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## Trace elements in a Mediterranean scorpaenid fish: Bioaccumulation processes and spatial variations

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### ABSTRACT

The black scorpionfish *Scorpaena porcus*, a carnivorous sedentary species, was studied as an indicator of local trace element (TE) contamination of coastal fish species in the northwestern Mediterranean Sea. Fish were collected in seagrass meadows in winter 2012 at four sampling sites: three were located near Marseille, a highly urbanized and industrial area that is subjected to various types of contaminant inputs, and one was located near Hyères, a less urbanized area. Size, sex, age and diet of fish were determined before stable isotope and TE analyses. C and N isotopic compositions and concentrations of nine TEs (As, Ba, Cd, Cu, Cr, T-Hg, Ni, Pb, and Zn) were determined in dorsal white muscle. Most TEs did not bioaccumulate in *S. porcus*, as no increase in TE concentrations with increasing size, age or trophic level of fish was evident. Total mercury (T-Hg) was the only element to bioaccumulate and biomagnify, displaying strong positive correlations of its concentration with age ( $r = 0.88$ ,  $p < 0.01$ ), length ( $r = 0.84$ ,  $p < 0.01$ ) and  $\delta^{15}\text{N}$  value ( $r = 0.68$ ,  $p < 0.01$ ). Compared with the reference site, higher concentrations of As, Ba, Cd, T-Hg, Pb, and Zn were recorded in fish collected at one or all of the Marseille site. In contrast, no difference was observed for Cr, Cu, and Ni concentrations with site. Spatial differences in TE concentrations in *S. porcus* were related to differences in isotopic composition and to the primary environmental characteristics and human activities prevailing at each site. Concentrations of Cd, T-Hg and Pb in fish muscle (priority substances for monitoring) were all below the maximum permitted European values.

### 1. Introduction

Coastal marine environments represent high value eco-socio-systems, but they are also discharge and accumulation areas of anthropogenic compounds, such as trace elements (TEs) (Matthai et al., 2002; Cobelo-García et al., 2004). Such chemical contamination leads to the alteration of marine ecosystems, with an impact at individual, species, population and community levels (Fleeger et al., 2003; Halpern et al., 2008; Tartu et al., 2013). The Mediterranean Sea being a semi-enclosed sea, it is predicted to be a particularly sensitive system to any change in its hydrographic conditions due to either climatic or anthropogenic forcings (Bethoux et al., 1999; The MERMEX Group, 2011). The biogeochemical cycling of TEs at the scale of the Mediterranean Sea is largely governed by atmospheric inputs and air-water exchanges (Migon et al., 2002; Heimbürger et al., 2010; The MERMEX Group, 2011). However, in coastal zones and on continental shelves, rivers, such as the Rhône River in the Gulf of Lions, constitute the main input

of terrigenous dissolved organic carbon (DOC) (Sempéré et al., 2000) and particulate metals (Elbaz-Poulichet, 2005; Cathalot et al., 2013; Cossa et al., this issue). In addition, TE input sources include submarine volcanoes, cold seepages and sub-marine freshwater inflows, as well as inputs from large coastal cities, industries, effluents, runoff and marine traffic (Oursel et al., 2013).

Certain TEs have the capability to bioaccumulate within aquatic organisms from the environment because of their persistent nature, chemical speciation and bioavailability (MacDonald et al., 2002; Castro-González and Méndez-Armenta, 2008; The MERMEX Group, 2011, Vieira et al., 2011). Certain TEs have unknown functions in biological systems and are considered to be potentially toxic to organisms at low concentrations, such as barium (Ba), cadmium (Cd), total mercury (T-Hg) and lead (Pb) (Schroeder and Darrow, 1972; Velusamy et al., 2014). Other TEs are essential, such as arsenic (As), copper (Cu), chromium (Cr), nickel (Ni), and zinc (Zn), as they play a specific role in metabolism (Authman et al., 2012; Bosch et al., 2016) and are required

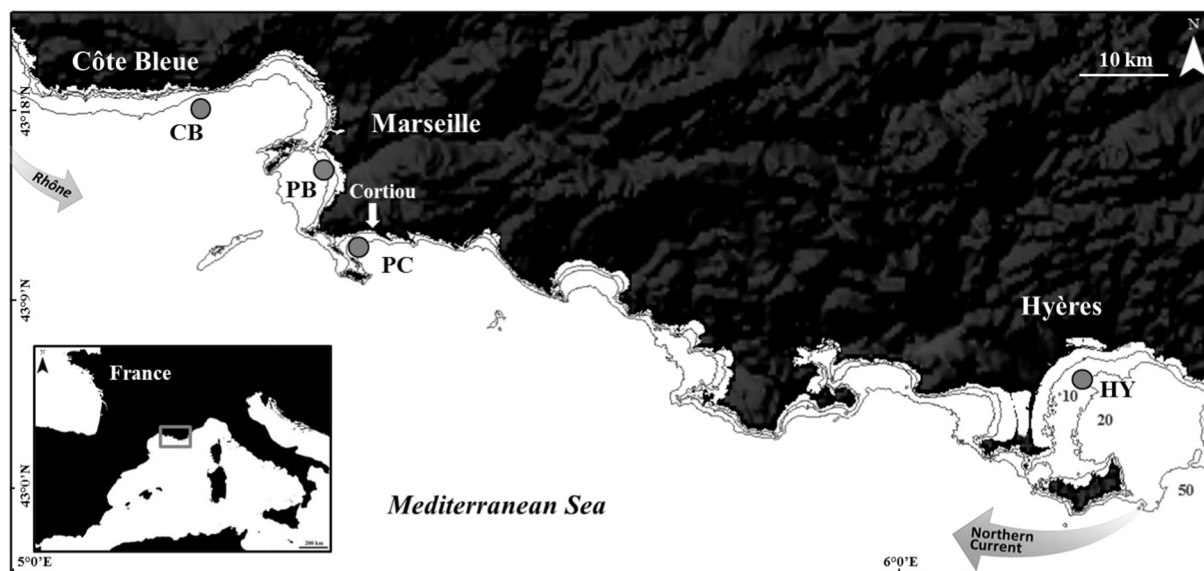


Fig. 1. Location of the sampling sites along the northwestern Mediterranean Sea (gray points) (CB: Côte Bleue; PB: Prado Bay; PC: Plateau des Chèvres; and HY: Hyères). Cortiou: location of Marseille sewage output. Bathymetric data from SHOM, 2010.

by living organisms in small concentrations to achieve a normal ontogenetic development (Merciai et al., 2014). However, essential TEs may also have toxic effects at elevated exposure levels (Wood, 2012; Vrhovnik et al., 2013). The sorption of TEs is affected by chemical (e.g., chemical speciation; Vrhovnik et al., 2013), environmental (e.g., pH, temperature and turbidity; Castro-González and Méndez-Armenta, 2008) and biological factors. Contaminants can bioaccumulate during the life of organisms (increases with size or age) and biomagnify with increasing trophic level, but different patterns are observed depending on the contaminant and the organism (Harmelin-Vivien et al., 2012). While the contamination of fish by TEs can come directly from the water through respiration or directly through the skin and membranes, the main way of TE inputs into fish is food through the consumption of contaminated prey (Hall et al., 1997). Bioaccumulation in fish is a complex process that depends on various biological parameters (species, size, age, sex, diet, trophic level and metabolism) (Castro-González and Méndez-Armenta, 2008; Harmelin-Vivien et al., 2012; Wood, 2012), and differs among tissues due to differences in TE absorption, detoxification and storage mechanisms (Has-Schön et al., 2006; Storelli, 2008; Metian et al., 2013). Contaminants, such as TEs, emerged as useful ecotracers of trophic patterns and environmental parameters when used in combination with other chemical tracers, such as C and N isotopic compositions (Dierking et al., 2009; Chouvelon et al., 2014; Cresson et al., 2014b, 2015b), as they provide complementary time- and space-integrated information on bioaccumulation events and trophic positions of fish in the food web.

Environmental monitoring programs for coastal ecosystems are developing in the Mediterranean Sea for providing scientific knowledge in the assessment of the health and sustainability of ecosystems (Tomasello et al., 2012; Copat et al., 2013; Miniero et al., 2014; Personnic et al., 2014; Naccari et al., 2015; Bonito et al., 2016). Studies carried out on TE bioconcentration and/or biomagnification in Mediterranean species investigated magnoliophytes (Pergent-Martini, 1998; Pergent and Pergent-Martini, 1999; Lafabrie et al., 2007, 2009), invertebrates (Perez et al., 2005; Lafabrie et al., 2007; Angeletti et al., 2014), and fish (Canli and Atli, 2003; Copat et al., 2012, 2013). However, most studies on fish concern continental shelf species and are focused on Hg contamination (Harmelin-Vivien et al., 2009, 2012; Cossa et al., 2012; Cresson et al., 2014a, 2015a, 2015b). Thus, there is a lack of knowledge on TE concentrations in fish species living in coastal waters of the northwestern Mediterranean Sea (Uluozlu et al., 2007).

In France, the annual per capita fish consumption ranges from 30 to

60 kg (FAO, 2012; Vieira et al., 2015) and seafood has always been an important part of the diet of Mediterranean populations (Faget, 2009). Given the potential deleterious effects to human health related to the consumption of contaminated fish (Domingo et al., 2007; Martorell et al., 2011; Vieira et al., 2011), it is crucial to better understand the processes of TE accumulation in Mediterranean coastal fishes and to evaluate the subsequent risk due to fish consumption. The black scorpionfish, *Scorpaena porcus* Linnaeus, 1758, while not the most consumed species, is largely used in traditional recipes (fish soup, fried scorpionfish fillets and the famous “bouillabaisse”) and is a common component of local fisheries all year round (Leleu et al., 2014). This species is common in all of the Mediterranean Sea between 5 and 30 m depth, but may be occasionally recorded deeper. This a sedentary macrocarnivore living on rocky substrates and seagrass beds (Harmelin-Vivien et al., 1989). It plays an important role in the trophic functioning of these ecosystems, being one of the most common predators and accounting for 18–35% of the total fish biomass (Bell and Harmelin-Vivien 1982; Ourgaud et al., 2015). For these characteristics, *S. porcus* could be used as an indicator of TE contamination in Mediterranean coastal fish species.

Thus, the aim of the present study was to provide some baseline information on the concentration levels of different TEs (As, Ba, Cd, Cr, Cu, Ni, T-Hg, Pb, and Zn) susceptible to revealing anthropic influences in coastal marine fishes or being a threat to health security. For this purpose, *S. porcus* was collected from different locations subjected to different degrees of anthropic inputs. We used a multi-tracer approach (TEs, C and N stable isotopes, stomach contents and otolith analyses) and analyzed muscles that are the main portion of the fish eaten by consumers. The main questions addressed are (1) Did TE concentrations differ among sampling sites and were they related to differences in the biological parameters of fish or characteristics of the environment? (2) Were these differences similar for all TEs analyzed? (3) Could bioaccumulation and/or biomagnification processes be evident for some TEs? (4) Are TE concentrations recorded below European regulations for fish flesh commercialization?

## 2. Materials and methods

### 2.1. Study area and sample preparation

This study was carried out in *Posidonia oceanica* seagrass meadows in three sampling sites of the Bay of Marseille, in the vicinity of one of

the largest cities on the Mediterranean Sea, and in one site of the Gulf of Hyères (HY), a less urbanized or industrialized area, considered as a reference site (Fig. 1). The three Marseille sites were subjected to different anthropic and freshwater inputs: Côte Bleue (CB), located in the northern area of the bay and not directly influenced by Marseille urban sewage, but sporadically exposed to the influence of Rhône River waters (Petrenko et al., 2005); Prado Bay (PB), subjected to episodic inputs from the small coastal Huveaune River and to constant urban and harbor inputs; and Plateau des Chèvres (PC), located in the southern area of the bay close to the outlet of the Cortiou wastewater treatment plant, and thus directly exposed to nutrient-rich urban sewage (Bellan, 1970; Arfi et al., 2000).

Fish were sampled by fishermen operating a small beam trawl (1.5 × 0.5 m, 8 mm stretched mesh size) (Harmelin-Vivien, 1981) during both day and night as diel differences occur in the activity rhythms, feeding behavior and position of fish associated with *P. oceanica* seagrass meadows (Harmelin-Vivien, 1982). Five trawls of 10 min duration at a constant towing speed of 2.8 km h<sup>-1</sup> (combined surface area covered of 3500 m<sup>2</sup>) were carried out between 8 and 18 m depth. A total of 588 individuals of *S. porcus* were collected. Once caught, fish were immediately stored on ice in polyethylene bags. In the laboratory, fish were measured to the nearest mm (total length, TL) before dissection and sex determination. Dissections were performed on an acid (10% HCl) cleaned glass worktop with ceramic scalpels and forceps, wearing powder free single use nitrile gloves. All instruments were cleaned with 10% HCl, rinsed with Milli-Q water between each sample and dried at 100 °C for 2 h. Dorsal white muscle samples of fish without skin were individually put into polyethylene bags and kept frozen at -20 °C until analysis. The number of fish processed per type of analysis is given in Table 1.

## 2.2. Stomach content analysis

Stomach contents were removed and stored in 95% ethanol before analysis. As ontogenic variations of diet are high in *S. porcus* (Harmelin-Vivien et al., 1989), individuals were separated into three size/age classes (see Section 2.1). Prey items in stomachs were sorted under a stereo microscope to the lowest possible taxonomic group and counted. The different prey categories were grouped according to fish size class, sampling site and period (day or night). Prey items were dried for 24 h at 50 °C and weighed (± 0.01 mg dry weight, dw). The importance of prey categories in the *S. porcus* diet was assessed by their percentage by number (%N), percentage by weight based on digested non-reconstituted prey weight (%W), percentage of occurrence (%O) and index of relative importance (%IR; Pinkas et al., 1971), calculated as % IRI = (IRI/Σ IRI) × 100, where IRI = %O × (%N + %W).

## 2.3. Otolith analysis

The age (in years) of the individuals was estimated by the otolith analysis of individuals covering all size/age classes. Sagittal otoliths

**Table 1**

Number of individuals of the black scorpionfish *Scorpaena porcus* by sampling site (CB: Côte Bleue; PB: Prado Bay; PC: Plateau des Chèvres; and HY: Hyères), size/age class (C1: juveniles, < 70 mm total length (TL), aged 0+; C2: sub-adults 71–130 mm TL, 1–3 years; C3: and adults, > 131 mm TL, 3–6 years), and sex (U: undetermined; F: female; and M: male) for analyses of stomach contents, isotopic compositions (C and N) and trace elements (TEs).

	Site				Class			Sex		
	CB	PB	PC	HY	C1	C2	C3	U	F	M
Stomach contents	78	110	209	191	45	458	85	128	234	226
Stable isotopes	18	16	13	21	17	34	17	34	18	16
Trace elements	7	16	19	77	40	72	7	64	30	25

were removed, cleaned, weighed (± 0.1 mg) and their length (± 0.1 mm) was measured. As weight and length did not differ between right and left otoliths (Wilcoxon tests,  $p > .05$ ), the left otolith was arbitrarily chosen for age reading. Sagittae were immersed in distilled water and observed by transparency using a stereo microscope under reflected light without any additional preparation (Fabien Morat, pers. comm.). Reading was done from the nucleus to the anterior extremity of the rostrum by counting annual annuli. One annulus was the combination of an opaque mark and a hyaline mark, as already observed in other Scorpaenidae (Massutí et al., 2000; La Mesa et al., 2005; Demirhan and Can, 2009; Scarcella et al., 2011). Each otolith was analyzed by three different observers to obtain the fairest estimate of age. Correlation was established between the total otolith length (T<sub>oL</sub>, mm) and the age of the individuals (years).

## 2.4. Isotopic and trace element analyses

Samples of dorsal white muscle were freeze-dried and ground to a fine powder before analysis (Pinnegar and Polunin, 1999). Measurements of C and N isotopic compositions were performed at the individual level with an elemental analyzer (Flash EA 1112, Thermo Scientific, Milan, Italy) coupled with an isotope ratio mass spectrometer (Delta V Advantage with a ConFlo IV interface, Thermo Scientific, Bremen, Germany) at the LIENSs stable isotope facility of the University of La Rochelle, France. Briefly, samples of 0.40 ± 0.01 mg of freeze-dried powder were weighed and sealed in tin capsules for analysis. The results are expressed following the delta (δ) notation, as deviations from standards (Vienna Pee Dee Belemnite for δ<sup>13</sup>C, N<sub>2</sub> in air for δ<sup>15</sup>N):  $\delta X (\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$ , where X is <sup>13</sup>C or <sup>15</sup>N and R is <sup>13</sup>C/<sup>12</sup>C or <sup>15</sup>N/<sup>14</sup>N, respectively. Analytical precision, based on the analyses of acetanilide (Thermo Scientific), used as laboratory internal standard was < 0.15‰ for carbon and nitrogen. Calibration was done using reference materials (USGS-24, IAEA-CH6, -600 for carbon; and IAEA-N2, -NO-3, -600 for nitrogen). The C/N ratio provided by the elemental analyzer was used as a proxy of the lipid content. A value of the C/N ratio lower than 3.7 indicates that the lipid content is low and does not affect the isotopic composition of carbon (Sweeting et al., 2006). The δ<sup>15</sup>N value of individuals was used as a proxy of their trophic position, as commonly done (Newsome et al., 2007; Cossa et al., 2012; Sebastiano et al., 2016).

The analysis of As, Ba, Cd, Cr, Cu, T-Hg, Ni, Pb and Zn in fish was performed at the individual level for the biggest fish and on pools of individuals (from the same site, size/age class and sex) for the smallest ones. The required mineralization of samples was performed on aliquots of ~250 mg digested in 3 mL (for masses lower than 100 mg) or 5 mL (for masses higher than 100 mg) of a 3:1 (v:v) mixture of 65% HNO<sub>3</sub> (6 mL) and 37% HCl (2 mL) (Merck Suprapur® quality). Acid mineralization was performed overnight, at room temperature, and then in a microwave for 30 min with increasing temperature up to 120 °C, and 15 min at 120 °C (1200 W). After the mineralization process, each sample was diluted to 50 mL with Milli-Q water, according to the volume of acid added to the mineralization. Elements were analyzed using an ICP-OES (Vista-Pro, Varian, Mulgrave, Victoria Australia) or an ICP-MS (X SeriesII, Thermo Fisher Scientific, Bremen, Germany). T-Hg does not require a mineralization step and so it was directly analyzed from the dried samples by atomic absorption spectrometry with an Advanced Mercury Analyzer (AMA 254, Altec, Prague, Czech Republic) as described by Bustamante et al. (2006).

Precision and repeatability of TE analyses were assessed with certified reference materials (dogfish liver DOLT-4 and lobster hepatopancreas TORT-2; NRCC), treated and analyzed in the same way as the samples. The results were in good agreement with the certified values, and the relative standard deviations were always below 15%, proving good repeatability of the method. Blanks were tested in each analytical batch. All element concentrations were given on a dry weight basis (μg g<sup>-1</sup> dw). The detection limits (μg g<sup>-1</sup>) were 0.0008 (T-Hg), 0.003 (Cd

and Pb), 0.01 (Cr, Cu, and Ni), 0.03 (As and Ba), and 0.52 (Zn).

## 2.5. Statistical analyses

Spatial differences in diet composition were tested using Spearman's rank correlations coefficient on the %IRI of prey categories. Correlations between size and carbon and nitrogen isotopic compositions in fish muscle were assessed using generalized linear models (GLMs; Legendre and Legendre, 2012). GLMs were also used to analyze the correlation between fish size/age classes  $\delta^{15}\text{N}$  values or TE concentrations.

The variability in size, age, isotopic compositions and TE levels were analyzed independently using a univariate PERMANOVA (Anderson, 2001). The PERMANOVA routine was performed for testing the simultaneous responses of one ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and each TE) or more (nine TEs) variables to all factors (site, class and sex) in an analysis of variance (ANOVA and MANOVA) based on an experimental design of any resemblance measure using permutation methods (Anderson, 2001; McArdle and Anderson, 2001; Anderson et al., 2008). The variables were normalized prior to analyses. Euclidean distance similarity matrices were generated. This method is equivalent to a parametric MANOVA, but free from the assumptions of normality and the homoscedasticity of residuals (Anderson, 2001). *P*-values were obtained using 9999 random unrestricted permutations of raw data and Type I sums of squares. Monte Carlo *P*-values were considered when there were not enough possible permutations (< 2000). Significance was set at *P*-value less than 0.05 (\* = 0.05, \*\* = 0.01, and \*\*\* = 0.001). The differences in mean TE concentrations between site, class and sex were described by a non-metric Multi-Dimensional Scaling ordination (nMDS; Clarke 1993; Clarke and Gorley 2006) calculated by the dissimilarity matrix among samples on the basis of the binomial dissimilarity measure (Anderson, 2001). If the stress levels from 2-dimensional ordination plots were lower than 0.2, the analyses were validated (Clarke 1993). Then, correlation vectors were calculated with the Spearman rank correlation index. Lastly, a multivariate PERMANOVA (Anderson, 2001) was performed, based on the binomial deviance (scaled) dissimilarity measures. Analyses were performed using PRIMER 6 software (6.1.16) and the PERMANOVA add-on (1.06) (Plymouth Marine Laboratory, Plymouth, United Kingdom) (Clarke and Warwick, 2001).

## 3. Results

### 3.1. Size, age and sex ratio

A total of 588 individuals were sampled (78 at CB, 110 at PB, 209 at PC, and 191 at HY). Fish were classified into three classes corresponding to three age and size classes: (C1) juveniles (aged 0+, < 70 mm TL); (C2) sub-adults (1–3 years, 71–130 mm TL); and (C3) adults (3–6 years, > 131 mm TL) (Table 2). The average total length for all individuals was 109 mm ( $\pm$  29 mm) for a mean age of 2 years ( $\pm$  1 year). A significant difference among sampling sites was observed, with larger and older individuals at HY than at the three Marseille (i.e., PB, CB, PC) sites (PERMANOVA,  $F = 14.19$ ,  $p < .001$ ,  $\text{HY} > \text{PB} = \text{CB} = \text{PC}$ , for TL; and PERMANOVA,  $F = 17.25$ ,  $p < .001$ ,  $\text{HY} > \text{PB} = \text{PC} = \text{CB}$ , for age). The sex ratio was well balanced (51% females; 49% males) and independent of sampling site (PERMANOVA,  $F = 1.28$ ,  $p > .05$ ). Fish with indeterminate sex represented 20% of samples, with the predominance of juvenile individuals in the smallest size/age class.

### 3.2. Diet

Out of the 588 individuals analyzed, 103 had empty stomachs (27%) and 18 contained prey items at an advanced state of decomposition (3%) that did not allow correct taxonomic identification. The results were therefore obtained from the analysis of 467 stomach

**Table 2**

Mean ( $\pm$  SD) size and age of *Scorpaena porcus* according to sampling site and size/age class.

Site	Class	Total length (mm)	Age (year)
Côte Bleue (CB)	Juveniles	54 $\pm$ 12	0 <sup>+</sup>
	Sub-adults	105 $\pm$ 19	1.58 $\pm$ 0.79
	Adults	160 $\pm$ 27	3.67 $\pm$ 1.15
Prado Bay (PB)	Juveniles	41 $\pm$ 5	0 <sup>+</sup>
	Sub-adults	86 $\pm$ 20	1.14 $\pm$ 0.69
	Adults	161 $\pm$ 8	3.75 $\pm$ 0.50
Plateau des Chèvres (PC)	Juveniles	51 $\pm$ 10	0 <sup>+</sup>
	Sub-adults	100 $\pm$ 14	1.40 $\pm$ 0.55
	Adults	159 $\pm$ 21	4.00 $\pm$ 0.82
Hyères (HY)	Juveniles	67 $\pm$ 2	0.20 $\pm$ 0.45
	Sub-adults	101 $\pm$ 10	1.50 $\pm$ 0.53
	Adults	152 $\pm$ 25	3.67 $\pm$ 1.03

contents. Diet composition differed significantly between size/age classes (Spearman  $r_s$ , all  $p < .05$ ), indicating a gradual change in *S. porcus* diet with the growth of individuals and an increase in mean prey size (Table 3). Juveniles preyed mainly on small crustaceans, such as small Caridea, isopods and amphipods, while subadults and adults consumed more diversified and larger prey, such as Brachyura and Teleostei. However, Caridea remained the principal prey in all size/age classes. Subadults consumed less Isopoda and Amphipoda and more Brachyura, Galatheididae and Teleostei than juveniles. The diet of adults mainly consisted of Caridea, Teleostei and Brachyura. If the prey categories consumed by *S. porcus* were the same at all sampling sites, with Caridea always preferentially consumed, their relative proportions in the fish diet differed significantly among sites, with individuals from PB and PC differing from those of CB and HY. *S. porcus* from PB and PC presented similar diets ( $r_s = 0.79$ ,  $p > .05$ ), with higher proportions of Caridea (65.0 and 81.7%IRI, respectively) and Amphipoda (14.0 and 10.0% IRI, respectively) consumed, than at CB and HY (Caridea: 33.2 and 57.4%IRI, respectively; and Amphipoda: < 3% IRI). In contrast, the diet of *S. porcus* was similar at CB and HY ( $r_s = 0.71$ ,  $p > .05$ ), where the species ingested higher proportions of Isopoda (15.5% and 25.9% IRI, respectively), Galathea (> 3.0%IRI) and Teleostei (8.7 and 3.0% IRI, respectively), than at PB and PC (Isopoda: < 2.0%IRI; Galathea: < 1.0%IRI; and Teleostei: < 2.0%IRI).

### 3.3. Stable isotope compositions

In this study, the mean C/N ratio of *S. porcus* muscle was  $3.2 \pm 0.1$  (Table 4), so no correction was applied on  $\delta^{13}\text{C}$  values for lipids. A wide range of  $\delta^{13}\text{C}$  values was observed among all *S. porcus* analyzed (from  $-18.8$  to  $-16.5\text{‰}$ ,  $\Delta \delta^{13}\text{C} = 2.3\text{‰}$ ) and  $\delta^{15}\text{N}$  values (from 6.4 to 12.0‰,  $\Delta \delta^{15}\text{N} = 5.6\text{‰}$ ). Such variations were related to differences among both sites and size/age classes (Fig. 2). Carbon isotopic compositions were significantly less enriched for individuals collected at PC and PB than at CB and HY (PERMANOVA,  $F = 37.43$ , for  $\delta^{13}\text{C}$ ;  $p < .001$ ), and  $\delta^{15}\text{N}$  values were significantly lower at HY than at CB, PB and PC (PERMANOVA,  $F = 57.74$ , for  $\delta^{15}\text{N}$ ,  $p < .001$ ) (Table 4). In addition, juveniles exhibited significantly lower  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values than adults, regardless of the site (PERMANOVA,  $F = 4.79$ ,  $p < .01$ , for  $\delta^{13}\text{C}$ ;  $F = 21.06$ ,  $p < .001$  for  $\delta^{15}\text{N}$ ). Significant correlations between length and  $\delta^{15}\text{N}$  values were observed at CB ( $R^2 = 0.55$ ,  $p < .001$ ), PB ( $R^2 = 0.34$ ,  $p < .05$ ), PC ( $R^2 = 0.64$ ,  $p < .01$ ) and HY ( $R^2 = 0.69$ ,  $p < .001$ ), while all correlations between TL and  $\delta^{13}\text{C}$  values were not significant ( $p > .05$  at all sampling sites) (Fig. 3).

### 3.4. TE concentrations

All TEs analyzed were found in varying concentrations (Table 5). Ranges of detected concentrations for the nine elements quantified in *S.*

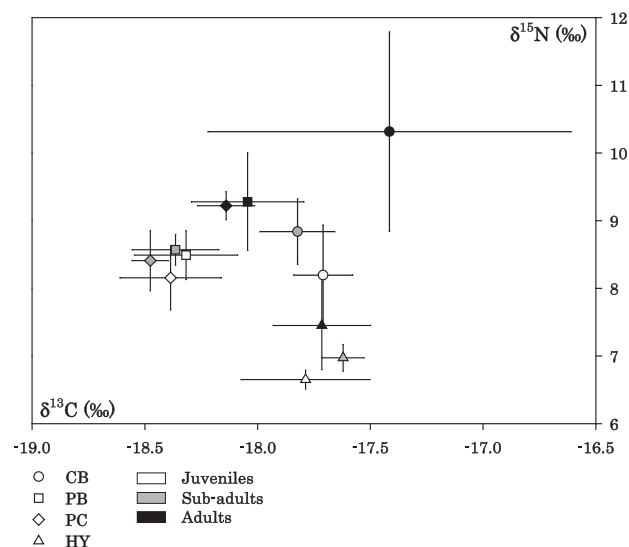
**Table 3**

Diet composition of *Scorpaena porcus* according to size/age classes. Dietary indices: % frequency of occurrence (%FO), % by number (%N), % by dry weight (%W) and % index of relative importance (%IRI) of each prey category. Others: Stomatopoda, Mollusca, crustacean remains and particulate organic matter. Numbers of stomachs with prey are indicated in brackets.

Prey items	Juveniles (43)				Sub-adults (363)				Adults (79)			
	%FO	%N	%W	%IRI	%FO	%N	%W	%IRI	%FO	%N	%W	%IRI
Crustacea												
Amphipoda	25.0	22.2	5.1	15.6	9.5	7.5	1.4	3.6	0.6	0.5	< 0.1	< 0.1
Isopoda	26.3	22.8	25.4	24.5	12.9	10.3	4.9	7.3	7.6	7.8	2.8	4.2
Caridea	40.8	48.7	65.1	57.6	35.4	46.8	42.2	56.6	31.3	38.4	26.4	40.9
Brachyura	–	–	–	–	8.8	8.2	19.3	9.4	19.8	16.5	31.7	17.8
Galathea	1.2	0.7	2.5	0.5	8.1	6.0	13.0	7.4	7.9	7.2	3.6	4.0
Annelida	1.6	1.6	0.8	0.5	1.9	2.3	0.5	0.3	0.6	0.5	< 0.1	< 0.1
Teleostei	–	–	–	–	4.2	2.8	11.0	4.8	10.8	10.3	29.5	19.2
Others	5.0	4.0	1.0	1.4	19.3	16.0	7.8	10.7	21.9	19.0	6.0	13.7

*porcus* muscles were as follows: (min–max values): 11.15–144.50 µg As g<sup>-1</sup> dw; 0.18–0.37 µg Ba g<sup>-1</sup> dw; 0.02–0.04 µg Cd g<sup>-1</sup> dw; 0.15–8.73 µg Cr g<sup>-1</sup> dw; 0.35–1.04 µg Cu g<sup>-1</sup> dw; 0.24–2.19 µg T-Hg g<sup>-1</sup> dw; 0.10–4.69 µg Ni g<sup>-1</sup> dw; 0.02–0.07 µg Pb g<sup>-1</sup> dw; and 13.01–76.56 µg Zn g<sup>-1</sup> dw. The nMDS ordination revealed a large variation of element concentrations in the muscle of *S. porcus* among sampling sites and size classes of individuals (PERMANOVA, F = 12.50, p < .05, for site; F = 19.37, p < .05, for class) (Fig. 4a and b).

As, Ba, Cd, T-Hg, Pb, and Zn concentrations displayed significant differences among the four sampling sites (Table 5, Fig. 5). Pair-wise tests showed that the highest TE concentrations were not always measured in the same site. As concentration was significantly higher at PC than at the three other sites. Higher Ba concentrations were found at CB and PB than PC and HY, while the highest T-Hg concentrations were recorded in the Bay of Marseille at PB and PC. Concentrations of Cd, Pb and Zn were all higher at the three Marseille sites than at HY. Cr, Cu and Ni were the only elements that did not statistically differ in concentration with site. TE concentrations also displayed significant differences among fish size classes, except for Cr, Ni and Zn (PERMANOVA, F = 16.73, p < .05 for As; F = 103.82, p < .01 for Ba; F = 5.85, p < .01 for Cd; F = 26.62, p < .05 for Cu; F = 2115.00, p < .01 for T-Hg; and F = 1408.40, p < .01 for Pb), while sex had no effect on TE concentrations (results not shown, PERMANOVA, F = 5.46, p > .05). Pair-wise tests revealed that adults always presented the highest concentrations in As and T-Hg (Fig. 4). In contrast, Ba, Cd, Cu and Pb concentrations were significantly higher in either juveniles or in sub-adults. The potential trophic transfer of TEs was estimated using the relationships between trace element concentrations and δ<sup>15</sup>N values of individuals. Generally, a significant positive correlation between them indicates that the element is biomagnified through the food chain, whereas a significant negative correlation suggests that



**Fig. 2.** C and N isotopic compositions (‰) in the muscle of *Scorpaena porcus* according to sampling site (○ CB: Côte Bleue; □ PB: Prado Bay; ◇ PC: Plateau des Chèvres; and △ HY: Hyères) and size/age classes of individuals (white: juveniles < 70 mm total length, aged 0+; gray: sub-adults 71–130 mm, 1–3 years; and black: adults > 131 mm, 3–6 years).

biodiminution has occurred. In the present study, for all individuals combined, significant correlations between age, size and δ<sup>15</sup>N values were observed for T-Hg (r = 0.88, p < .001, r = 0.84, p < .001, and r = 0.68, p < .01, respectively), but not for the eight other TEs analyzed. Positive, but not significant, correlations (p > .05) were observed for As and negative, but not significant, correlations (p > .05) for the other TEs.

**Table 4**

C and N isotopic compositions and C/N (Mean ± SD) of *Scorpaena porcus* muscles according to sampling site and class.

Site	Class	Total length (mm)	Age (year)	δ <sup>13</sup> C (‰)	δ <sup>15</sup> N (‰)	C/N
Côte Bleue (CB)	Juveniles	54 ± 12	0 <sup>+</sup>	-17.7 ± 0.1	8.2 ± 0.7	3.2 ± < 0.1
	Sub-adults	105 ± 19	1.58 ± 0.79	-17.8 ± 0.2	8.8 ± 0.5	3.2 ± < 0.1
	Adults	160 ± 27	3.67 ± 1.15	-17.4 ± 0.8	10.3 ± 1.5	3.2 ± < 0.1
Prado Bay (PB)	Juveniles	41 ± 5	0 <sup>+</sup>	-18.3 ± 0.2	8.5 ± 0.4	3.2 ± < 0.1
	Sub-adults	86 ± 20	1.14 ± 0.69	-18.4 ± 0.2	8.6 ± 0.2	3.2 ± < 0.1
	Adults	161 ± 8	3.75 ± 0.50	-18.0 ± 0.3	9.3 ± 0.7	3.1 ± < 0.1
Plateau des Chèvres (PC)	Juveniles	51 ± 10	0 <sup>+</sup>	-18.4 ± 0.2	8.2 ± 2.9	3.2 ± < 0.1
	Sub-adults	100 ± 14	1.40 ± 0.55	-18.5 ± 0.1	8.4 ± 0.4	3.2 ± < 0.1
	Adults	159 ± 21	4.00 ± 0.82	-18.1 ± 0.1	9.2 ± 0.2	3.2 ± 0.1
Hyères (HY)	Juveniles	67 ± 2	0.20 ± 0.45	-17.8 ± 0.3	6.7 ± 0.1	3.2 ± < 0.1
	Sub-adults	101 ± 10	1.50 ± 0.53	-17.6 ± 0.1	7.0 ± 0.2	3.2 ± < 0.1
	Adults	152 ± 25	3.67 ± 1.03	-17.7 ± 0.2	7.5 ± 0.7	3.2 ± 0.1

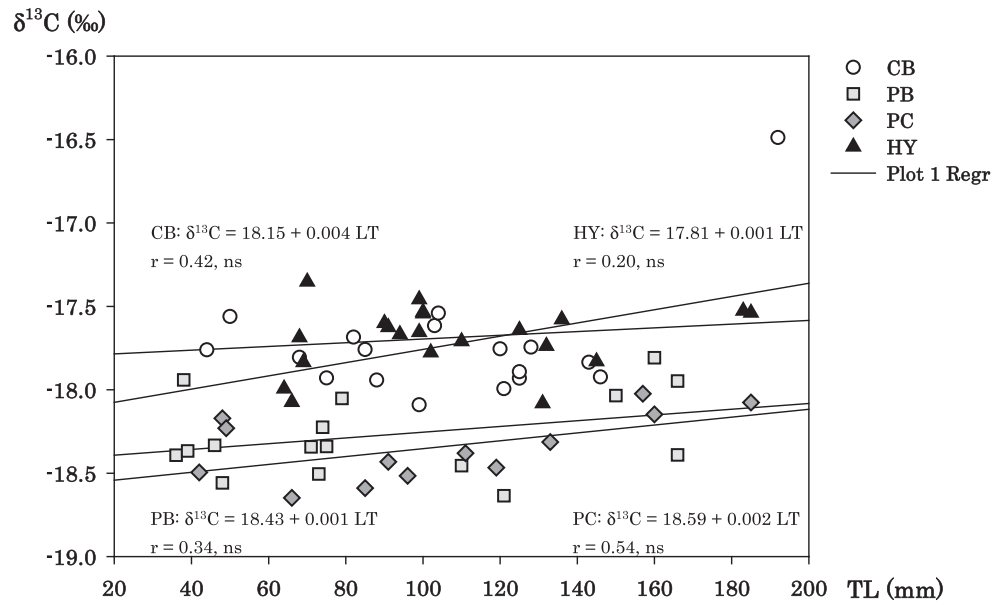
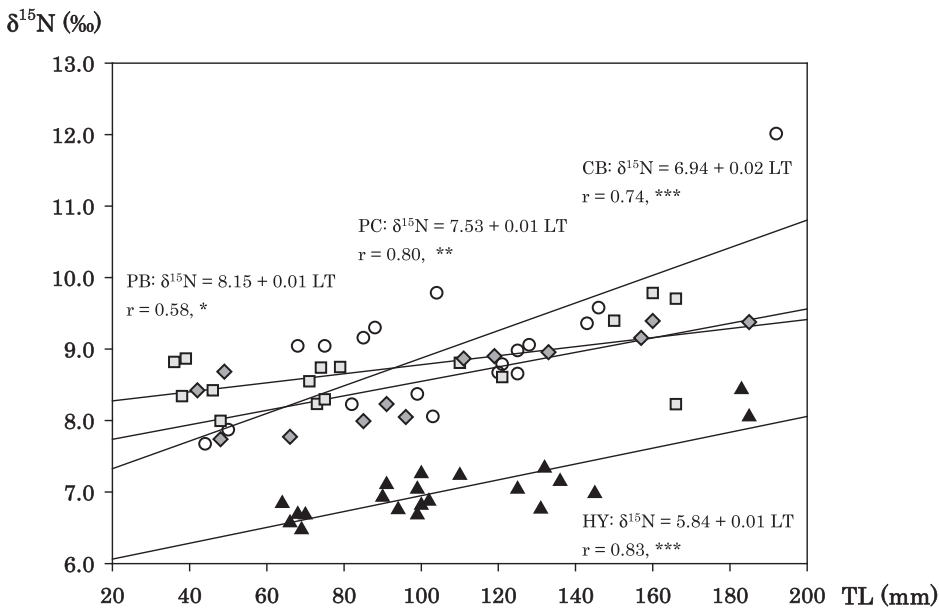


Fig. 3. Linear correlation between total length (TL, mm), and C and N isotopic compositions (‰) of *Scorpaena porcus* at the four sampling sites (white circle: CB; light gray square: PB; gray diamond: PC; and black triangle: HY). \*\*\*:  $p < .001$ ; \*\*:  $p < .01$ ; \*:  $p < .05$ ; and ns (non-significant):  $p > .05$ .



#### 4. Discussion

This study provided baseline information on the concentration status of nine TEs in the black scorpionfish *Scorpaena porcus*, a common sedentary fish representative of the spatial variation of coastal environments in the northwestern Mediterranean Sea. Three elements (Cd, Hg and Pb) have been more studied as they are subjected to international regulation (EC, 2006), while the others, (As, Cr, Cu, Ba, Ni, and Zn) have rarely been previously investigated in this region.

##### 4.1. Influence of site

Contamination patterns are driven by local contamination sources, environmental factors and the biology of the species (Cossa et al., 2012; Cresson et al., 2015a), and assessing a site-specific contamination pattern requires a good understanding of all these parameters. The quantification of TE concentrations in the muscle of *S. porcus* collected in seagrass beds, combined with the measurement of C and N isotopic compositions, allowed the discrimination of local populations from four coastal sites investigated in the northwestern Mediterranean Sea. It was

also demonstrated that spatial patterns of TE concentrations differed among trace elements, but As and Zn were always the most concentrated TEs, and Ba, Pb, and Cd the least concentrated ones. Cr, Cu, and Ni were the only three TEs that displayed similar concentrations in *S. porcus* muscle at all sites. This could likely be related to the similar concentrations of these elements in the main crustacean prey species ingested by *S. porcus* at these sites (Ourgaud, unpublished data), as food is the main route of TE inputs in fish (Hall et al., 1997). On the reverse, the other TEs analyzed exhibited significant spatial variations. The highest As concentration was observed at PC, while higher T-Hg levels were recorded at PB and PC, and higher Ba levels at CB and PC. Cd, Pb and Zn were all observed in higher concentrations at the three sites of the Bay of Marseille compared to the site located at HY. These results reflected a higher anthropic influence in Marseille Bay than at Hyères, due to the size and scale of the activity of this large city, as TEs are commonly used in many industrial, harbor and urban activities (The MERMEX Group, 2011; Bonnard et al., 2012; Oursel et al., 2013; Falcy et al., 2014). Moreover, the Bay of Marseille is subjected also to additional TE inputs related to the sporadic intrusions of Rhône River waters in the north of the bay (Sempéré et al., 2000; Frayse et al., 2014).

**Table 5**

Comparison of trace element concentrations (Mean  $\pm$  SD,  $\mu\text{g}\cdot\text{g}^{-1}$  dw) in the muscle of *Scorpaena porcus* among sampling sites. Comparisons were performed by PERMANOVA and the Pair-wise column refers to the sites compared. \*\*\*:  $p < .001$ ; \*\*:  $p < .01$ ; \*:  $p < .05$ ; and ns (non-significant):  $p > .05$ .

	Côte Bleue (CB)	Prado Bay (PB)	Plateau des Chèvres (PC)	Hyères (HY)	PERMANOVA	$p$	Pair-wise test (site)
As	<b>25.63 <math>\pm</math> 14.19</b> (17.44–42.01)	<b>31.27 <math>\pm</math> 23.77</b> (11.15–57.50)	<b>89.53 <math>\pm</math> 48.16</b> (54.72–144.5)	<b>45.59 <math>\pm</math> 11.72</b> (26.37–66.30)	F = 15.99	*	PC > HY – PB – CB
Ba	<b>0.30 <math>\pm</math> 0.09</b> (0.19–0.35)	<b>0.26 <math>\pm</math> 0.10</b> (0.19–0.37)	<b>0.23 <math>\pm</math> 0.06</b> (0.18–0.30)	<b>0.22 <math>\pm</math> 0.01</b> (0.20–0.23)	F = 84.23	**	CB – PB > PC – HY
Cd	<b>0.029 <math>\pm</math> 0.010</b> (0.02–0.04)	<b>0.025 <math>\pm</math> 0.012</b> (0.02–0.04)	<b>0.021 <math>\pm</math> 0.014</b> (0.02–0.03)	<b>&lt; 0.020</b> –	F = 4.97	**	CB – PB – PC > HY
Cr	<b>0.64 <math>\pm</math> 0.28</b> (0.48–0.96)	<b>2.72 <math>\pm</math> 4.16</b> (0.18–7.53)	<b>1.75 <math>\pm</math> 0.94</b> (0.83–2.70)	<b>2.78 <math>\pm</math> 3.32</b> (0.15–8.73)	F = 0.93	ns	
Cu	<b>0.58 <math>\pm</math> 0.01</b> (0.57–0.58)	<b>0.50 <math>\pm</math> 0.15</b> (0.35–0.65)	<b>0.60 <math>\pm</math> 0.10</b> (0.49–0.68)	<b>0.56 <math>\pm</math> 0.16</b> (0.38–1.04)	F = 3.44	ns	
Hg	<b>0.70 <math>\pm</math> 0.47</b> (0.42–1.24)	<b>1.03 <math>\pm</math> 1.01</b> (0.44–2.19)	<b>0.93 <math>\pm</math> 0.86</b> (0.30–1.91)	<b>0.48 <math>\pm</math> 0.27</b> (0.24–1.35)	F = 196.07	**	PB – PC > CB – HY
Ni	<b>0.33 <math>\pm</math> 0.05</b> (0.28–0.36)	<b>0.36 <math>\pm</math> 0.39</b> (0.10–0.81)	<b>0.52 <math>\pm</math> 0.32</b> (0.23–0.87)	<b>1.33 <math>\pm</math> 1.87</b> (0.10–4.69)	F = 0.38	ns	
Pb	<b>0.029 <math>\pm</math> 0.010</b> (0.02–0.04)	<b>0.025 <math>\pm</math> 0.012</b> (0.02–0.04)	<b>0.028 <math>\pm</math> 0.019</b> (0.02–0.07)	<b>0.023 <math>\pm</math> 0.005</b> (0.02–0.03)	F = 1090.70	**	PC – CB – PB > HY
Zn	<b>48.66 <math>\pm</math> 5.45</b> (45.52–54.95)	<b>61.62 <math>\pm</math> 12.95</b> (53.96–76.56)	<b>46.89 <math>\pm</math> 15.85</b> (29.48–60.50)	<b>15.57 <math>\pm</math> 1.92</b> (13.01–19.07)	F = 619.20	***	PB – CB – PC > HY

High concentrations of As and Zn are often recorded in marine fish and shellfish (Kim et al., 2007a; Copat et al. 2013; Vieira et al., 2015). The high As concentrations observed in *S. porcus* at PC could be related to As inputs in the marine environment by Cortiou sewage waters (Oursel et al., 2013) but also by high industrial effluents dumped into the Cassidaigne canyon located eastward, not far from PC and upstream from the main current (Millot, 1999). The higher concentrations of Zn compared to those of other essential elements, partly resulted from its crucial importance for living organisms, which is well studied in fish (Watanabe et al., 1997; Bury et al., 2003; Pouil et al., 2017). However, the high concentrations of Zn recorded in *S. porcus* in Marseille sites could also be attributed to its high level in the local environment. Zn is considered as an anthropogenic component, used, for example, in Zn based sacrificial anodes of boat traffic and in other industrial activities (Matthiessen et al., 1999; Hu et al., 2013). As with most contaminants, As and Zn are predominantly assimilated and bioaccumulated from dietary sources and surrounding waters (Xu and Wang 2002; Bury et al., 2003; Mathews and Fisher 2009).

Spatial variations of TE concentrations in *S. porcus* could be related, in part, to differences in their isotopic compositions, themselves linked to differences in the isotopic values of organic matter (OM) sources among sites. First, the lower  $\delta^{13}\text{C}$  values found in *S. porcus* individuals at PC and PB in Marseille Bay could be explained by a higher influence of riverine OM inputs at these sites, as continental OM is known to be depleted in  $^{13}\text{C}$  compared to marine OM (Riera and Richard, 1997; Kim et al., 2007a, 2007b; Harmelin-Vivien et al. 2008). Second, the higher  $\delta^{15}\text{N}$  values of fish from the three Marseille sites reflected an organic enrichment of the environment due to higher anthropogenic inputs in this area (Darnaude et al., 2004; Cresson et al., 2012). The similar lower  $\delta^{13}\text{C}$  values of *S. porcus* at PC and PB could be related to local inputs of freshwater from the Cortiou sewage treatment plant and the Huveaune River, as already measured in coastal OM and phytoplankton at these sites by Rastorgueff et al. (2011) and Cresson et al. (2012). Fish from PC were exposed daily to the sewage waters of Cortiou, composed of the treated effluent from the Marseille conurbation and the waters of the Huveaune, Prado, and Jarret rivers. During heavy rains, the Huveaune River waters were directly discharged into Prado Bay and had an impact on fish from PB seagrass meadows (Oursel et al., 2013). At these sites, *S. porcus* presented particularly high concentrations of As, Pb, T-Hg and Zn, all TEs mainly related to anthropic activities but also Ba, which traces from a continental influence. In the area, the Rhône River

is the most important source of Ba (Salminen et al., 2005), which may explain the highest Ba concentration in *S. porcus* recorded at CB, a site more subjected to the sporadic influence of this river than PB and PC (Frayssé et al., 2014). Thus, differences in the proportions of terrestrial and marine organic matter inputs at the base of seagrass food webs between Marseille Bay and Hyères were reflected in the differences of isotopic compositions observed in *S. porcus* from the two areas (i.e., lower  $\delta^{13}\text{C}$  values and higher  $\delta^{15}\text{N}$  values at Marseille, higher  $\delta^{13}\text{C}$  values and lower  $\delta^{15}\text{N}$  values at Hyères), and in their concentrations of some trace elements (generally higher As, Ba, Cd, T-Hg, Pb, and Zn at Marseille linked to higher human and industrial pressures).

#### 4.2. Influence of diet and trophic level

The dietary uptake is the dominant entry pathway of contaminants in most organisms (Zhao et al., 2001) and the main route for TE bioaccumulation (Willis and Sunda, 1984; Hall et al., 1997; Xu and Wang 2002). Contaminant concentration in organisms depends on many factors: trophic group, biological characteristics (e.g., species, swimming behavior, metabolic activities), uptake and elimination kinetics, chemical speciation and bioavailability of contaminants, local environmental conditions (salinity, temperature, pH, alkalinity, etc.), and concentrations in water and sediments (Canli and Atli, 2003). Bioavailability of TEs from ingested food is strongly influenced by the nature of the food and varies considerably among species (e.g., Luoma et al., 2002). As *S. porcus* is a sedentary species (Bell and Harmelin-Vivien, 1983), isotopic compositions and TE concentrations measured in its muscles reflect its local feeding in *Posidonia oceanica* seagrass meadows. Its diet is mainly composed of Decapoda crustaceans and Teleostei. Spatial variability in fish diet could also be linked to spatial differences (nature and frequency) of organic matter inputs, and contributed to explain spatial differences in TE concentrations. The high similarity of fish diet at PC and PB could be related to the chronic inputs of terrestrial and sewage water POM (Oursel et al., 2013), which favored the populations of detritivorous benthic invertebrates (Amphipoda, Caridea, Brachyura, Anomura) (Bellan et al., 1980), found in large numbers in the stomach contents of *S. porcus* at these sites. The higher consumption of these detritivorous prey at PC and PB contributed in turn to a higher concentrations in some trace elements, such as Hg, in fish from these sites.

A significant increase of  $\delta^{15}\text{N}$  values with size and age was observed



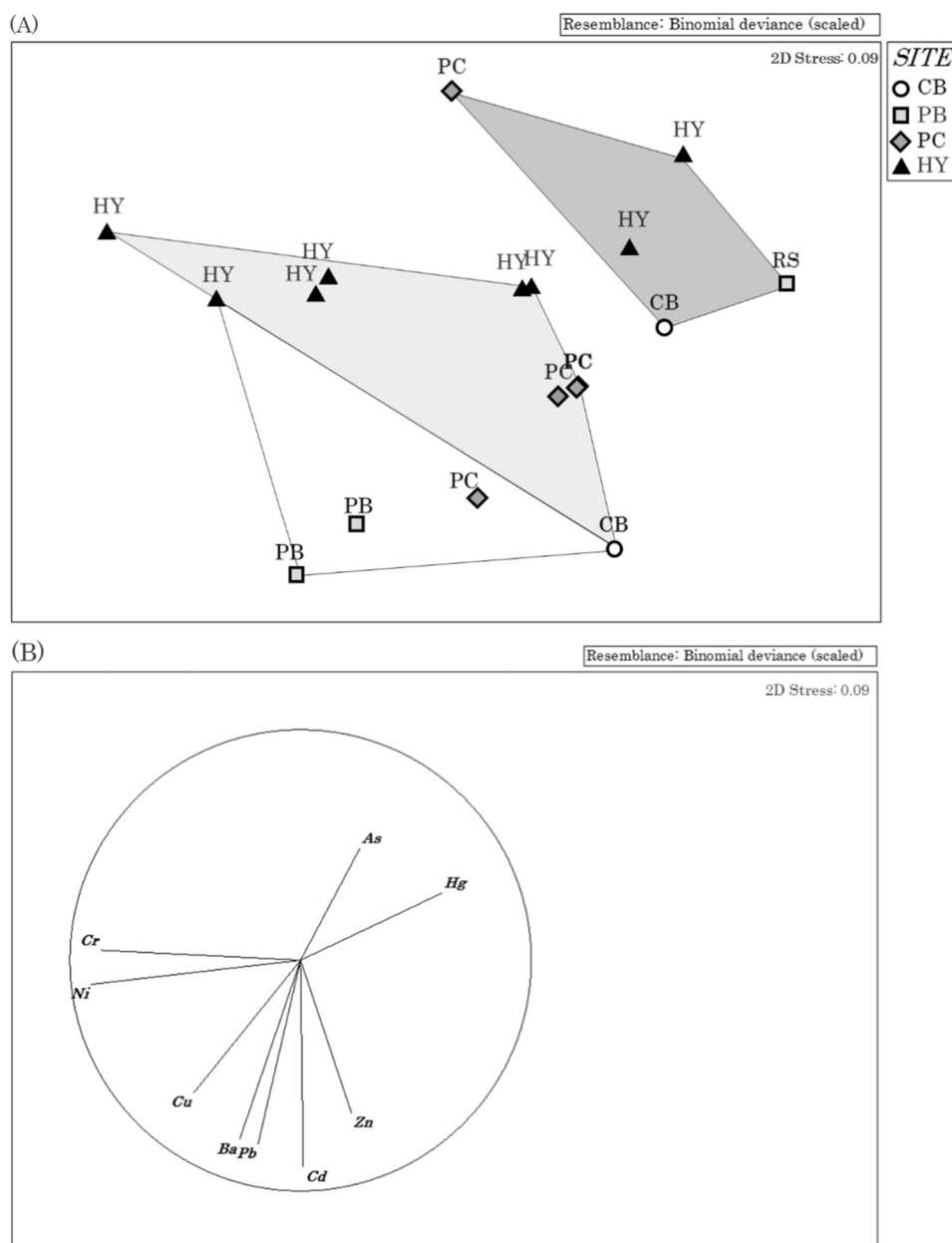


Fig. 4. (A) Two-dimensional nMDS ordination plot of trace element concentrations in the muscle of *Scorpaena porcus* according to sampling site (CB: Côte Bleue; PB: Prado Bay; PC: Plateau des Chèvres; and HY: Hyères); and (B) correlation vectors (binomial deviance resemblance) and 2D stress (0.09; good as < .2). Polygon ellipse lines are drawn for each size/age-class (white: juveniles; light gray: sub-adults; and dark gray: adults). Trace elements are indicated by sampling site symbols (white circle: CB; light gray square: PB; gray diamond: PC; and black triangle: HY).

in this species at all sampling sites, while  $\delta^{13}\text{C}$  values did not differ. The trophic level increase (estimated by  $\delta^{15}\text{N}$ ) in larger and older individuals was linked to ontogenetic modifications of their diet, with the consumption of prey of increasing size and trophic level when growing (Harmelin-Vivien et al., 1989, this paper), along with morphological, physiological and growth rate differences between juvenile and adult fish (Perga and Grey, 2010). The increasing size of the mouth allows a broadening of the captured prey spectrum, resulting in the consumption of larger and more diverse prey, such as larger Caridea, Brachyoura and Teleostei (Bell and Harmelin-Vivien, 1983; Stergiou and Karpouzi, 2002; Chauvelon et al., 2014; Cresson et al., 2014b) in order to satisfy the increasing energy needs of the individuals (Harmelin-Vivien et al., 1989; Karpouzi and Stergiou, 2003).

The present study revealed major differences in TE concentrations with fish size/age class for most elements analyzed, while sex had no influence. Higher concentrations of As and T-Hg were recorded in adults, and higher concentrations of Ba, Cd, Cu, and Pb in juveniles and sub-adults, while no effect of fish size was observed for Cr, Ni, and Zn. However, significant correlations between TE concentrations and size,

age and  $\delta^{15}\text{N}$  values of individuals were evident only for T-Hg, indicating that bioaccumulation during the life of *S. porcus* and biomagnification with its trophic level occurred only for T-Hg. No significant correlations with size, age or trophic level was observed for all the other elements.

Our results on T-Hg bioaccumulation and biomagnification were consistent with many studies that report positive correlations between  $\delta^{15}\text{N}$  values or trophic level, and total or organic Hg in various marine organisms and food webs (Campbell et al., 2005; Magalhães et al., 2007; Ikemoto et al., 2008; Dierking et al., 2009; Nfon et al., 2009; Cossa et al., 2012; Harmelin-Vivien et al., 2012; Hao et al., 2013; Chauvelon et al., 2014). The absence of correlation between the concentrations of the other TEs and *S. porcus* descriptors ( $\delta^{15}\text{N}$  values, TL, age) indicated that they were neither biomagnified nor biodiminished during the fish ontogenetic development or through the food web. Similar results are reported in several studies (Ikemoto et al., 2008; Zhang and Wang, 2012; Hao et al., 2013), while other ones indicate a biodiminution of As, Cd and Pb in freshwater and marine ecosystems (Asante et al., 2008). Some laboratory studies suggest that low assimilation

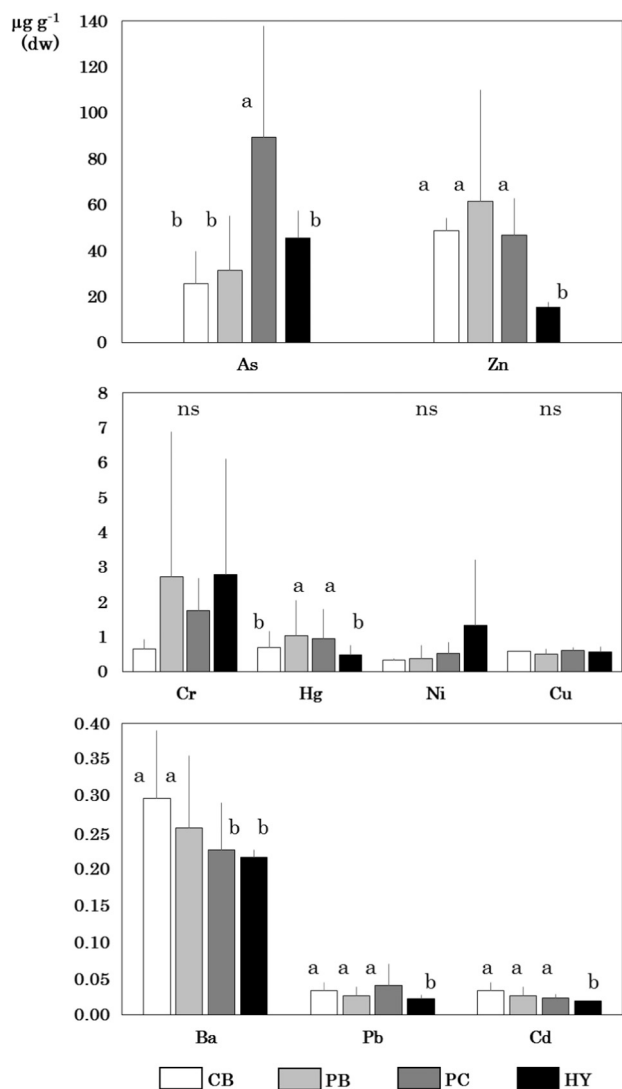


Fig. 5. Concentrations (Mean  $\pm$  SD) of the nine trace elements (TEs,  $\mu\text{g g}^{-1}$  dw) in the muscle of *Scorpaena porcus* in the four sampling sites (in white, CB: Côte Bleue; in light gray, PB: Prado Bay; in dark gray, PC: Plateau de Chèvres; and in black, HY: Hyères). For convenience, TEs are ranked according to concentration ranges. Significant differences among sites are indicated by different letters (ns: not-significant,  $p > .05$ ).

efficiencies and high efflux rates of Cd and Pb in marine organisms decrease their biomagnification potentials in food webs (Wang, 2002), while others find evidence of Cd biomagnification in food webs (Dietz et al., 2000). The same controversy is observed for the relationship of As concentration with  $\delta^{15}\text{N}$  values in the North Pacific Ocean (Asante et al., 2008: biominution, versus Asante et al., 2010: biomagnification).

#### 4.3. Sanitary risk assumption

Among the analyzed TEs, the European Commission only has set a limit for Cd, T-Hg and Pb (EC, 2006) in seafood (Cd:  $0.05 \mu\text{g g}^{-1}$  wet weight (ww); Hg:  $0.5 \mu\text{g g}^{-1}$  ww; and Pb:  $0.3 \mu\text{g g}^{-1}$  ww). T-Hg is of priority concern because of its neurotoxic, immunotoxic and teratogenic effects in vertebrates (Schlacher-Hoenlinger and Schlacher, 1998; Tartu et al., 2013). The values found in *S. porcus* from northwestern coastal sites in the present study for Cd ( $0.135 \pm 0.092 \mu\text{g g}^{-1}$  ww), Hg ( $0.004 \pm 0.001 \mu\text{g g}^{-1}$  ww) and Pb ( $0.005 \pm 0.003 \mu\text{g g}^{-1}$  ww) are lower than the limits defined by the European Commission in fish muscle (EC, 2006).

Comparison with data available in the literature indicates that TE

concentrations found in the muscle of *S. porcus* at Marseille Bay and Hyères seagrass meadows were lower than or similar to the values reported for this species by Ulzuolu et al. (2007) in the Aegean Sea or other marine fish (predators of invertebrates and small fish) in the Mediterranean Sea (Harmelin-Vivien et al., 2009; Ennouri et al., 2013; Cresson et al., 2014a) and other regions (e.g., Monteiro et al., 1991; Miao et al., 2001; Metian et al., 2013; Briand et al., 2014). Concentrations in the muscle of *S. porcus* were not alarming. However, in order to assess the level of contamination for this species and the associated risk of consumption, additional analyses, i.e. speciation analyses of As and Hg, and TEs in different parts of fish (e.g., liver), are required.

## 5. Conclusion

The analysis of the concentrations of nine trace elements (TEs), combined with the determination of diet, age, isotopic compositions and trophic level of the black scorpionfish *Scorpaena porcus* enabled the separation of local populations at four sampling sites submitted to different level of anthropic pressures in the northwestern Mediterranean Sea. Thus, *S. porcus* appears to be a good candidate for the monitoring of environmental contamination in coastal waters, given its sedentary, predatory feeding behavior, high trophic level and its high abundance in Mediterranean coastal habitats. The risk of potential adverse effects through the consumption of *S. porcus* in the south of France appears to be limited, because the concentrations recorded are lower than the toxicological reference values (Cd, Pb and Hg). However, significant bioaccumulation and bioamplification processes were observed for T-Hg and were nearly significant for As but not for the other TEs analyzed. Large-scale monitoring studies of *S. porcus* could thus represent an appropriate tool to assess the contamination status of the environment by these two elements (Hg and As), as well as their speciation, which present particular sanitary risks to human health associated with the consumption of seafood (Nadal et al., 2008; Lloret et al., 2016).

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