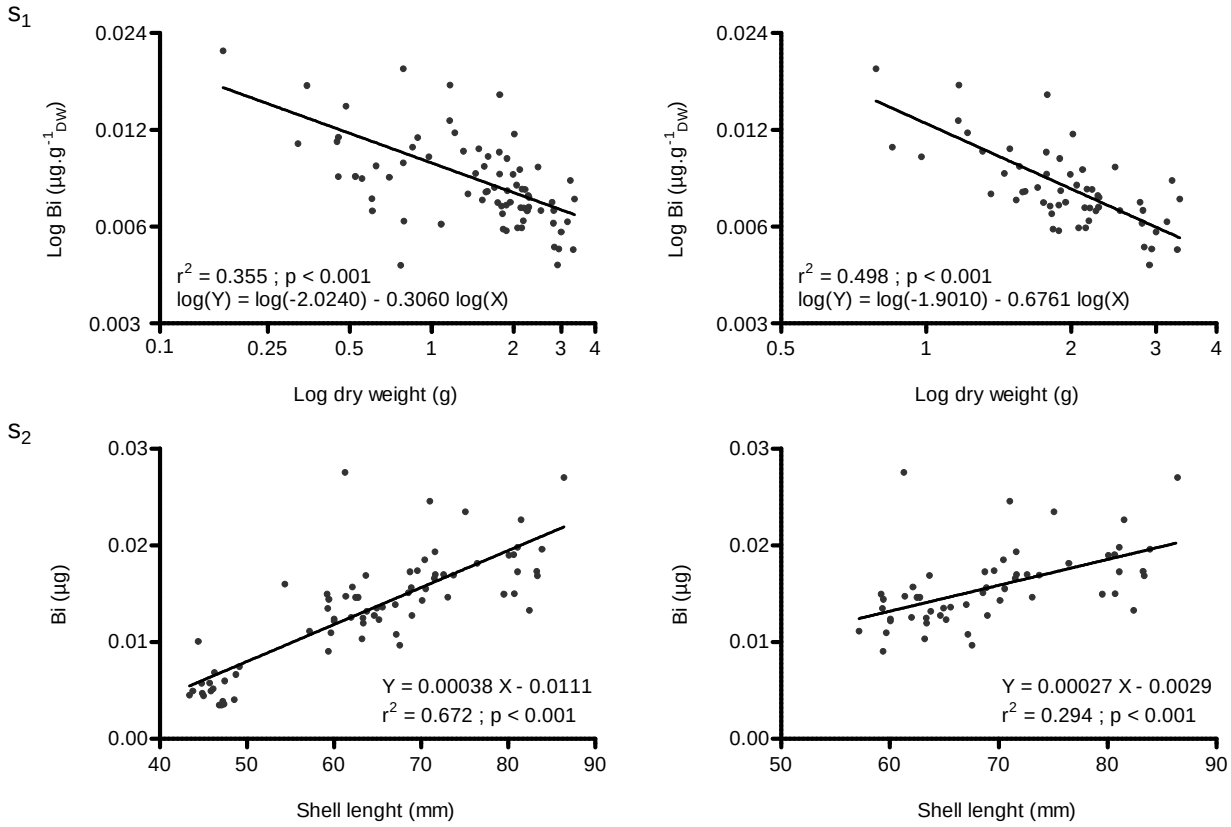
Annex A (***Continued*****).** Double log transformed power functions modelling relationships between m_1-n_1) the mussel soft tissue dry weight (g) and Sn or Sb concentrations ($\mu g.g^{-1}_{DW}$) and linear regressions modelling relationships between m_2-n_2) the mussel shell length (mm) and Sn or Sb total contents (µg), all individuals together (4 left graphs) or limited to mussels larger than 55 mm (4 right graphs). Equations and their corresponding fitting parameters (r^2 and p-levels) are reported on graphs.



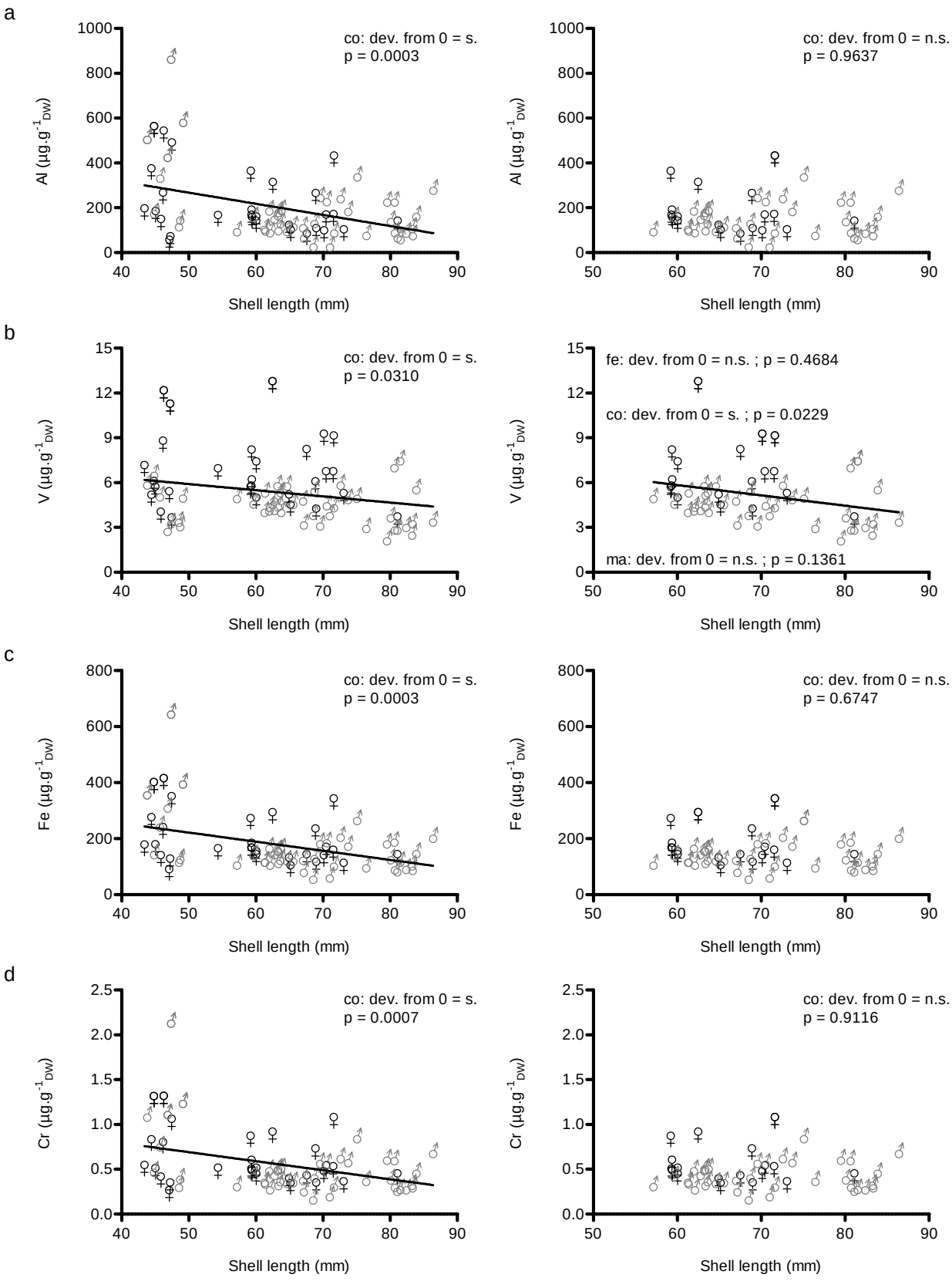
Annex A (*Continued*). Double log transformed power functions modelling relationships between o_1-p_1) the mussel soft tissue dry weight (g) and As or Mo concentrations ($\mu g.g^{-1}_{DW}$) and linear regressions modelling relationships between o_2-p_2) the mussel shell length (mm) and As or Mo total contents (µg), all individuals together (4 left graphs) or limited to mussels larger than 55 mm (4 right graphs). Equations and their corresponding fitting parameters (r^2 and p-levels) are reported on graphs.



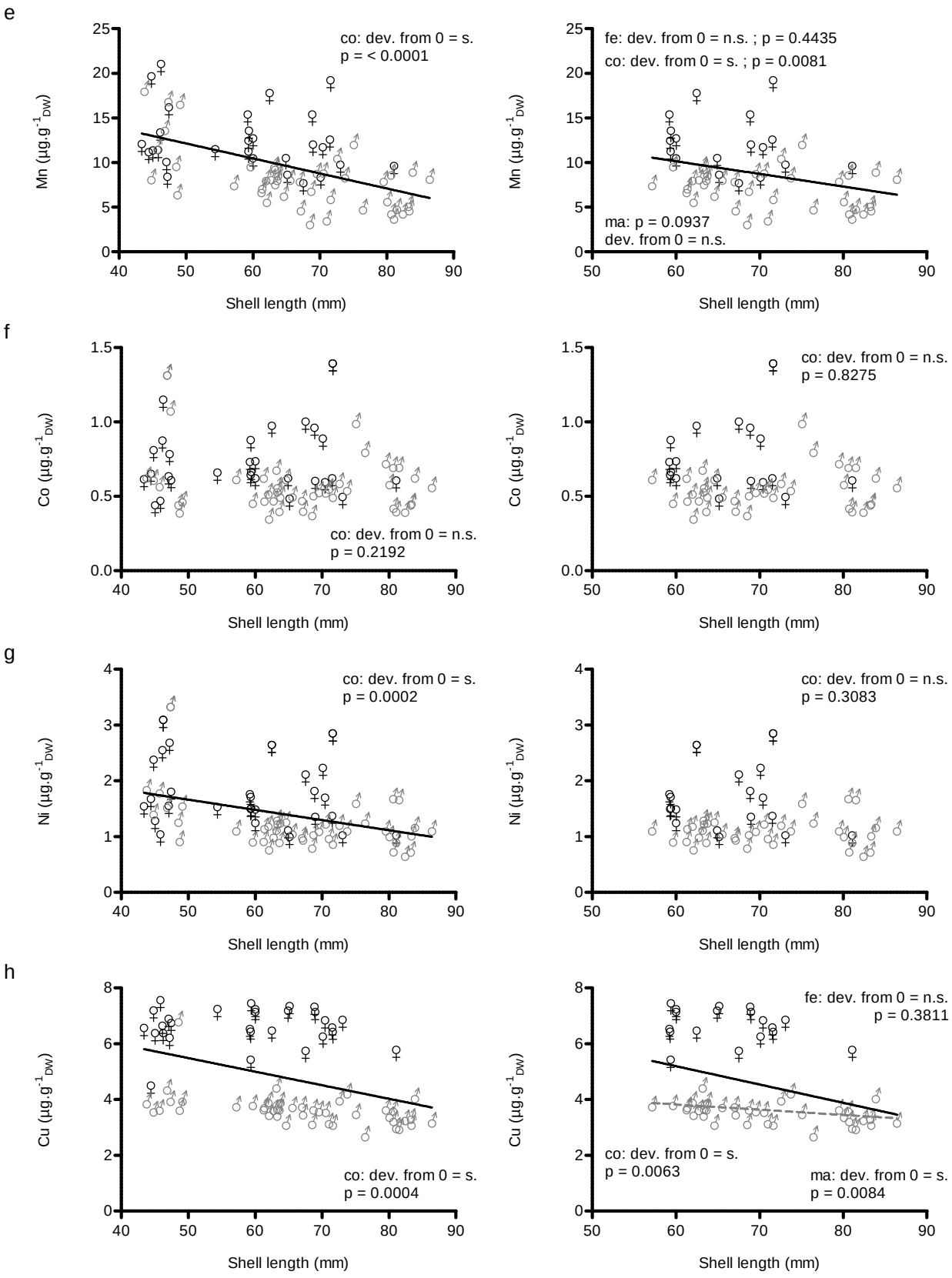
Annex A (*Continued*). Double log transformed power functions modelling relationships between q_1-r_1) the mussel soft tissue dry weight (g) and Be or Pb concentrations ($\mu g.g^{-1}_{DW}$) and linear regressions modelling relationships between q_2-r_2) the mussel shell length (mm) and Be or Pb total contents (µg), all individuals together (4 left graphs) or limited to mussels larger than 55 mm (4 right graphs). Equations and their corresponding fitting parameters (r^2 and p-levels) are reported on graphs.



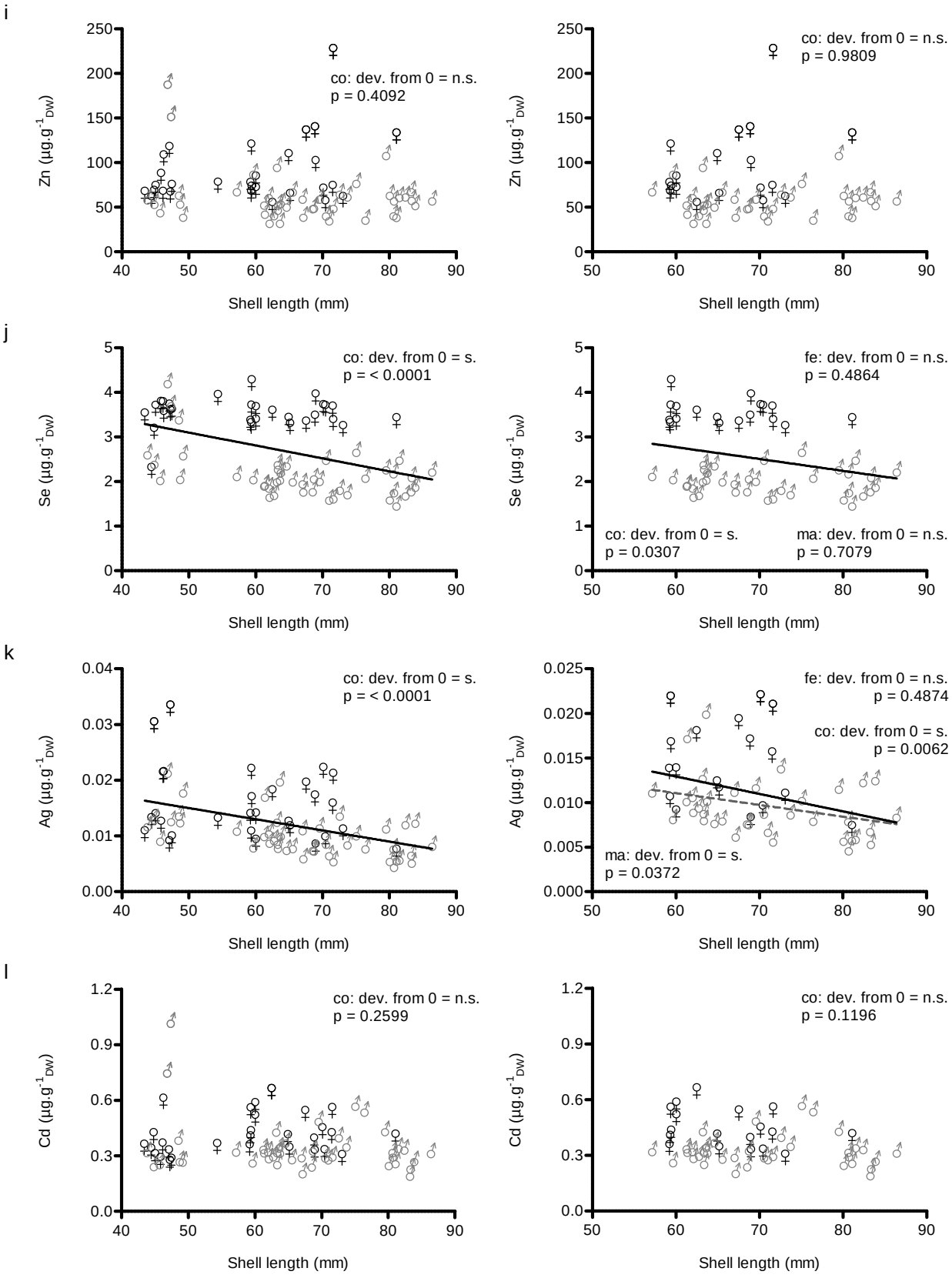
Annex A (*Continued*). Double log transformed power functions modelling relationships between s_1) the mussel soft tissue dry weight (g) and Bi concentrations ($\mu g.g^{-1}_{DW}$) and linear regressions modelling relationships between s_2) the mussel shell length (mm) and Bi total contents (µg), all individuals together (4 left graphs) or limited to mussels larger than 55 mm (4 right graphs). Equations and their corresponding fitting parameters (r^2 and p-levels) are reported on graphs.



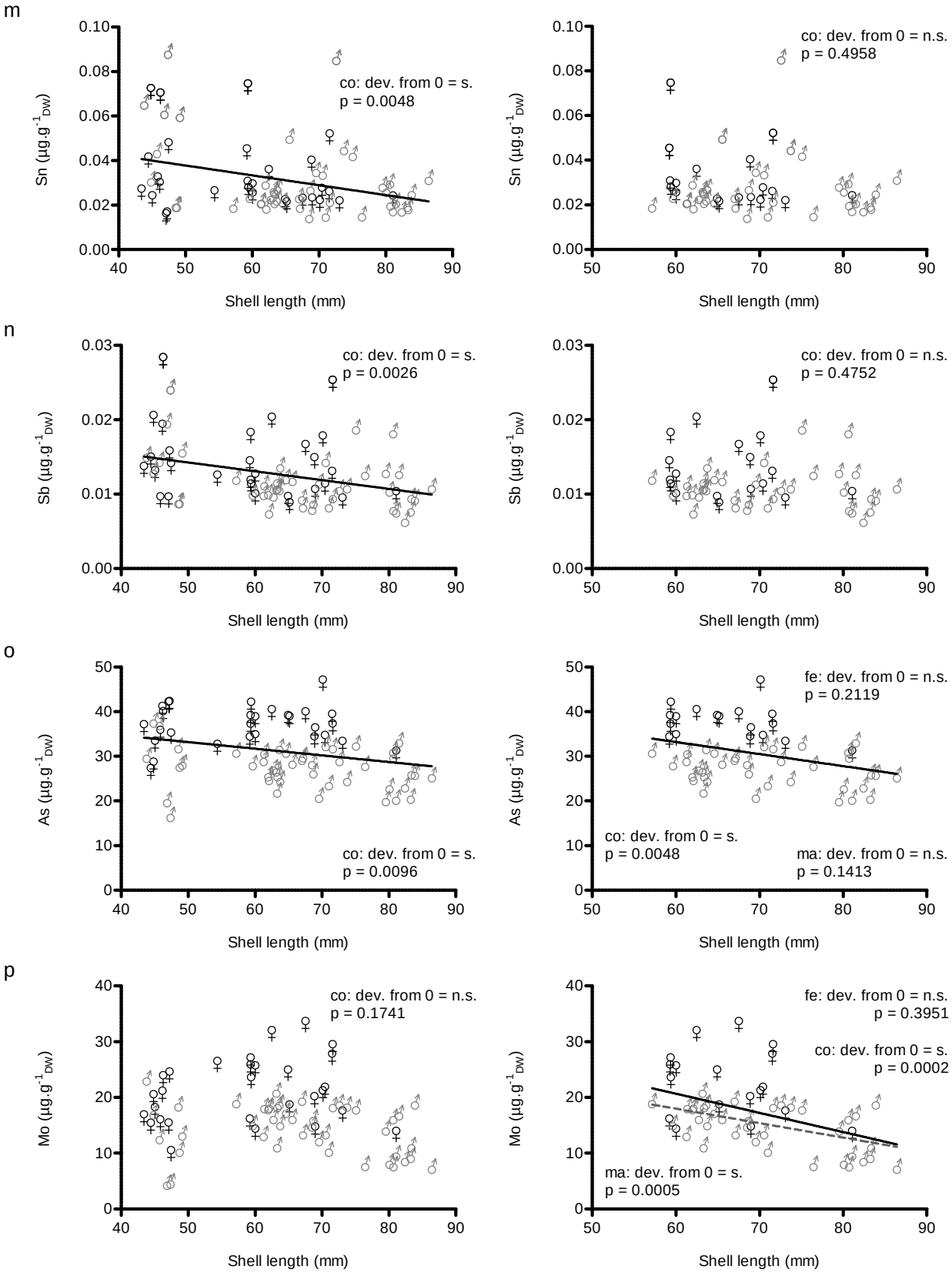
Annex B. Linear regressions modelling relationships between the mussel shell length and a) Al, b) V, c) Fe and d) Cr concentrations ($\mu g.g^{-1}_{DW}$). Females and males are represented by their gender symbol. Probabilities of linear slopes to deviate (dev.) from 0 are given (s. = significant, n.s. = non-significant). The regression common (co.) to females and males, significant ($p < 0.05$) for Al, V, Fe and Cr when considering all individuals (left graphs), underlines the determining effect played by small-size mussels. The regression common (co.) to females and males for V, significant ($p < 0.05$) when considering only mussels larger than 55 mm (right graphs), underlines the difference in V accumulation linked to sex prior to spawning, superimposed on the size effect; regressions specific to each sex (fe., ma.) are moreover not significant ($p > 0.05$).



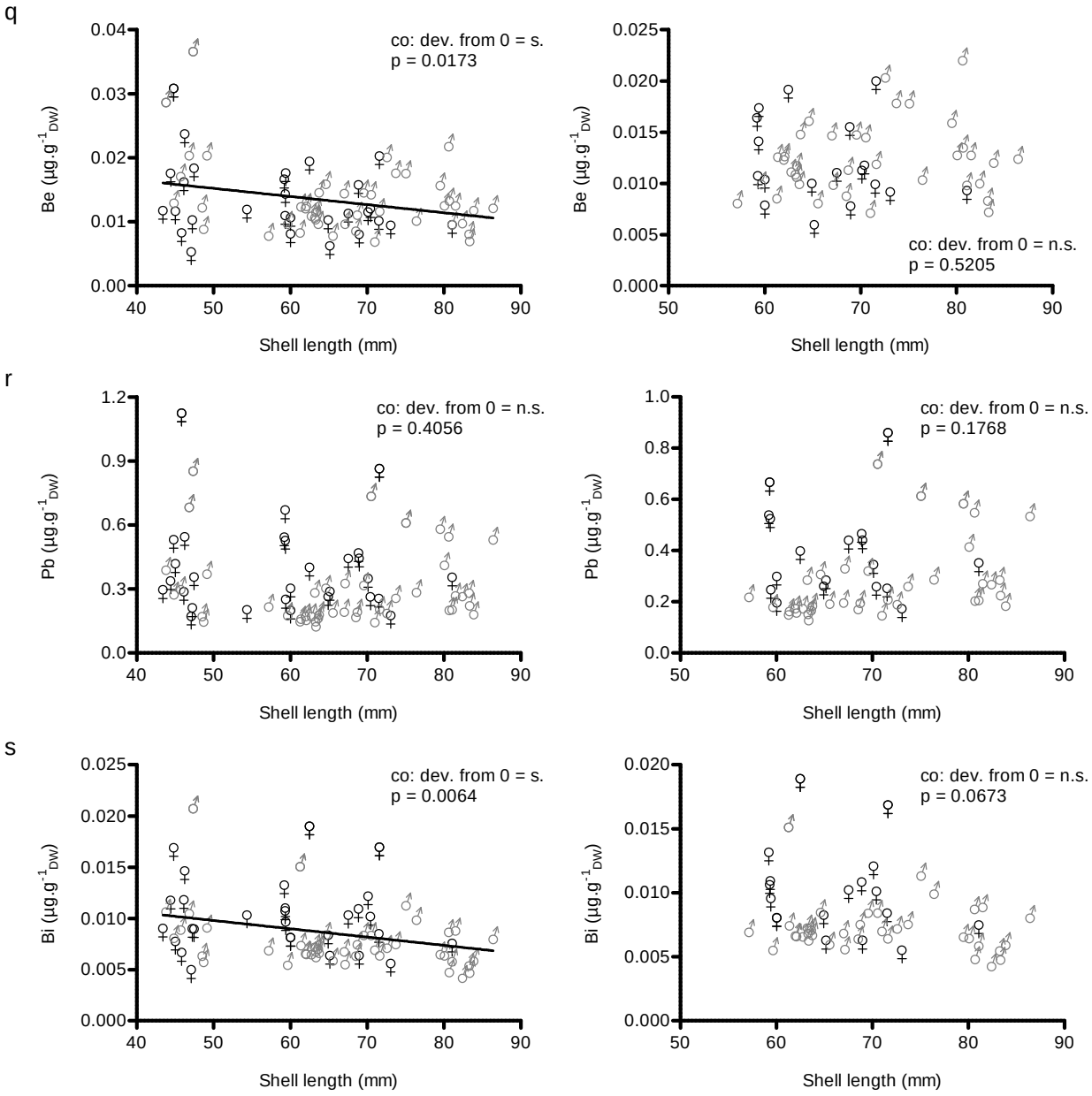
Annex B (*Continued*). Linear regressions modelling relationships between the mussel shell length and e) Mn, f) Co, g) Ni and h) Cu concentrations ($\mu g.g^{-1}_{DW}$). Females and males are represented by their gender symbol. Probabilities of linear slopes to deviate (dev.) from 0 are given (s. = significant, n.s. = non-significant). The regression common (co.) to females and males, significant ($p < 0.05$) for Mn, Ni and Cu when considering all individuals (left graphs), underlines the determining effect played by small-size mussels. The regression common (co.) to females and males for Mn and Cu, significant ($p < 0.05$) when considering only mussels larger than 55 mm (right graphs), underlines the difference in Mn and Cu accumulation linked to sex prior to spawning, superimposed on the size effect; regressions specific to each sex (fe., ma.) are moreover not significant ($p > 0.05$), except for Cu accumulation in males.



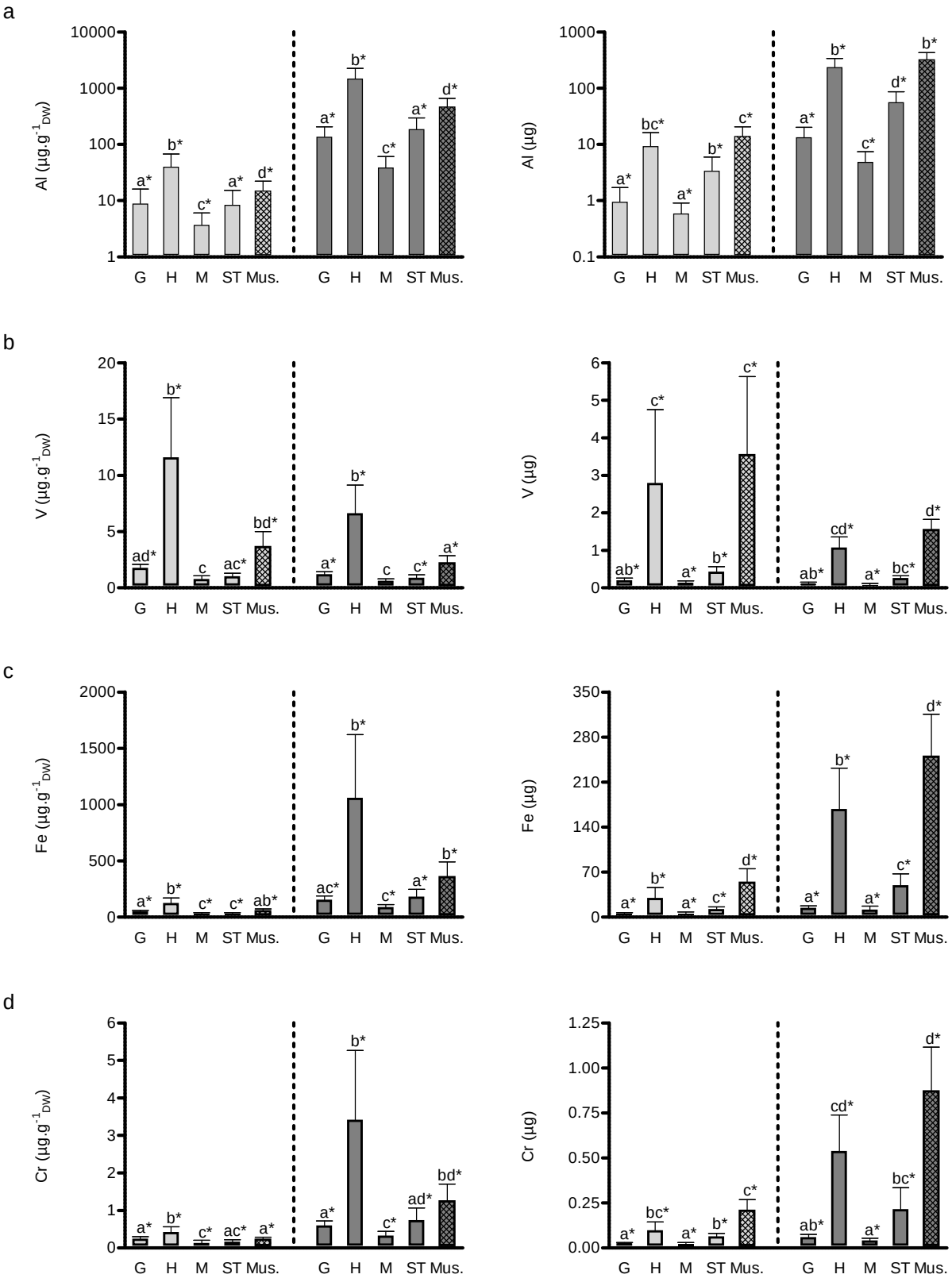
Annex B (*Continued*). Linear regressions modelling relationships between the mussel shell length and i) Zn, j) Se, k) Ag and l) Cd concentrations (µg.g $^{-1}_{DW}$). Females and males are represented by their gender symbol. Probabilities of linear slopes to deviate (dev.) from 0 are given (s. = significant, n.s. = non-significant). The regression common (co.) to females and males, significant ($p < 0.05$) for Se and Ag when considering all individuals (left graphs), underlines the determining effect played by small-size mussels. The regression common (co.) to females and males for Se and Ag, significant ($p < 0.05$) when considering only mussels larger than 55 mm (right graphs), underlines the difference in Se and Ag accumulation linked to sex prior to spawning, superimposed on the size effect; regressions specific to each sex (fe., ma.) are moreover not significant ($p > 0.05$), except for Ag accumulation in males.



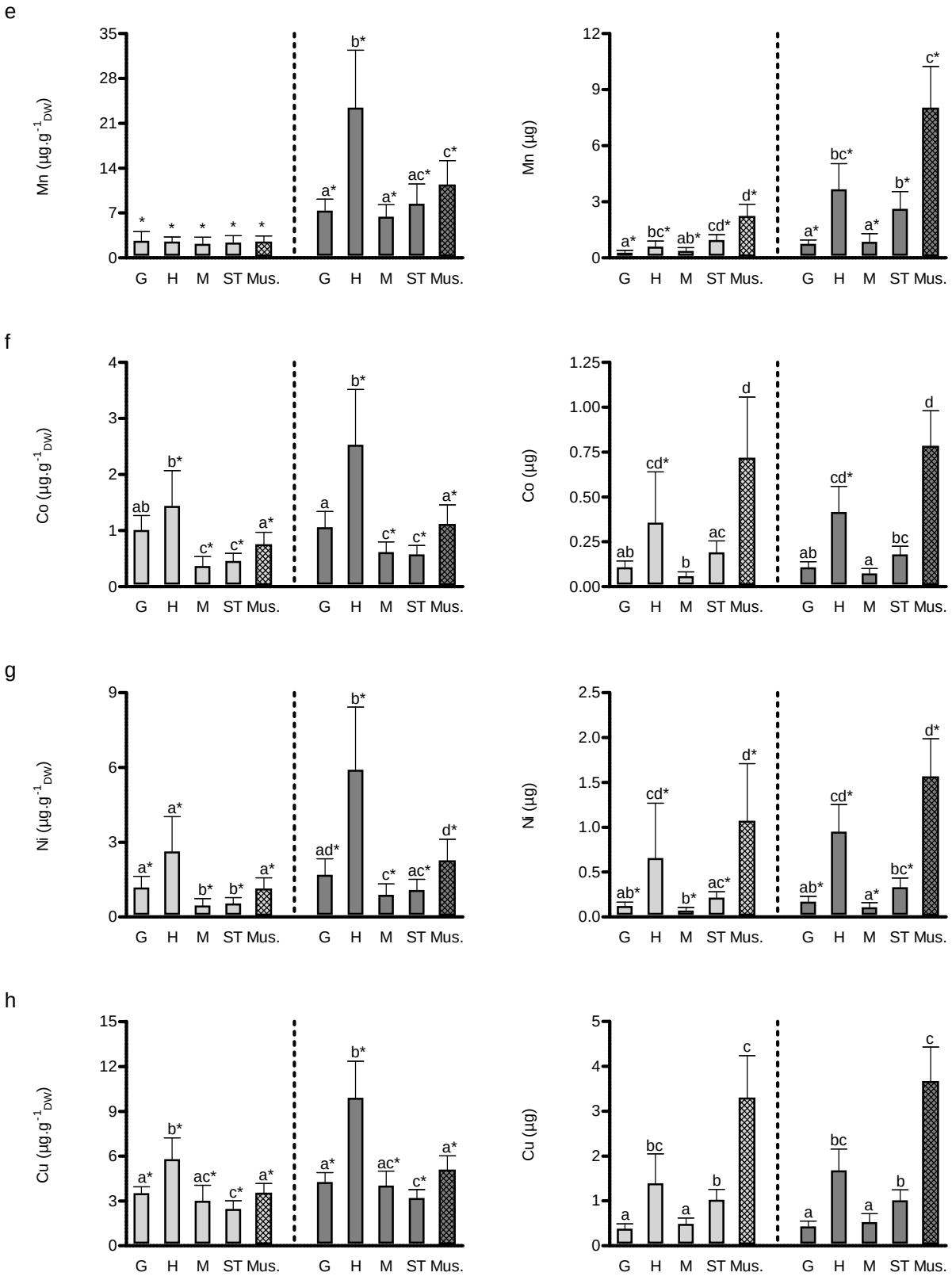
Annex B (*Continued*). Linear regressions modelling relationships between the mussel shell length and m) Sn, n) Sb, o) As and p) Mo concentrations ($\mu g.g^{-1}_{DW}$). Females and males are represented by their gender symbol. Probabilities of linear slopes to deviate (dev.) from 0 are given (s. = significant, n.s. = non-significant). The regression common (co.) to females and males, significant ($p < 0.05$) for Sn, Sb and As when considering all individuals (left graphs), underlines the determining effect played by small-size mussels. The regression common (co.) to females and males for As, significant ($p < 0.05$) when considering only mussels larger than 55 mm (right graphs), underlines the difference in As accumulation linked to sex prior to spawning, superimposed on the size effect; regressions specific to each sex (fe., ma.) are moreover not significant ($p > 0.05$). The regression common (co.) to females and males for Mo, significant ($p < 0.05$) when considering only mussels larger than 55 mm (right graphs), relies on the diminution in Mo concentrations in large size males; the relationship between the size of males larger than 55 mm and their Mo concentrations is moreover very highly significant ($p < 0.001$).



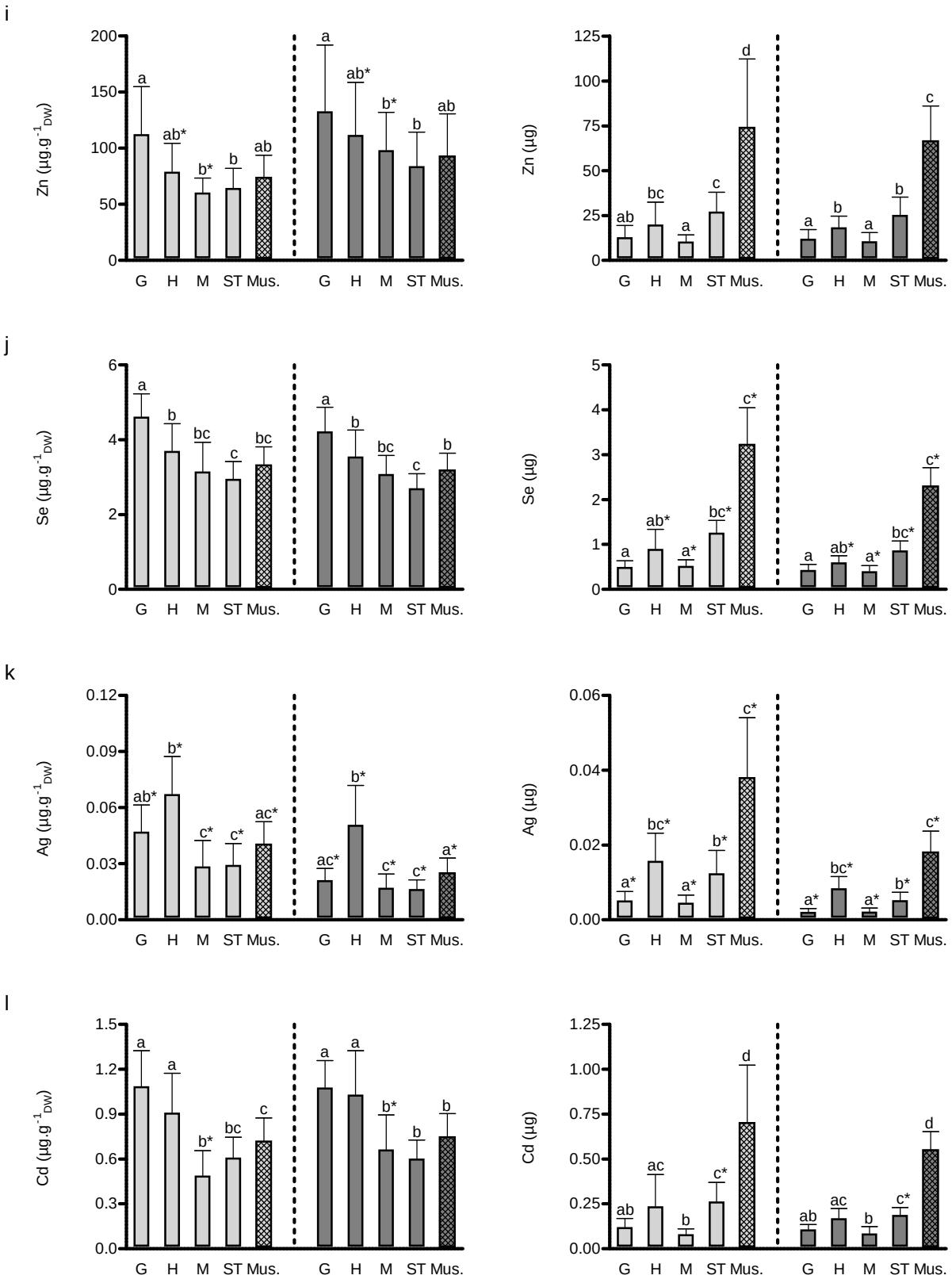
Annex B (*Continued*). Linear regressions modelling relationships between the mussel shell length and q) Be, r) Pb and s) Bi concentrations (µg.g $^{-1}_{DW}$). Females and males are represented by their gender symbol. Probabilities of linear slopes to deviate (dev.) from 0 are given (s. = significant, n.s. = non-significant). The regression common (co.) to females and males, significant ($p < 0.05$) for Be and Bi when considering all individuals (left graphs), underlines the determining effect played by small-size mussels. There is no significant ($p > 0.05$) regression common (co.) to females and males when considering only mussels larger than 55 mm (right graphs).



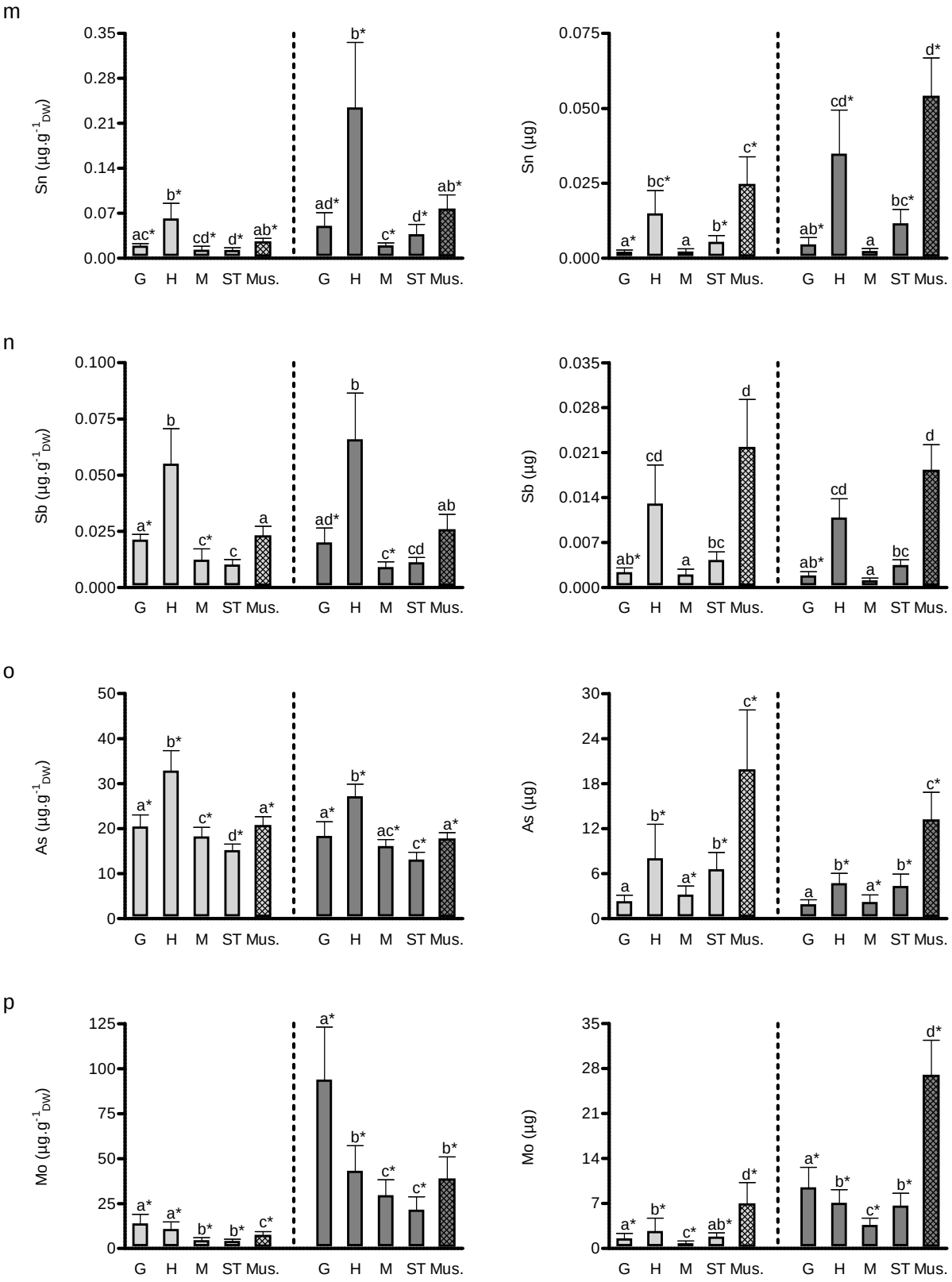
Annex C. a) Al, b) V, c) Fe and d) Cr levels in gills (G), hepatopancreas (H), mantle (M), remaining soft tissues (ST) and whole mussels (Mus.) sampled before (light gray) or after (dark grey) spawning. Levels are expressed as concentrations ($\mu g.g^{-1}_{DW}$ - 4 left graphs) or total contents (µg - 4 right graphs). Letters represent significant differences ($p < 0.05$) between the 4 body compartments and whole mussels of a same gametogenic status (*i.e.* multiple comparison tests of means); * represent significant differences ($p < 0.05$) for a same body compartment or between mussels before or after spawning (*i.e.* pairwise comparison tests of means), respectively.



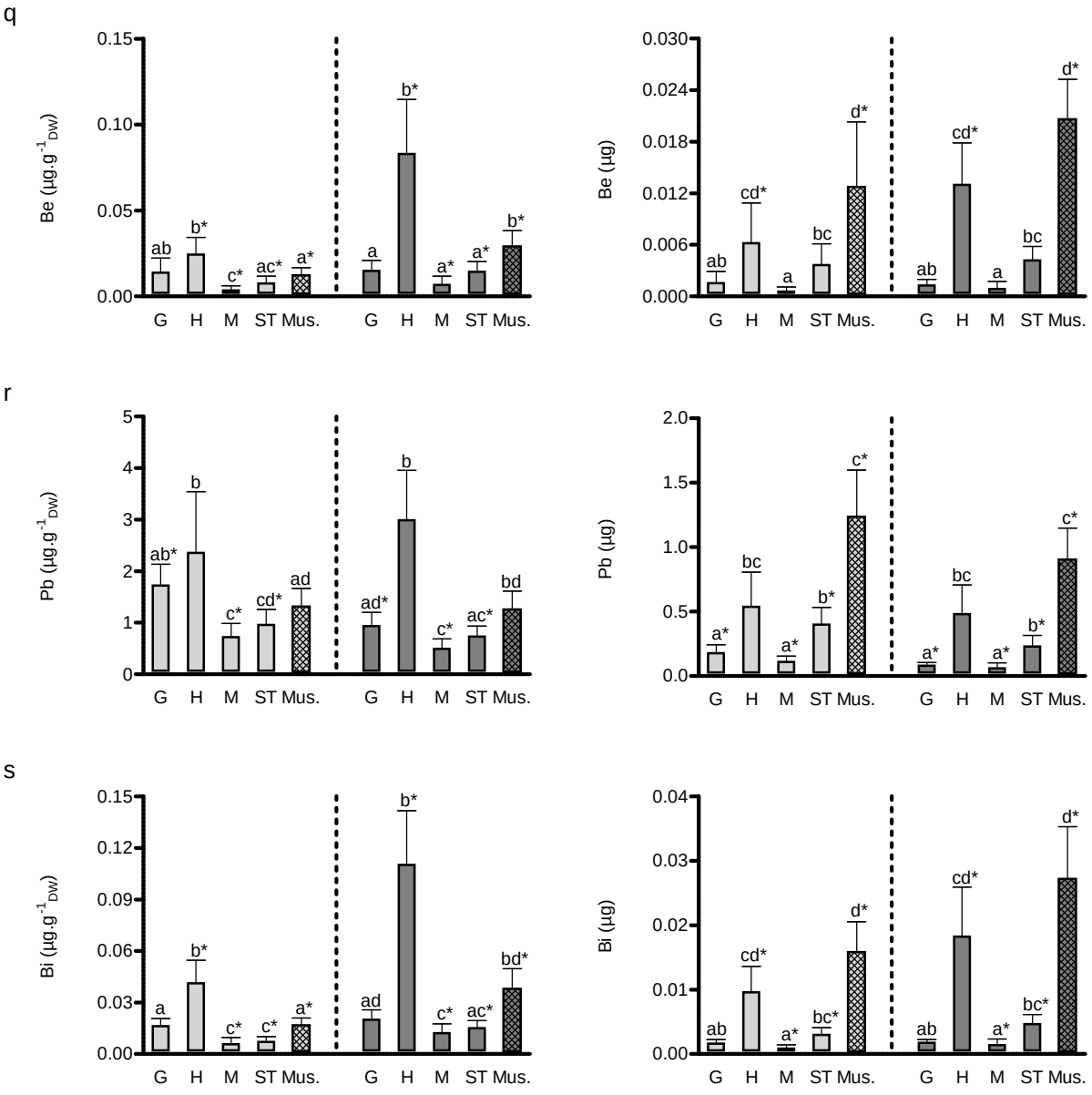
Annex C (*Continued*). e) Mn, f) Co, g) Ni and h) Cu levels in gills (G), hepatopancreas (H), mantle (M), remaining soft tissues (ST) and whole mussels (Mus.) sampled before (light gray) or after (dark grey) spawning. Levels are expressed as concentrations ($\mu g.g^{-1}_{DW}$ - 4 left graphs) or total contents (µg - 4 right graphs). Letters represent significant differences ($p < 0.05$) between the 4 body compartments and whole mussels of a same gametogenic status (*i.e.* multiple comparison tests of means); * represent significant differences ($p < 0.05$) for a same body compartment or between mussels before or after spawning (*i.e.* pairwise comparison tests of means), respectively.



Annex C (*Continued*). i) Zn, j) Se, k) Ag and l) Cd levels in gills (G), hepatopancreas (H), mantle (M), remaining soft tissues (ST) and whole mussels (Mus.) sampled before (light gray) or after (dark grey) spawning. Levels are expressed as concentrations ($\mu g.g^{-1}_{DW}$ - 4 left graphs) or total contents (µg - 4 right graphs). Letters represent significant differences ($p < 0.05$) between the 4 body compartments and whole mussels of a same gametogenic status (*i.e.* multiple comparison tests of means); * represent significant differences ($p < 0.05$) for a same body compartment or between mussels before or after spawning (*i.e.* pairwise comparison tests of means), respectively.



Annex C (*Continued*). m) Sn, n) Sb, o) As and p) Mo levels in gills (G), hepatopancreas (H), mantle (M), remaining soft tissues (ST) and whole mussels (Mus.) sampled before (light gray) or after (dark grey) spawning. Levels are expressed as concentrations ($\mu g.g^{-1}_{DW}$ - 4 left graphs) or total contents (µg - 4 right graphs). Letters represent significant differences ($p < 0.05$) between the 4 body compartments and whole mussels of a same gametogenic status (*i.e.* multiple comparison tests of means); * represent significant differences ($p < 0.05$) for a same body compartment or between mussels before or after spawning (*i.e.* pairwise comparison tests of means), respectively.



Annex C (*Continued*). q) Be, r) Pb and s) Bi levels in gills (G), hepatopancreas (H), mantle (M), remaining soft tissues (ST) and whole mussels (Mus.) sampled before (light gray) or after (dark grey) spawning. Levels are expressed as concentrations ($\mu g.g^{-1}_{DW}$ - 4 left graphs) or total contents (µg - 4 right graphs). Letters represent significant differences ($p < 0.05$) between the 4 body compartments and whole mussels of a same gametogenic status (*i.e.* multiple comparison tests of means); * represent significant differences ($p < 0.05$) for a same body compartment or between mussels before or after spawning (*i.e.* pairwise comparison tests of means), respectively.