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SHORT NOTES

An ecophysiological discussion of trace element bioaccumulation in cultured *Mytilus galloprovincialis*

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Monitoring programs conducted by the French Research Institute for the Exploitation of the Sea IFREMER have been using the mussel watch approach introduced by Goldberg [1] since 1974, initially on wild and cultured bivalve mollusks [2], leading to long time data series for several trace elements (TEs: Ag, Cd, Cr, Cu, Hg, Ni, Pb, V and Zn; http://envlit.ifremer.fr/). Since 1996, transplanted caged *Mytilus galloprovincialis* LAMARCK, 1819 have been used to characterize the chemical contamination of Mediterranean coastal waters even in locations where no native wild mussels were available. This project succeeded in assessing the natural background and the extent of the chemical contamination first at the scale of the French Mediterranean littoral [3,4], and more recently at the scale of the whole western Mediterranean Sea [5,6]. However, these programs have focussed on a limited number of metals. Nowadays, the development of very sensitive equipment allows the measurement of some TEs found at very low environmental levels. In parallel, recent technological developments have led to an increase in the extraction and industrial refinement of TEs previously of little concern. Therefore, the environmental monitoring of less studied, potentially toxic TEs of emerging environmental concern is relevant [7].

From data previously published by RICHIR & GOBERT [7], the first objective of this short note was to discuss the bioaccumulation profile of 19 TEs that have been either broadly (Cr, Ni, Cu, Zn, Cd, Pb, As, Ag and V) or little monitored (Be, Al, Fe, Mn, Co, Se, Mo, Sn, Sb and Bi) in the Mediterranean mussel *M. galloprovincialis*. The second objective was to test the relevance of the Trace Element Pollution Index of RICHIR & GOBERT [8] when modelling the effect of the shell length and flesh dry weight on the overall accumulation of these 19 TEs in rope-grown mussels. Because of the importance of gametogenesis in the physiological cycle of *M. galloprovincialis*, the third objective was to briefly discuss the deterministic effect of the sex and the reproductive status on the overall TE bioaccumulation and the TE-specific compartmentalization in that species.

Briefly, to realise these three objectives, *M. galloprovincialis* were purchased from the shellfish farm of the Diane pond on the eastern coast of Corsica, France (42°07'45.00''N, 9°31'01.00''E), in March 2010 (after mussel spawning) and February 2011 (before mussel spawning). Seventy four mussels sampled in February 2011 were used to investigate the bioaccumulation of the 19 TEs listed above. These 74 mussels were further segregated according to their sex to study differences between male and female TE bioaccumulation prior to spawning. Forty supplementary large-size (70-80 mm shell length) mussels purchased in March 2010

(n = 20) and February 2011 (n = 20) were used for the analysis of body compartmentalization after and before spawning, respectively, at one-year interval. Body compartments were sorted as follows: gills, hepatopancreas, mantle and remaining soft tissues. TE levels were determined by ICP-MS (ICP-MS ELAN DRC II, PerkinElmer®) after mineralization in a closed microwave digestion labstation (Ethos D, Milstone Inc.), using HNO_3 and H_2O_2 as reagents (Suprapur grade, Merck). Analytical accuracy was checked by analysing Certified Reference Materials: BCR 278, NIST 1566b and NIST 2976. The global mean recovery, all elements together, was 95 ± 9 %. For each TE, the analytical detection limit (L_D) was calculated according to Currie [9] or Grinzaid et al. [10].

The Trace Element Pollution Index (TEPI) of Richir & Gobert [8], which synthetizes the accumulated levels (concentrations or contents) of all the studied TEs into a single index value, was calculated for each of the 74 mussels sampled in February 2011 as follows: TEPI = $(Cf_1 * Cf_2 \ldots Cf_n)^{1/n}$, where Cf_n is the mean normalized concentration or content of the TE n of a given mussel [11,12]. The log-transformed power function: $\log_{10}Y = \log_{10}a + b\log_{10}X$, and the linear regression: $Y = bX + a$, were tested to model the relationships between the 74 mussel flesh dry weight (X; from 0.17 to 3.36 g) or shell length (X; from 43.40 to 86.41 mm) and TEPI values (Y) [13,14,15]. *b* is the slope of linear functions; $\log_{10}a$ and *a* are the Y-intercepts. To select the most adequate model that best described these relationships, the second order Akaike information criterion (AICc) was used [16]. Statistical analyses were performed with STATISTICA 10 (Stat-Soft Inc.) and GraphPad Prism 5 (GraphPad Software Inc.) software.

Results showed that rope-grown *M. galloprovincialis* from the Diane pond efficiently bioaccumulated the 10 little monitored TEs (Be, Al, Fe, Mn, Co, Se, Mo, Sn, Sb, Bi) in addition to the 9 TEs classically monitored (Cr, Ni, Cu, Zn, Cd, Pb, As, Ag and V) in that species (Table 1). Comparative graphs ordering TEs either broadly or little monitored in *M. galloprovincialis* by decreasing order of concentrations are given in Fig. 1. Concentrations ranged from 10^{-3} µg g_{DW}^{-1} for Bi to 10^{2} µg g_{DW}^{-1} for Al and Fe (Table 1; Fig. 1). Essential TEs classically monitored such as Zn, Cu, Ni and Cr appeared to be preferentially accumulated unlike non-essential toxic TEs such as Cd, Pb and Ag (Fig. 1a) [17]. With regard to As, this TE was reported to be naturally more bioaccumulated in *M. galloprovincialis* [3], mostly as organicated non-toxic species [18,19]. The mean V concentration was relatively high compared to data reported by the IFREMER (http://envlit.ifremer.fr/) for the Diane pond and could thus reflect a temporary moderate contamination of the pond by that specific element [7]. Bioaccumulation of TEs little monitored in *M. galloprovincialis* showed a similar graphic profile (Fig. 1b) to the previous one. Environmentally abundant and/or essential TEs such Fe, Al, Mo, Mn, Se and Co were bioaccumulated in a more important way, while concentrations of non-essential and potentially toxic Sn, Be, Sb and Bi remained low to very low [17]. The Diane pond has previously been considered little contaminated by TEs [7]; Fig. 1 thus presents the natural aptitude of rope-grown *M. galloprovincialis* to bioaccumulate TEs in clean environmental conditions. Although essential TEs appeared to be preferentially accumulated, unlike non-essential toxic ones, there is currently little physiological evidence about this preferential accumulation of essential versus other elements [20,21].

The overall concentration of the 19 studied TEs in mussel flesh, synthetized as TEPI values, decreased when the mussel flesh dry weight increased (Fig. 2a); their overall flesh content increased when the mussel shell length increased (Fig. 2b). According to AICc values, the relationships between the overall TE concentration of the 74 mussels, without size restriction, and their body dry weight, or between the overall TE content and the mussel shell length, were better modelled by the power function (Fig. 2a; Table 2a; AICc value of 93.08%) or the linear regression (Fig. 2b; Table 2b; AICc

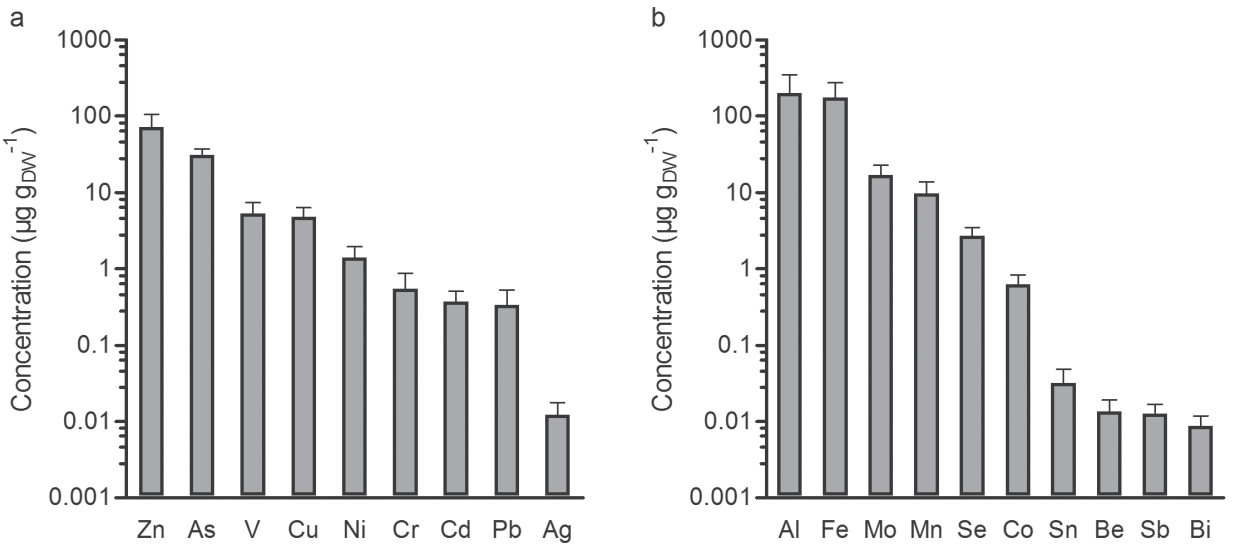

Fig. 1 – Concentration profiles (mean ± SD, in µg g_{DW}^{-1}; logarithmic scale) of trace elements (TEs) either (a) broadly or (b) little monitored in *Mytilus galloprovincialis* (n = 74).

value of 96.47%), respectively. In contrast, the relationship between the overall TE content and the mussel shell length was properly modelled by both the linear regression and the power function when only individuals larger than 55 mm were considered (Table 2b; AICc values of 47.90% and 52.10%). Small-size mussels (< 55 mm) thus had an antagonistic effect on the modelling of the overall TE bioaccumulation in rope-grown *M. galloprovincialis*: they led us to elect the linear regression to model the relationship between the mussel size and their overall TE content (AICc value rising from 47.90% to 96.47%), but sensibly diminished the significance of the power function modelling the relationship between the mussel flesh dry weight and their overall TE concentration (AICc value decreasing from 98.83% to 93.08%). These results corroborate the observations of Richir & Gobert [7] made for each TE considered separately and support

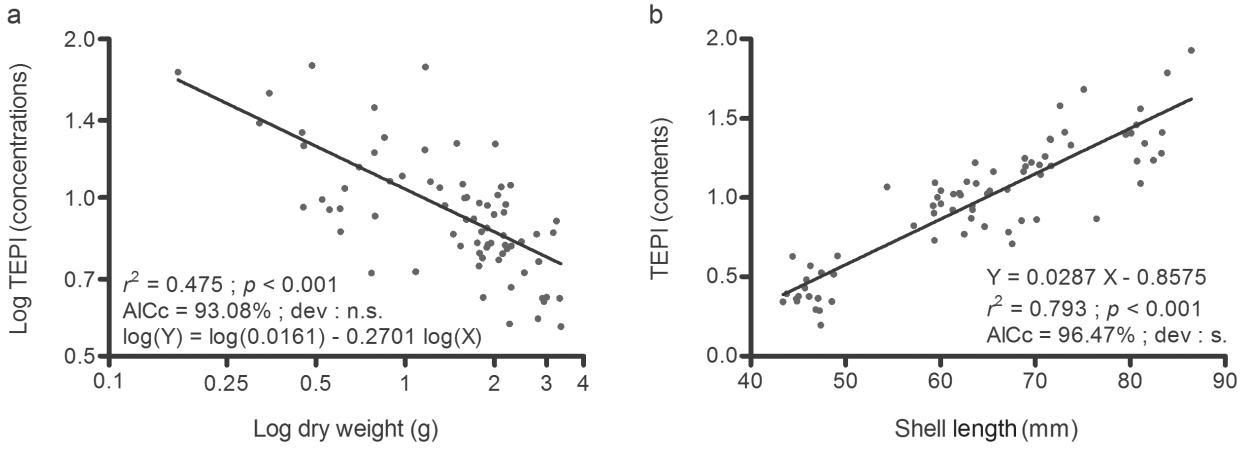

Fig. 2 – (a) Log transformed power function modelling the relationship between *Mytilus galloprovincialis* (n = 74) soft tissue dry weight and Trace Element Pollution Index (TEPI) values (no unit), calculated from mean normalized concentrations of the 19 studied trace elements (TEs), and (b) linear regression modelling the relationship between mussel shell length and TEPI values (no unit), calculated from mean normalized contents of the 19 studied TEs. Linear equations and their corresponding fitting parameters (r^2; p-value; deviation (dev.) from the model: s = significant, n.s. = non-significant; AICc) are reported on graphs.

TABLE 1

Trace element (TE) concentrations (mean ± SD, in µg g_{DW}^{-1}) in rope-grown *Mytilus galloprovincialis* purchased before they spawned, and Trace Element Pollution Index (TEPI) values (no unit) calculated from mean normalized concentrations of the 19 studied TEs. TE concentrations and TEPI values are given for all mussels together, independently of their size or sex, for mussels sorted by size-class (cl. 1 to cl. 4) and for mussels sorted by sex. Numbers between brackets are numbers of mussels. Letters represent significant differences ($p < 0.05$) between size-classes; * represent significant differences ($p < 0.05$) between sexes. Concentration data used to calculate TEPI values are from RICHIR & GOBERT [7].

	All mussels (74)	**Mussels sorted by size-class**								**Mussels sorted by sex**			
			cl. 1: 43-54 mm (19)		cl. 2: 55-64 mm (21)		cl. 3: 65-74 mm (20)		cl. 4: 75-87 mm (14)		Females (29)		Males (45)
Al	200 ± 150	a	323 ± 223	ab	162 ± 63	b	150 ± 95	ab	160 ± 88		204 ± 143		197 ± 156
V	5.35 ± 2.02	ab	5.80 ± 2.50	a	5.67 ± 1.84	ab	5.43 ± 1.67	b	4.11 ± 1.68	*	6.55 ± 2.40	*	4.57 ± 1.24
Fe	177 ± 97	a	255 ± 146	ab	156 ± 43	b	148 ± 63	b	146 ± 55		186 ± 89		172 ± 102
Cr	0.554 ± 0.320		0.803 ± 0.489		0.477 ± 0.146		0.462 ± 0.197		0.465 ± 0.181		0.581 ± 0.288		0.537 ± 0.341
Mn	9.86 ± 3.87	a	12.89 ± 4.03	b	9.84 ± 2.84	b	9.07 ± 3.62	b	6.88 ± 2.51	*	12.18 ± 3.48	*	8.36 ± 3.37
Co	0.634 ± 0.205		0.688 ± 0.255		0.605 ± 0.135		0.626 ± 0.235		0.616 ± 0.173	*	0.707 ± 0.215	*	0.587 ± 0.185
Ni	1.41 ± 0.54	a	1.81 ± 0.66	b	1.30 ± 0.37	b	1.34 ± 0.50	b	1.16 ± 0.34	*	1.68 ± 0.59	*	1.24 ± 0.43
Cu	4.82 ± 1.50	a	5.55 ± 1.40	a	4.86 ± 1.47	a	4.98 ± 1.55	b	3.56 ± 0.69	*	6.50 ± 0.67	*	3.74 ± 0.61
Zn	72.6 ± 33.6		79.7 ± 37.8		66.2 ± 22.2		75.7 ± 43.8		67.9 ± 25.2	*	86.3 ± 36.4	*	63.7 ± 28.8
Se	2.70 ± 0.78	a	3.24 ± 0.66	ab	2.64 ± 0.76	b	2.64 ± 0.81	b	2.17 ± 0.48	*	3.48 ± 0.34	*	2.21 ± 0.54
Ag	0.0123 ± 0.0054	a	0.0157 ± 0.0068	a	0.0124 ± 0.0040	ab	0.0116 ± 0.0048	b	0.0083 ± 0.0027	*	0.0151 ± 0.0064	*	0.0104 ± 0.0038
Cd	0.374 ± 0.131		0.390 ± 0.200		0.389 ± 0.100		0.357 ± 0.089		0.352 ± 0.111	*	0.397 ± 0.106	*	0.358 ± 0.144
Sn	0.0318 ± 0.0167		0.0413 ± 0.0222		0.0282 ± 0.0117		0.0312 ± 0.0170		0.0248 ± 0.0073		0.0323 ± 0.0160		0.0314 ± 0.0174
Sb	0.0126 ± 0.0042		0.0152 ± 0.0052		0.0119 ± 0.0028		0.0119 ± 0.0040		0.0113 ± 0.0038	*	0.0140 ± 0.0048	*	0.0118 ± 0.0036
As	31.2 ± 6.1	a	32.7 ± 6.8	ab	31.7 ± 5.5	ab	32.3 ± 5.9	b	26.9 ± 4.5	*	36.3 ± 4.3	*	27.8 ± 4.6
Mo	17.1 ± 5.8	ab	16.3 ± 6.0	a	19.5 ± 4.9	a	18.8 ± 5.6	b	12.3 ± 4.2	*	20.7 ± 5.8	*	14.8 ± 4.5
Be	0.0135 ± 0.0056		0.0169 ± 0.0085		0.0122 ± 0.0030		0.0121 ± 0.0042		0.0128 ± 0.0040		0.0127 ± 0.0056		0.0140 ± 0.0057
Pb	0.336 ± 0.192		0.400 ± 0.253		0.268 ± 0.138		0.324 ± 0.185		0.369 ± 0.154		0.378 ± 0.211		0.309 ± 0.175
Bi	0.0087 ± 0.0032		0.0100 ± 0.0039		0.0089 ± 0.0032		0.0082 ± 0.0026		0.0073 ± 0.0021	*	0.0097 ± 0.0034	*	0.0080 ± 0.0028
TEPI	0.959 ± 0.269	a	1.137 ± 0.307	ab	0.932 ± 0.198	b	0.920 ± 0.270	b	0.813 ± 0.190	*	1.094 ± 0.274	*	0.872 ± 0.229

the relevance of the TEPI to model, in a reduced number of synthesis equations, the relationships between the overall levels of bioaccumulated contaminants and the physiology of organisms.

The same was concluded when modelling the relationship between the shell length and the overall TE concentration of the 19 studied TEs in mussel flesh. Thus, the overall TE concentration in mussel flesh was linearly correlated (p = 0.0002) with the shell length when considering all the 74 mussels, without size restriction (Fig. 3). This significant relationship was to be attributed mainly to small-size mussels (43–54 mm) whose TE-specific and overall mean concentrations were always higher than for the mid-size individuals (55-64 mm and 65-74 mm), except for Mo, As and Cd (Table 1). Small-size mussels further showed a high inter-individual variability of their TE concentrations. These observations reflected unfavourable physical conditions of growth of small-size mussels found inside the rope [7]. When the linear regression model was run again for mussels larger than 55 mm only, the overall TE concentration in mussel flesh was no longer correlated (p = 0.1085) with the shell length (Fig. 3). For mid- to large-size *M. galloprovincialis* grown on ropes, the size did not significantly influence flesh concentrations of most TEs, although they were slightly lower on average in individuals larger than 75 mm (Table 1). As their culture begins synchronically, all mussels on a rope have the same age, but may differ in size according to individual growing conditions [14]. Thus, when sorting mussels for monitoring purposes, care should be taken to use neither small- (restrained growth and concentration effect) nor large-size (rapid growth and dilution effect) mussels, these individuals being not representative of the rope population [7].

Female and male mussels accumulated TEs unevenly during gametogenesis. As a result, mean TE concentrations in mussel dry flesh sampled prior to spawning differed significantly (p < 0.05) between sexes and were higher in females (from 3% for Al up to 74% for Cu, except for Be), for an overall difference (*i.e.* TEPI values) of 25% (Table 1). This different accumulation between individuals of opposite sexes resulted in the linear regression with slope still close to the significance threshold level of 0.05 when modelling the relationship between the shell length of mussels larger than 55 mm and their overall TE concentration (p = 0.1085; Fig. 3). The sex-related bioaccumulation of TEs during gametogenesis could depend on a functional role played by metallothioneins (MTs), as already suggested by Latouche & Mix [22] in the early 80s and supported by several subsequent experimental and field studies [23, 24, 25].

In *M. galloprovincialis*, up to 45 % of soft tissue weight can be lost during spawning [8]. Despite the importance of gametogenesis in the physiological cycle of *M. galloprovincialis*, the proportional distribution of TEs between main body compartments analysed respectively a few days before or after spawning, at one-year interval, remained the same: the 19 studied TEs were more accumulated in the hepatopancreas compared to the mantle and the gills (except for Mo in mussel having spawned), and only Zn, Se, Cd (at both reproductive states) and Mn (in mussels close to spawning) showed higher contents in the remaining soft tissues of mussels (Fig. 4). This very conservative character of TE compartmentalization (physiological and temporal constancy) is an argument in favour of some internal regulation of TE redistribution processes between organs [20,21], in addition to passive diffusion processes according to concentration gradients and tissue affinities.

To conclude, little monitored TEs as well as broadly monitored ones were efficiently bioaccumulated in rope-grown *M. galloprovincialis*, with a preferential accumulation for essential and abundant ones. The relevant use of the TEPI to model, in a reduced number of synthesis equations, the relationships between the overall levels of bioaccumulated contaminants and the physiology of organisms was described. The significant effect of the size of cultured mussels whose growth was above or below average on the accumulation of TEs in their flesh was pointed

TABLE 2

Comparison of linear regressions and log transformed power functions modelling (a) relationships between *Mytilus galloprovincialis* soft tissue dry weight and Trace Element Pollution Index (TEPI) values, calculated from mean normalized concentrations of the 19 studied trace elements (TEs), and (b) relationships between mussel shell length and TEPI values, calculated from mean normalized contents of the 19 studied TEs. Modelling was applied for all mussels together (n = 74) or for individuals large than 55 mm only (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*-value; deviation (dev.) from the model: s. = significant, n.s. = non-significant; AICc).

(a) Relationships between the overall TE concentration and the mussel flesh dry weight

	linear regression			log transformed power function	
	all mussels	mussels > 55 mm		all mussels	mussels > 55 mm
b	-0.2140	-0.2415	*b*	-0.2701	-0.5296
a	1.3190	1.3900	$\log_{10}a$	0.0161	0.0986
r^2	0.437	0.443	r^2	0.475	0.526
p	< 0.001	< 0.001	*p*	< 0.001	< 0.001
dev.	n.s.	n.s.	dev.	n.s.	n.s.
AICc	6.92%	1.17%	AICc	93.08%	98.83%

(b) Relationships between the overall TE content and the mussel shell length

	linear regression			log transformed power function	
	all mussels	mussels > 55 mm		all mussels	mussels > 55 mm
b	0.0287	0.0240	*b*	1.8530	1.4530
a	-0.8575	-0.5227	$\log_{10}a$	-3.3670	-2.6180
r^2	0.793	0.527	r^2	0.774	0.528
p	< 0.001	< 0.001	*p*	< 0.001	< 0.001
dev.	s.	n.s.	dev.	s.	n.s.
AICc	96.47%	47.90%	AICc	3.53%	52.10%

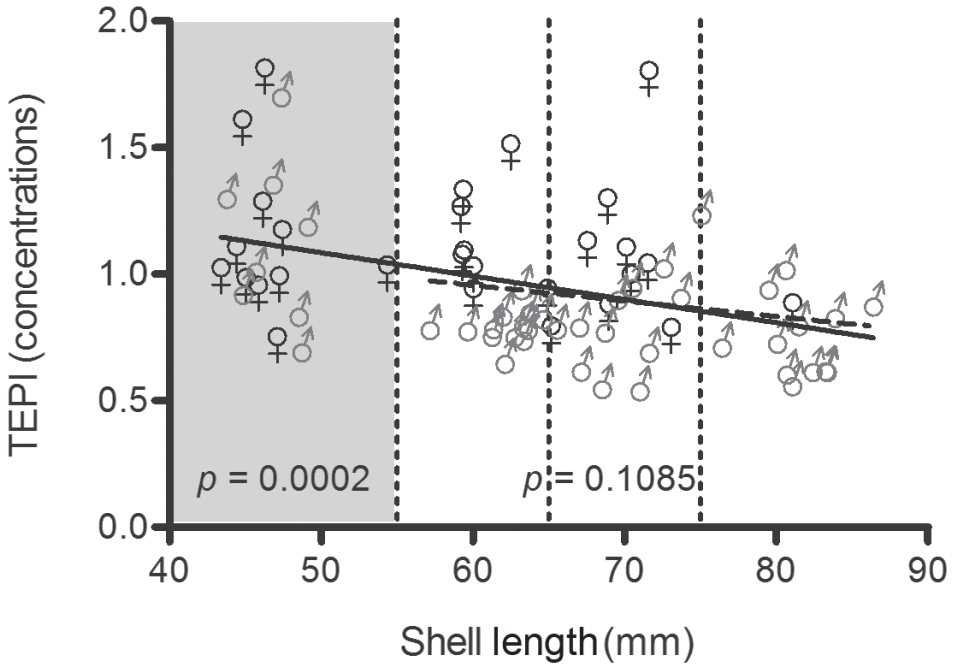

Fig. 3 – Linear regressions modelling the relationship between *Mytilus galloprovincialis* shell length and Trace Element Pollution Index (TEPI) values (no unit), calculated from mean normalized concentrations of the 19 studied trace elements (TEs), all mussels together (n = 74, shaded area included; full regression line) or restricted to mussels larger than 55 mm (n = 55, shaded area excluded; dashed regression line). Mussels were purchased before they spawned. ♀ and ♂ symbolize females and males, respectively. The dotted vertical lines separate mussels into 4 equivalent size-classes of about 10 mm. Model *p*-values (significant for all mussels together only) are given on the graph.

out, as was the effect of the sex of mussels close to spawning. Finally, the conservative character of TE compartmentalization regardless of the physiological status of sampled mussels, and their proportional redistribution between tissues, were suggested to rely on some internal regulatory processes that require further investigations.

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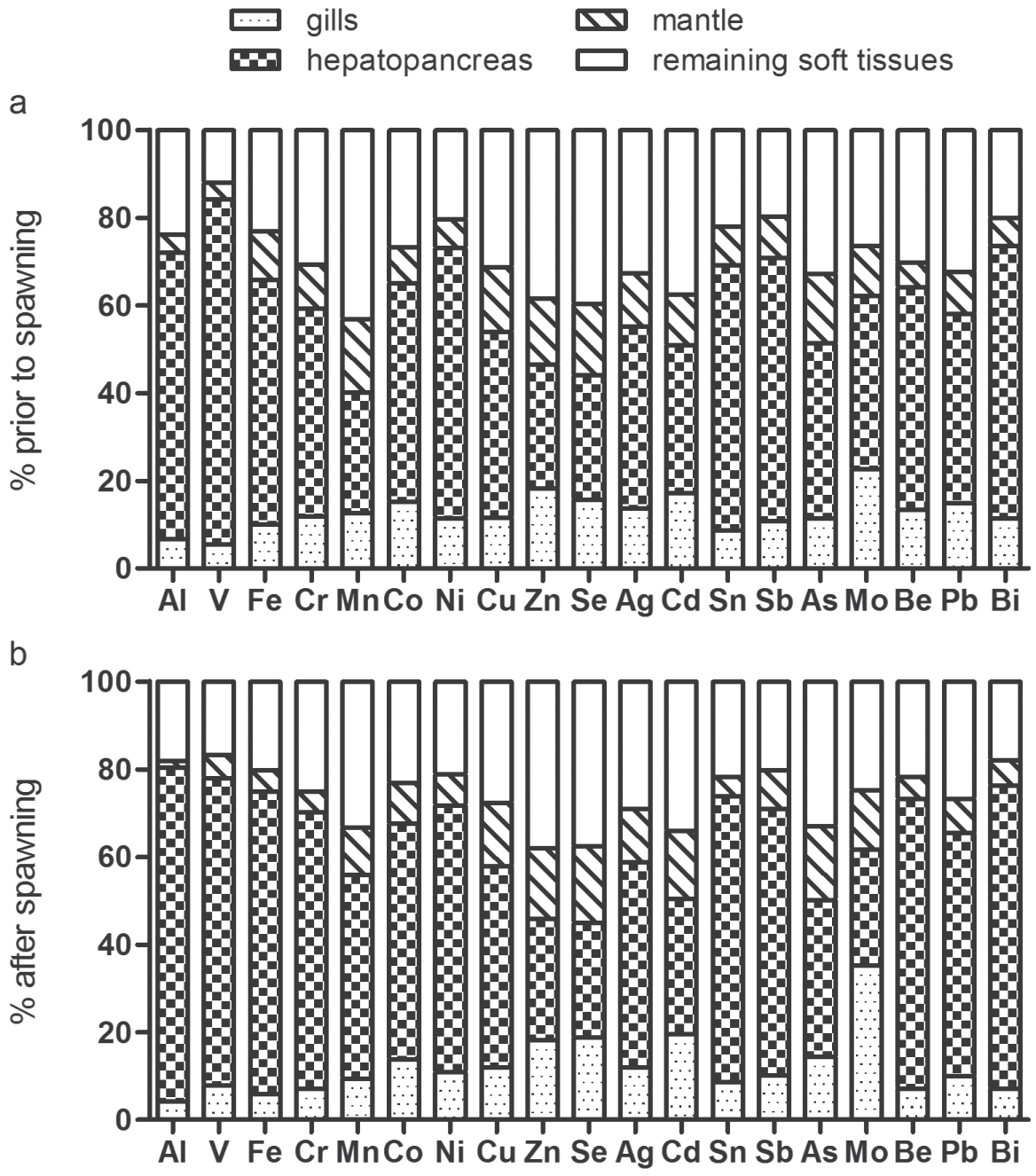

Fig. 4 – Proportional distribution of trace elements between main body compartments (gills, hepatopancreas, mantle and remaining soft tissues; in % of total contents) of *Mytilus galloprovincialis*. Mussels were purchased (a) a few days before they spawned (n = 20) and (b) a few days after they spawned (n = 20), at one-year interval.

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