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# High inter-species variability in elemental composition of the twilight zone fauna varies implications for predators and exploitation by humans

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## ABSTRACT

While the importance of oceanic micronektonic species in biogeochemical cycles and in the transfer of matter in food webs is globally recognized, specific knowledge on elemental concentrations and their variability within this community is still poorly documented. Here, we report for the first time in the Bay of Biscay, North-East Atlantic, the body composition in various biological parameters and chemical elements of a meso-to bathypelagic micronektonic community. Stable carbon and nitrogen isotope compositions ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ), C:N ratios, energy density, as well as the concentrations in 6 macro-minerals and 13 trace elements including essential (micro-nutrients) and non-essential elements (undesirables, with no known biological function) were measured in whole organisms of 4 crustacean and 11 fish species caught simultaneously around 800 m depth. The results showed a low variability of  $\delta^{13}\text{C}$  values, confirming that all studied species share the same habitat. On the contrary, large differences were observed among species for several elements. Trace elements showed the greatest variability (i. e. larger range of values), especially silver (Ag), arsenic (As), cadmium (Cd), cobalt and vanadium. Significant differences were also revealed among taxa for Ag, As, Cd, copper and strontium concentrations (with crustaceans > fish), as well as for  $\delta^{15}\text{N}$  values and phosphorus concentrations (with fish > crustaceans). Although concentrations varied greatly among species, they could be grouped according to their energy density and composition in 19 chemical elements, through hierarchical clustering analysis. Six functional groups of species have been thus identified, reflecting contrasted nutritional benefit and/or exposure to undesirables for predators feeding on this deep pelagic community. Finally, the concentrations measured for the potentially toxic trace elements (undesirables) exceeded the existing European thresholds for Cd and to a lesser extent mercury (Hg), which point out potential risks in the perspective of a future exploitation of these deep living resources by humans.

## 1. Introduction

Meso- and bathypelagic zones represent the largest marine habitat in the world (Sutton, 2013), containing almost 95% of the volume of oceans (Horn, 1972). However, their ecological functioning remains

largely unknown (Ramirez-Llodra et al., 2010; St. John et al., 2016; Webb et al., 2010), while increased interest in the ecosystem services (e. g. provisioning service) provided by deep-water resources emerges with the depletion of land and shallow-water resources (Ramirez-Llodra et al., 2010, 2011). Thus, real conservation versus exploitation

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challenges currently occur for the deep-sea, especially in the twilight zone (i.e. 200-1000 m depth pelagic areas; [Martin et al., 2020](#)).

Oceanic micronekton, which includes actively swimming fishes, crustaceans and cephalopods with body sizes generally between 2 and 20 cm concentrating in the deep scattering layer (DSL) ([Sutton, 2013](#)), is an ecologically key compartment of deep-pelagic ecosystems. Either feeding directly on zooplankton or on organisms that feed on zooplankton, like all primary consumers, micronekton plays a major trophic role in marine ecosystems by being at the interface between the lowest and upper trophic levels ([Saunders et al., 2019](#)). Oceanic micronekton is also known to play a central role in biogeochemical cycles, at least those of carbon, as an important component of the “biological carbon pump” (e.g. [Davison et al., 2013](#); [Hidaka et al., 2001](#); [Martin et al., 2020](#); [Ramirez-Llodra et al., 2010](#)). This is partly because most of micronektonic species perform diel vertical migrations, going up at night from the deepest to surface layers to feed.

Diel vertical migrations are considered Earth’s largest animal migrations when taken *in toto* ([Martin et al., 2020](#); [Ramirez-Llodra et al., 2010](#); [Sutton, 2013](#)), especially if we consider the quantity of migrating biomass over a 24-h cycle. These migrations ensure a large and rapid vertical transport of organic matter and nutrients between epipelagic feeding zones and deeper areas, but various contaminants (both inorganic and organic) are also probably transferred between both zones during these migrations. These inputs are then transformed (mineralized, excreted, etc.) and/or transferred to the numerous higher trophic levels (large fish including sharks, marine mammals, seabirds) feeding on oceanic micronekton ([Choy et al., 2013](#); [Danielsen et al., 2010](#); [Ochoa-Acuña et al., 2002](#); [Pusineri et al., 2008](#); [Robinson et al., 2010](#); [Spitz et al., 2011](#)). However, the role of oceanic micronekton in major marine biogeochemical cycles is still poorly assessed globally ([Martin et al., 2020](#)), especially in terms of energy, organic matter and potentially associated contaminants transfers.

Studies focusing on these transfers and on the contamination of pelagic deep-sea fauna remain scarce, due to the inherent difficulty of accessing samples from these environments ([Martin et al., 2020](#); [Ramirez-Llodra et al., 2010](#); [Webb et al., 2010](#)). Deep-water areas are generally considered remote from direct anthropogenic sources of pollutants in comparison with coastal areas. However, the few studies performed revealed that deep-sea organisms present non-negligible (or high levels) of inorganic elements such as trace metals ([Chauvelon et al., 2012](#); [Figueiredo et al., 2020](#); [Ridout et al., 1989](#); [Wiech et al., 2020](#)) and/or organic compounds such as persistent organic pollutants ([Koenig et al., 2013](#); [Romero et al., 2018](#); [Takahashi et al., 2010](#); [Unger et al., 2008](#); [Webster et al., 2014](#)). This emphasizes the importance of better understanding the role of micronekton in the storage and transfer of contaminants in the deep-pelagic water column, and towards deep-sea predators.

Besides its fundamental role in oceanic food webs and probable role in the biological carbon pump, oceanic micronekton biomass is nowadays recognized far from being negligible, especially for areas considered to be poorly productive. For example, the biomass of mesopelagic fish has recently been estimated at 7–10 billion tons (e.g. [Irigoien et al., 2014](#); [Kaartvedt et al., 2012](#)), that is one order of magnitude higher than the first estimates in the 1980’s ([Gjøsæter and Kawaguchi, 1980](#)). In a context of dwindling neritic marine resources, the oceanic micronekton has thus become a potential target for industrial fishing (for the production of animal feed, for instance; [Alvheim et al., 2020](#); [Grimaldo et al., 2020](#); [Hidalgo and Browman, 2019](#); [Olsen et al., 2019](#); [Wiech et al., 2020](#)). However, oceanic micronekton exploitation may drastically impact the predatory species it supports, the structure of deep pelagic ecosystems as well as the ecosystem services these ecosystems provide (e.g. provisioning and regulating services) given the likely role of this compartment in biogeochemical cycles.

In the context of increasing (fishing) pressure on micronekton for exploitation, the understanding of the functional diversity of this community and its role in ecosystem functioning appear crucial ([Martin](#)

[et al., 2020](#)). The quality of matter transfers and of biogeochemical dynamics such as the biological carbon pump should indeed depend on the composition of the micronekton itself, both in terms of i) taxonomic composition (i.e. species composition of the micronektonic communities) and ii) biological parameters and elemental composition of the different species (e.g. energy density, major and trace elements’ concentrations). We can then hypothesize that within oceanic micronekton communities, species are not interchangeable in maintaining the current dynamics of mesopelagic ecosystems.

In the present study, the intrinsic (body) composition of 15 species (including 11 fish and 4 crustaceans) of a micronektonic mesopelagic community sampled during a single fishery in the twilight zone of the Bay of Biscay, North-East Atlantic, was analysed for the first time. This composition included i) 4 biological parameters corresponding to trophic markers and markers of body condition, and ii) 19 chemical elements: 6 major constitutive elements and 13 trace elements, comprising both elements considered essential for biological organisms and non-essential elements (i.e. with currently no known biological role). The objectives of this study were: i) to identify if certain biological parameters or chemical elements are more variable than others within the community considered (i.e. identifying the parameters or elements with a larger range of values compared to other parameters or elements, through different indexes of data dispersion); ii) to assess inter-taxa (crustaceans vs. fish) and inter-species differences for each parameter or element studied; iii) to propose functional groups of species displaying similar patterns in terms of global intrinsic composition, which would bring contrasted nutritional benefit and/or exposure to undesirables for predators according to the species groups they feed on within the micronektonic community considered; iv) to compare the concentrations measured to existing European environmental and health thresholds for some undesirables (namely the non-essential trace elements cadmium, mercury and lead), which should help to point out the implications of an exploitation of these deep pelagic resources by humans.

## 2. Materials and methods

### 2.1. Sampling

Organisms were collected in a canyon of the slope of the Bay of Biscay (North-East Atlantic) in October 2017, during the EVHOE fishery survey (“Evaluation Halieutique de l’Ouest de l’Europe”; <https://doi.org/10.17600/17002300>) conducted each autumn by the “Institut Français de Recherche pour l’Exploitation de la Mer” (Ifremer) on R/V Thalassa. A total of 266 individuals belonging to 11 fish species and 4 crustacean species was collected at night using a 25 m vertical opening pelagic trawl in the deep scattering layer (ca. 800 m depth in the water column; 1330 m bottom floor). All organisms were collected during one haul of 60 min, at a speed of approximately 4 knots (geographical coordinates at the beginning of the turn/end of the fishing: 45.103°N, –3.543° W). For small fish and crustaceans, organisms (whole bodies) belonging to the same species were pooled by individuals of similar sizes to obtain sufficient material for the different analyses (energy density, carbon (C) and nitrogen (N) stable isotopes and chemical elements; [Table 1](#)). The size of each individual (total length (in cm) for fish, cephalothorax length (in mm) for crustaceans) as well as the total fresh weight of individuals or pools (to the nearest 0.5 g wet mass) were determined on board, and the individuals were rinsed with ultrapure water before storage. Samples (individuals or pools of individuals, N = 39 in total) were finally stored at –20 °C until further treatment in the laboratory.

In clean and contamination-free conditions of the laboratory, whole organisms were briefly thawed and the digestive tracts of fish (i.e. stomachs and intestines) were emptied and put back in individuals. Before grinding whole organisms for major and trace element analyses, a small piece of white muscle (typically <3% of individual total weight)

**Table 1**

Characteristics of the samples collected in the twilight zone of the Bay of Biscay, NE Atlantic, and analysed for biological parameters and major and trace chemical elements (species names, migratory pattern, depth range, number of individuals, size of individuals, and moisture percentage of whole organisms). SD = Standard Deviation.

Taxa/Family	Species	Migratory pattern <sup>a</sup>	Depth range (m) <sup>a</sup>	Individuals per sample (number of samples)	Average length of individuals in samples (min-max) <sup>b</sup>	Moisture % (mean ± SD)
<b>Crustaceans</b>						
Oplophoridae	<i>Ephyrina figueirai</i>	(NA)	(NA)	7 (n = 1)	16.7 mm	69.1
	<i>Systellaspis debilis</i>	DVM	0–3700	15 (n = 1)	12.6 mm	73.4
Pasiphaeidae	<i>Pasiphaea sivado</i>	DVM	0-870 (usually 100–300)	31-48 (n = 3)	20.3–22.3 mm	78.2 ± 0.2
Sergestidae	<i>Sergia robusta</i>	DVM	100–5000	12 (n = 1)	21.0 mm	76.4
<b>Fish</b>						
Alepocephalidae	<i>Xenodermichthys copei</i>	DVM	100-2650 (usually 100–1230)	5 (n = 3)	14.1–16.9 cm	87.4 ± 0.4
Myctophidae	<i>Lampanyctus crocodilus</i>	DVM	700-1000 (d), 45–250 (n)	5 (n = 3)	12.3–13.3 cm	79.5 ± 0.8
	<i>Myctophum punctatum</i>	DVM	225-750 (d), 0–125 (n)	5-8 (n = 3)	6.5–8.3 cm	70.2 ± 1.7
	<i>Notoscopelus kroeyeri</i>	DVM	325-1000 (d), 0–125 (n)	3-9 (n = 3)	7.9–12.3 cm	67.5 ± 3.4
Paralepididae	<i>Arctozenus risso</i>	No DVM	0-2200 (usually 200–1000)	4-5 (n = 3)	18.3–21.0 cm	74.9 ± 2.7
Platyroctidae	<i>Searsia koefoedi</i>	Probably no DVM <sup>c</sup>	450–1500	3 (n = 1)	15.0 cm	85.2
Serrivomeridae	<i>Serrivomer beanii</i>	DVM	0–6000	1-4 (n = 5)	52.3–72.0 cm	87.4 ± 1.2
Sternoptychidae	<i>Argyropelecus olfersii</i>	Short DVM	200-800 (d), 0–600 (n)	3 (n = 3)	7.5–10.0 cm	74.5 ± 0.7
Stomiidae	<i>Chauliodus sloani</i>	DVM	200-4700 (usually 500–1000)	1 (n = 3)	26.0 cm	85.2 ± 1.7
	<i>Stomias boa</i>	DVM	200–2200	1-3 (n = 3)	27.7–35.0 cm	84.0 ± 1.5
Trichiuridae	<i>Aphanopus carbo</i> (juveniles)	DVM	200-2300 (usually 700–1300, with juveniles mesopelagic)	1 (n = 3)	61.0–62.0 cm	77.2 ± 2.8

<sup>a</sup> Migratory patterns and depth ranges from [www.fishbase.org](http://www.fishbase.org), Lusher et al. (2016) and references therein. (NA) = information Not Available; DVM = Diel Vertical Migration; (d) = day; (n) = night.

<sup>b</sup> Cephalothorax length for crustaceans, total length for fish.

<sup>c</sup> No DVM, at least towards epipelagic waters at night for feeding; but possible migrations deeper into the bathypelagic zone for Platyroctidae species (Novotny, 2018).

was collected on each individual and frozen at  $-20^{\circ}\text{C}$  for the subsequent analysis of C and N stable isotopes (the muscle being generally recommended in the literature for food web studies inferred from stable isotope analyses; Pinnegar and Polunin, 1999). Whole individuals were then cut into small pieces and a first “fresh” grinding of individuals (or pools of individuals for small fish and crustaceans) was carried out using an Ultra Turrax® type grinder with stainless steel arms. Samples were finally refrozen at  $-20^{\circ}\text{C}$  in acid pre-cleaned and calcined ( $450^{\circ}\text{C}$ ) glass jars, lyophilized during 72 h, and ground again into a fine and homogeneous powder using a stainless-steel knife mill. If necessary, this was completed by ball milling (MM400 Retsch®) using bowls and marbles with zirconium oxide coating. Each material was conscientiously rinsed with a succession of ultrapure water/ethanol/ultrapure water between each sample.

## 2.2. Energy density analyses

The energy density (ED) of organisms can be considered as a good proxy of the energy available in a given ecosystem and may represent a pertinent indicator of organisms' body condition and of the quality of matter fluxes, especially in terms of lipid transfers from zooplankton to upper trophic levels (Spitz et al., 2010; Wuenschel et al., 2019).

Energy density was estimated on dried homogenised samples (whole bodies of individuals or pools of individuals) following Spitz et al. (2010), using a Parr® 1266 semi-micro-oxygen bomb calorimeter and an adiabatic bomb-calorimetry in which gross energy is determined by measuring heat of combustion. Values are presented in  $\text{kJ g}^{-1}$  dry mass (dm) and are means of duplicate determination (deviation between two

assays <2%).

## 2.3. Carbon and nitrogen elemental and stable isotope analyses

Over the last decades, stable isotope analyses of C ( $\delta^{13}\text{C}$ ) and N ( $\delta^{15}\text{N}$ ) in biological tissues have been largely used as trophic markers to study the trophic ecology of marine organisms.  $\delta^{13}\text{C}$  values are generally considered a conservative tracer of the primary producer (or of the heterotrophic food source in some environments) at the base of the food web supporting consumers, and consequently a tracer of their foraging habitat (France, 1995; Hobson, 1999). Alternatively,  $\delta^{15}\text{N}$  values are generally used as a proxy of their relative trophic position in a given ecosystem (Post, 2002).

Stable isotope analyses were done on the muscle of individuals and/or pooled muscle samples corresponding to the individuals or pools of individuals analysed for major and trace element analyses (i.e. N = 39; Table 1). Muscle subsamples were homogenised manually into a fine powder. An aliquot of this powder ( $0.40 \pm 0.05$  mg dry mass) was weighed in tin cups. Analyses were finally performed with an isotope ratio mass spectrometer (Delta V Advantage with a ConFlo IV interface, Thermo Scientific) coupled to an elemental analyser (Flash EA, 2000; Thermo Scientific). The results are presented in the usual  $\delta$  notation relative to the deviation from international standards (Vienna Pee Dee Belemnite for  $\delta^{13}\text{C}$  values, and atmospheric nitrogen for  $\delta^{15}\text{N}$  values), in parts per thousand (‰). Based on replicate measurements of USGS-61 and USGS-62 used as laboratory internal standards, experimental analytical precision was <0.10‰ and <0.15‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. With the elemental analyser, bulk C:N ratios in muscle

could be also determined. Because lipids are highly depleted in  $^{13}\text{C}$  relative to other tissue components (DeNiro and Epstein, 1977), significant variations in lipids (especially between species) can affect  $\delta^{13}\text{C}$  signatures even if trophic sources are similar. However, samples were not lipid-extracted in order to have access to bulk C:N ratios as a proxy of the lipid content or body condition of organisms (Hoffman et al., 2015; Post et al., 2007), in addition to ED measurements. All the  $\delta^{13}\text{C}$  values obtained and further presented were thus mathematically corrected for the potential effect of lipids according to the formula proposed by Post et al. (2007) using bulk C:N ratios:

$$\delta^{13}\text{C}_{\text{(corrected)}} = \delta^{13}\text{C}_{\text{(bulk)}} - 3.32 + 0.99 \times \text{C:N ratio}$$

$\delta^{15}\text{N}$  values were not corrected and correspond to those obtained on bulk muscle samples.

#### 2.4. Chemical analyses (major and trace elements)

Total concentrations of calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P) and strontium (Sr), as major constitutive chemical elements (macro-minerals) in biological organisms, were determined by inductively coupled plasma atomic emission spectrometry (ICP-OES, Vista-Pro Varian) according to an in-laboratory approved method. Briefly with this method, aliquots of samples (~250 mg dry mass of homogenised powder) were digested using a 6:2 (v/v) mixture with nitric acid ( $\text{HNO}_3$ , 69%, Trace Metal Grade®, FisherScientific) and hydrochloric acid (HCl, 34%, Trace Metal Grade®, FisherScientific). Acidic digestion of the samples was performed overnight at room temperature and then in a microwave oven (START-D, Milestone). The digests were finally diluted to 50 mL with ultrapure water before analyses with ICP-OES.

Total concentrations of essential – arsenic (As), cobalt (Co), copper (Cu), Iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), vanadium (V), zinc (Zn) – and non-essential – silver (Ag), cadmium (Cd), lead (Pb) – trace elements were determined by inductively coupled plasma mass spectrometry (ICP-MS, ICAP-Qc ThermoFisher) according to an in-laboratory approved method. Briefly with this method, aliquots of samples (~200 mg of homogenised powder) were placed in Teflon bombs and mineralized with a mixture of ultrapure  $\text{HNO}_3$  acid (PlasmaPure Plus grade, SCP Science®) and ultrapure water using a microwave (ETHOS-UP, Milestone). The digests were then diluted to 50 mL with ultrapure water before analyses with ICP-MS.

Finally, total mercury (Hg) concentrations (a non-essential-element) were determined by atomic absorption spectrophotometry using an Advanced Mercury Analyser (ALTEC AMA-254, Altec Ltd), on aliquots of homogenised powder ( $50 \pm 5$  mg), according to the standard operating procedure described in the US-EPA method N°7473 (U.S. Environmental Protection Agency, 1998).

The quality assurance of all metal analyses relied on blank and internal standard controls, and on the accuracy and reproducibility of data relative to the certified reference materials (CRMs) used in each analytical run. Blank values were systematically below the detection limits and CRM values concurred with certified concentrations. Details on CRM results (recovery rates) as well as limits of quantification (LOQ) for each element analysed are reported in Table S1.

#### 2.5. Data treatment and statistical analyses

To assess the variability of biological parameters or chemical elements analysed within each taxon (crustaceans vs. fish) of the micro-nektonic community sampled, several indexes were calculated based on individual sample data or on species means (Table 2). For this data treatment focusing on parameter or element variability, crustaceans and fish were indeed systematically considered separately to limit bias due to i) unequal number of samples analysed between taxa (i.e.  $N = 6$  for crustaceans and  $N = 33$  for fish), and ii) documented differences

**Table 2**

Synthesis of the different indexes considered (see 2.5 for detailed calculations) to assess the variability of biological parameters or chemical elements measured on the community of micronekton collected in the twilight zone of the Bay of Biscay, NE Atlantic. VC = Variation Coefficient (in %); RIC = Relative Interquartile Coefficient; FC = Fold-Change value; Species RDT = Species Relative Difference with the Taxon; N = number of values.

Index	Values used for the calculation and associated N within each taxon	Information brought by the index and level of calculation (individual sample data- and/or species-based index)
VC	- Individual sample data (with $N = 6$ for crustaceans vs. $N = 33$ for fish) - Absolute dispersion index: standard deviation - Central value: mean	Dispersion of values within each taxon and for a given parameter or element, using the mean and the standard deviation in the calculation (individual sample data-based index)
RIC	- Individual sample data (with $N = 6$ for crustaceans vs. $N = 33$ for fish) - Absolute dispersion index: interquartile range - Central value: median	Dispersion of values within each taxon and for a given parameter or element, using the median and the interquartile range in the calculation (individual sample data-based index)
FC	- Species means (with $N = 4$ for crustaceans vs. $N = 11$ for fish)	Inter-species variability within a taxon and for a given parameter or element (species-based index)
Species RDT	- Species mean for each species - Individual sample data for taxon mean values (with $N = 6$ for crustaceans vs. $N = 33$ for fish)	Relative position of a species in comparison with the taxon to which it belongs (individual sample data- and species-based index)

between taxa (but see below) in terms of bioaccumulation capacities for various chemical elements (Asante et al., 2008; Cipro et al., 2018; Eisler, 2010a, 2010b). Firstly, as parameters or elements were in different units and/or of very different orders of magnitude, two relative indexes of data dispersion were calculated: the variation coefficient (VC) and the relative interquartile coefficient (RIC). These indexes consisted in the calculation of a ratio between an absolute dispersion index of data (standard deviation or interquartile range) and a central value (the mean or the median of data). More precisely, these indexes were calculated as follows (using all individual sample data, i.e.  $N = 6$  for crustaceans and  $N = 33$  for fish):  $\text{CV} (\%) = (\text{standard deviation}/\text{taxon mean value}) * 100$ ;  $\text{RIC} = (\text{quartile } 3 - \text{quartile } 1)/\text{quartile } 2$  (with quartile 2 corresponding to the median). Secondly, for each taxon, we calculated a fold-change (FC) value corresponding to the ratio between the maximum species mean value and the minimum species mean value observed within a taxon, thus based on species means (Table 2). Thirdly, for each species and each parameter analysed, we calculated the relative difference between the mean value of the given species and the mean value of the taxon to which it belongs (hereafter called “species RDT” for “species Relative Difference with the Taxon”). To fix the taxon mean value at 0 and therefore obtain positive or negative species RDT relative to the taxon, the formulae used to calculate these species RDT depended on the mean value obtained for the species compared to the mean value for the taxon, as follows: for species with a mean value > taxon mean value,  $\text{species RDT} = \text{species mean value}/\text{taxon mean value}$ ; for species with a mean value < taxon mean value,  $\text{species RDT} = -(\text{taxon mean value}/\text{species mean value})$ .

For each parameter, non-parametric Mann-Whitney-Wilcoxon (MWW) tests were then used (due to the relatively low number of samples for crustaceans,  $N = 6$ ) to assess differences between taxa. To identify any biomagnification or biodilution patterns of trace elements through the food web, Spearman correlation coefficient tests were also applied between  $\delta^{15}\text{N}$  values and trace elements (Ag, As, Cd, Co, Cu, Fe, Hg, Mn, Mo, Pb, Se, V, Zn). These statistical tests used individual sample data ( $N = 39$  in total) and the level of significance was always set at  $\alpha = 0.05$ .

Groups of species that had similar patterns in terms of intrinsic body



composition were identified by hierarchical clustering analysis (HCA), using Ward's minimum variance method (Ward, 1963). This method is based on the linear model criterion of least squares and allows to define groups that minimize the within-group sum of squares, while the computation of within-group sums of squares is based on a Euclidean model. After clustering, the optimal number of groups was first assessed by visual inspection of the resulting dendrogram, and was confirmed by the computation of the simple structure index criterion generally used in K-means partitioning (e.g. Spitz et al., 2014). The HCA was performed using the 20 variables corresponding to a concentration per unit mass and measured on whole organisms (i.e. ED among biological parameters, 6 major elements and 13 trace elements), including therefore all the parameters analysed except C:N ratios and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values measured in the muscle tissue.

Finally, in the context of increasing (fishing) pressure on deep pelagic resources, we estimated interesting to compare our data with some existing guidelines and thresholds, especially those related marine biological resources for Cd, Hg and Pb. For these non-essential trace elements, recommendations (i.e. threshold concentrations) are indeed given by the OSPAR Commission in the NE Atlantic (OSPAR Commission, 2009a, 2009b) and/or used by France in the assessment of marine waters' status within the frame of the European Marine Strategy Framework Directive (European Commission, 2008; Ifremer, 2018). The concentrations we measured were thus first compared to the Background Assessment Concentrations (BACs) defined by the OSPAR Commission for biota, especially fish, with a "background concentration" being defined as "the concentration of a contaminant at a pristine or remote site based on contemporary or historical data" (OSPAR Commission, 2009a, 2009b). These BACS are provided on a wet mass (wm) basis and specifically concern Hg concentrations in the fish muscle (BAC =  $0.035 \text{ mg kg}^{-1} \text{ wm}$ ), and Cd and Pb concentrations in the fish liver (BACs =  $0.026 \text{ mg kg}^{-1} \text{ wm}$  for both elements). The Cd, Hg and Pb concentrations we measured were then compared to the existing health thresholds defined by the European Commission for these elements in seafood (European Commission, 2006). These thresholds (also on wm basis) are defined for the "edible flesh" of seafood products and are: Cd threshold for undefined crustacean species =  $0.5 \text{ mg kg}^{-1} \text{ wm}$ ; Cd threshold for undefined fish species =  $0.05 \text{ mg kg}^{-1} \text{ wm}$ ; Pb threshold for undefined crustacean species =  $0.3 \text{ mg kg}^{-1} \text{ wm}$ ; Pb threshold for undefined fish species =  $0.5 \text{ mg kg}^{-1} \text{ wm}$ ; Hg threshold for both undefined crustacean and fish species =  $0.5 \text{ mg kg}^{-1} \text{ wm}$ .

All data used in the present study are available under the depository system PANGAEA (Chauvelon et al., 2021; DOI: <https://doi.org/10.1594/PANGAEA.937345>).

### 3. Results

#### 3.1. Stable isotope compositions (trophic markers)

$\delta^{13}\text{C}$  values presented a relatively narrow range of values, less than 2‰ (Fig. 1). The crustacean species *Ephyrina figueirai* had the highest value ( $-18.79\text{‰}$ ) and the fish species *Myctophum punctatum* the lowest value ( $-20.07 \pm 0.22\text{‰}$ ).  $\delta^{15}\text{N}$  values varied over a larger range, by slightly more than 4‰ (Fig. 1, Table 3). Overall, crustacean species presented lower  $\delta^{15}\text{N}$  value than fish species. The crustacean *Systelaps debilis* had the lowest  $\delta^{15}\text{N}$  value ( $8.05\text{‰}$ ) and the fish *Searsia koefedi* the highest one ( $12.22\text{‰}$ ).

#### 3.2. Levels and variability of biological parameters and chemical elements

Within the studied community (including both crustaceans and fish and considering individual sample data), C:N ratios varied between 3.17 and 8.50 and ED values between 16.7 and  $28.0 \text{ kJ g}^{-1} \text{ dm}$  (Table 3). With the exception of some samples at the upper end of the range of concentrations, the concentrations of the different chemical elements varied over the following order of magnitudes: Ag, Co, Hg, Pb and V

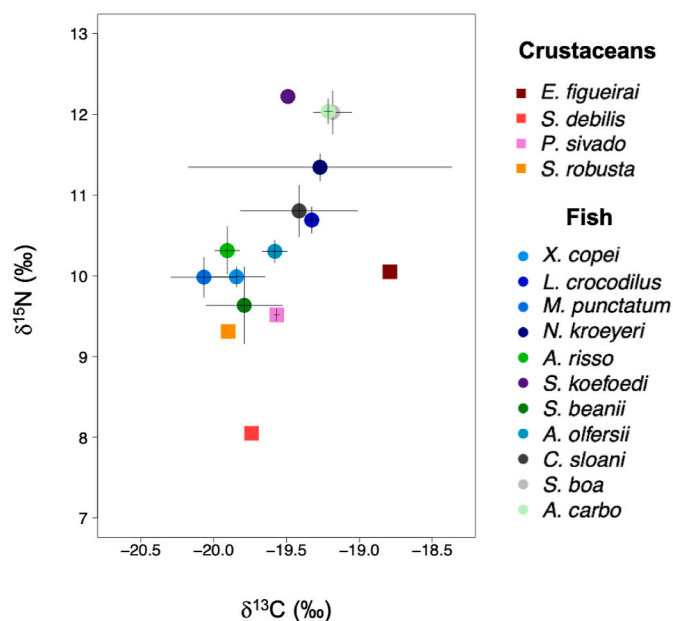


Fig. 1. Muscle  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (means  $\pm$  standard deviations per species, in ‰) determined in micronektonic species collected in the twilight zone of the Bay of Biscay, NE Atlantic, in October 2017 (corrected values for  $\delta^{13}\text{C}$ , see 2.1). Crustaceans are represented by squares and fish by circles. Species full names are listed in Table 1.

concentrations were mostly below  $1 \text{ mg kg}^{-1} \text{ dm}$  (all less than  $3 \text{ mg kg}^{-1} \text{ dm}$ ) while Cd, Mn, Mo and Se concentrations varied up to  $10 \text{ mg kg}^{-1} \text{ dm}$  (i.e. order of magnitude of the maximum concentrations); As and Cu concentrations varied up to  $100 \text{ mg kg}^{-1} \text{ dm}$ ; Sr, Fe and Zn concentrations ranged between ca.  $20 \text{ mg kg}^{-1}$  and  $650 \text{ mg kg}^{-1} \text{ dm}$ , with most samples having concentrations below  $200 \text{ mg kg}^{-1} \text{ dm}$ ; finally, Ca, K, Mg, Na and P concentrations ranged between ca.  $1000 \text{ mg kg}^{-1} \text{ dm}$  and  $60\,000 \text{ mg kg}^{-1} \text{ dm}$ , with most concentrations lower than  $30\,000 \text{ mg kg}^{-1} \text{ dm}$  (Tables 3 and 4).

Compared to trace elements, biological parameters and major elements showed the lowest variability within the community. Variation coefficients (VC) for biological parameters were all  $<30\%$  and followed the order:  $\delta^{13}\text{C} < \delta^{15}\text{N} < \text{C:N ratio}$  and/or ED, depending on the taxon for the last two parameters (Fig. 2, Table S2). Variation coefficients for major chemical elements were all  $<40\%$ , whatever the taxon considered. On the contrary, trace elements (except Se) had higher and a large range of VC, whatever the taxon considered (Fig. 2). More precisely in crustaceans, Cu, Hg, Mn, Se and Zn presented VC  $<50\%$ , while Ag, As, Cd, Co, Fe, Mo and Pb had VC comprised between 50% and 100%. Vanadium (V) was the only element presenting a VC  $>100\%$  in crustaceans (Fig. 2, Table S2). In fish, as in crustaceans, the element Se was those presenting the lowest VC (21%) and the only element with VC  $<50\%$ . Then, Co, Cu, Fe, Hg, Mn, Mo, Pb and Zn presented VC comprised between 50% and 100%, while Ag, As, Cd and V had VC  $>100\%$  (Fig. 2, Table S2).

Results for the three other indexes of variability calculated – namely RIC, FC values and species RDT – showed similar patterns to VC. Whatever the taxon considered, biological parameters and major elements had the lowest to intermediate variability, while trace elements (especially non-essential ones) presented the largest range of values and more broadly, the higher variability (Figs. 2 and 3, Table S2 and Figs. S1–S4). Fish also generally presented a higher variability than crustaceans. Finally, whether considering the indexes of data dispersion calculated from individual sample data (CV, RIC) or species-based indexes (FC values, species RDT), the elements that presented the highest variability in the 15 studied species were Ag, As, Cd, Co, V and to lesser extent Pb and Mo in crustaceans and fish, respectively.

**Table 3**

Values obtained per species for biological parameters and major chemical elements (in alphabetical order for the later).  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  (in ‰) and bulk C:N ratios were measured on muscle subsamples (corrected values for  $\delta^{13}\text{C}$ , see 2.1), energy density (ED, in  $\text{kJ g}^{-1}$  dry mass (dm)) and major elements (in  $\text{mg kg}^{-1}$  dm) on whole organisms. Values are means  $\pm$  standard deviations (when appropriate). Species are in the same order as in Table 1. Elements are listed in alphabetical order for major elements.

Species	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	C:N ratio	ED	Ca	K	Mg	Na	P	Sr
<b>Crustaceans</b>										
<i>Ephyrina figueirai</i>	−18.79	10.05	5.15	28.0	9 676	10 028	1 850	10 419	7 926	174.7
<i>Styellaspis debilis</i>	−19.74	8.05	3.32	20.5	31 608	11 397	3 136	16 724	9 937	538.2
<i>Pasiphaea sivado</i>	−19.57 $\pm$ 0.02	9.52 $\pm$ 0.08	3.36 $\pm$ 0.02	17.3 $\pm$ 0.5	24 283 $\pm$ 383	14 257 $\pm$ 59	3 493 $\pm$ 65	16 919 $\pm$ 119	12 459 $\pm$ 82	406.8 $\pm$ 17.1
<i>Sergia robusta</i>	−19.90	9.31	3.26	18.4	31 451	14 569	2 411	15 759	11 575	509.9
<b>Fish</b>										
<i>Xenodermichthys copei</i>	−19.84 $\pm$ 0.20	9.99 $\pm$ 0.12	3.39 $\pm$ 0.17	18.6 $\pm$ 0.2	16 486 $\pm$ 102	22 106 $\pm$ 547	2 626 $\pm$ 131	25 318 $\pm$ 824	16 948 $\pm$ 131	145.3 $\pm$ 1.5
<i>Lampanyctus crocodilus</i>	−19.33 $\pm$ 0.06	10.69 $\pm$ 0.16	3.25 $\pm$ 0.04	19.3 $\pm$ 1.1	35 668 $\pm$ 3812	16 988 $\pm$ 500	2 531 $\pm$ 86	20 879 $\pm$ 1643	19 533 $\pm$ 1830	161.4 $\pm$ 23.3
<i>Myctophum punctatum</i>	−20.07 $\pm$ 0.22	9.98 $\pm$ 0.25	5.35 $\pm$ 0.70	23.0 $\pm$ 0.7	21 515 $\pm$ 747	9 867 $\pm$ 380	1 703 $\pm$ 347	12 919 $\pm$ 4079	14 063 $\pm$ 909	89.7 $\pm$ 7.6
<i>Notoscopelus kroeyeri</i>	−19.27 $\pm$ 0.90	11.34 $\pm$ 0.17	6.31 $\pm$ 1.91	23.9 $\pm$ 1.3	20 005 $\pm$ 5084	9 606 $\pm$ 1547	1 437 $\pm$ 307	9 938 $\pm$ 2311	11 931 $\pm$ 1591	72.3 $\pm$ 12.1
<i>Arctozenus risso</i>	−19.90 $\pm$ 0.09	10.31 $\pm$ 0.29	3.99 $\pm$ 0.60	21.8 $\pm$ 2.1	22 463 $\pm$ 4820	14 654 $\pm$ 1624	2 058 $\pm$ 464	14 820 $\pm$ 4390	14 990 $\pm$ 2587	62.8 $\pm$ 18.2
<i>Searsia koefoedi</i>	−19.49	12.22	4.34	19.7	14 478	16 711	2 537	26 101	13 032	116.5
<i>Serrivomer beanii</i>	−19.79 $\pm$ 0.26	9.64 $\pm$ 0.48	3.29 $\pm$ 0.06	18.8 $\pm$ 1.4	27 012 $\pm$ 4299	20 438 $\pm$ 750	2 825 $\pm$ 475	29 387 $\pm$ 1179	17 419 $\pm$ 1661	113.6 $\pm$ 17.9
<i>Argyropelecus olfersii</i>	−19.58 $\pm$ 0.08	10.30 $\pm$ 0.14	3.26 $\pm$ 0.02	18.3 $\pm$ 0.2	49 792 $\pm$ 4633	13 454 $\pm$ 130	2 686 $\pm$ 179	21 334 $\pm$ 1717	27 215 $\pm$ 1598	142.4 $\pm$ 20.1
<i>Chauliodus sloani</i>	−19.41 $\pm$ 0.40	10.80 $\pm$ 0.32	3.50 $\pm$ 0.26	19.0 $\pm$ 0.9	28 440 $\pm$ 6465	19 852 $\pm$ 986	2 996 $\pm$ 255	28 264 $\pm$ 1318	18 187 $\pm$ 2858	109.5 $\pm$ 49.1
<i>Stomias boa</i>	−19.18 $\pm$ 0.13	12.02 $\pm$ 0.26	4.62 $\pm$ 1.13	19.5 $\pm$ 2.3	24 700 $\pm$ 4141	19 296 $\pm$ 2398	3 215 $\pm$ 427	28 333 $\pm$ 1085	16 082 $\pm$ 2156	103.5 $\pm$ 10.2
<i>Aphanopus carbo</i> (juveniles)	−19.21 $\pm$ 0.03	12.03 $\pm$ 0.15	3.17 $\pm$ 0.00	18.8 $\pm$ 0.4	32 181 $\pm$ 934	19 207 $\pm$ 829	1 911 $\pm$ 25	12 903 $\pm$ 816	21 036 $\pm$ 48	102.1 $\pm$ 4.9

### 3.3. Difference between taxa and species

Among biological parameters, significant differences (MWW tests) were found between taxa for  $\delta^{15}\text{N}$  only ( $p = 0.001$ ), with fish > crustaceans. There was no significant difference between taxa for  $\delta^{13}\text{C}$ , C:N ratio and ED (all  $p$ -values >0.05). Among chemical elements, crustaceans had significantly higher mean concentrations than fish for Sr ( $p < 0.001$ ), Ag ( $p < 0.001$ ), As ( $p = 0.002$ ), Cd ( $p < 0.001$ ) and Cu ( $p < 0.001$ ), while fish had significantly higher mean concentrations than crustaceans for P only ( $p < 0.001$ ). There was no significant difference between taxa for Ca, K, Mg, Na, Co, Fe, Hg, Mn, Mo, Pb, Se, V and Zn (MWW tests, all  $p$ -values >0.05).

Some species within each taxon presented particularly extreme (high or low) mean concentrations for certain chemical elements compared to other species, highlighting contrasted patterns of elemental composition at the species level (Tables 3 and 4; Fig. 3, S1–S4). Among fish, for instance, *Argyropelecus olfersii* had high concentrations in Ca, P and As when compared to other fish species. As such, the mean As concentration of this species was nearly 3 times higher (hereafter noted as “3 x” and corresponding to the species RDT) than the fish taxon mean value (i. e. species RDT of *A. olfersii* for As = 2.9 x). *Xenodermichthys copei* had the highest mean concentrations in K (species RDT = 1.3 x), As (3.1 x), Pb (2.4 x), Se (1.3 x) and V (4.4 x), and had also a relatively high

concentration in Zn (1.7 x). The two Stomiidae species *Stomias boa* and *Chauliodus sloani* had the highest mean concentrations in Fe (species RDT of *S. boa* and *C. sloani* for Fe = 2.2 x and 2.1 x, respectively). *S. boa* also presented the highest concentrations in Cd (3.4 x), Hg (3.4 x) and Zn (1.9 x), while *C. sloani* had the highest mean concentrations in Co (1.8 x) and Mo (2.5 x). Within the Myctophidae family, differences also occurred (Tables 3 and 4; Fig. 3, S1–S4). *Lampanyctus crocodilus* had higher mean concentrations than the two other myctophids *Notoscopelus kroeyeri* and *Myctophum punctatum* in all major elements (Ca, K, Mg, Na, P, Sr), but lower mean concentrations in several trace elements (compared to other fish species also), especially in As (species RDT for *L. crocodilus* = −4.8 x), Cu (−3.2 x), Mo (−16.3 x) and Pb (−2.1 x).

### 3.4. Species clustering according to their elemental composition

Among the 20 variables measured and included in the clustering analysis, 14 contributed mainly to the formation of groups (i.e. >50% of the variance explained by these variables in the definition of groups). These variables were: among i) biological parameters: ED (64%); ii) major elements: Na (83%), Sr (82%), K (78%), P (76%) and Ca (65%); iii) trace elements: Cu (95%), Ag (93%), Cd (82%), As (75%), Mg (72%), Pb (63%), Se (62%) and V (51%) (Table S3).

The clustering analysis subdivided the analysed samples into 2

**Table 4**

Values obtained per species for trace chemical elements (in alphabetical order, and in  $\text{mg kg}^{-1}$  dry mass), measured on whole organisms. Values are means  $\pm$  standard deviations (when appropriate). Species are in the same order as in Table 1. Elements are listed in alphabetical order.

Species	Ag	As	Cd	Co	Cu	Fe	Hg	Mn	Mo	Pb	Se	V	Zn
<b>Crustaceans</b>													
<i>Ephyrina figueirai</i>	1.32	22.16	1.26	0.24	28.55	121.2	0.184	4.01	3.12	0.06	1.40	2.37	31.1
<i>Stellaspis debilis</i>	1.39	18.12	11.57	0.52	61.75	194.2	0.195	7.31	3.83	0.26	2.33	0.26	46.4
<i>Pasiphaea sivado</i>	0.39 $\pm$ 0.01	75.06 $\pm$ 2.05	12.19 $\pm$ 0.09	0.10 $\pm$ 0.02	86.61 $\pm$ 9.94	70.0 $\pm$ 18.1	0.248 $\pm$ 0.020	3.36 $\pm$ 0.53	1.63 $\pm$ 0.48	0.05 $\pm$ 0.01	2.37 $\pm$ 0.08	0.10 $\pm$ 0.02	55.6 $\pm$ 1.5
<i>Sergia robusta</i>	0.76	35.01	2.57	0.40	25.07	219.5	0.469	6.50	5.34	0.08	2.91	0.32	103.1
<b>Fish</b>													
<i>Xenodermichthys copei</i>	0.04 $\pm$ 0.00	55.95 $\pm$ 6.30	0.91 $\pm$ 0.19	0.20 $\pm$ 0.01	2.36 $\pm$ 0.33	66.3 $\pm$ 14.1	0.254 $\pm$ 0.049	5.19 $\pm$ 0.51	1.03 $\pm$ 0.46	0.18 $\pm$ 0.01	2.91 $\pm$ 0.11	1.18 $\pm$ 0.51	94.3 $\pm$ 19.3
<i>Lampanyctus crocodilus</i>	0.02 $\pm$ 0.00	3.83 $\pm$ 0.47	0.30 $\pm$ 0.08	0.04 $\pm$ 0.00	0.92 $\pm$ 0.02	47.9 $\pm$ 10.3	0.419 $\pm$ 0.092	1.98 $\pm$ 0.17	0.12 $\pm$ 0.02	0.04 $\pm$ 0.01	2.83 $\pm$ 0.03	0.21 $\pm$ 0.03	45.4 $\pm$ 7.2
<i>Myctophum punctatum</i>	0.12 $\pm$ 0.03	6.76 $\pm$ 1.13	0.12 $\pm$ 0.01	0.19 $\pm$ 0.04	8.04 $\pm$ 2.87	120.6 $\pm$ 38.2	0.113 $\pm$ 0.054	6.55 $\pm$ 1.65	2.12 $\pm$ 1.06	0.09 $\pm$ 0.02	2.49 $\pm$ 0.23	0.14 $\pm$ 0.04	39.6 $\pm$ 4.2
<i>Notoscopelus kroeyeri</i>	0.01 $\pm$ 0.00	7.26 $\pm$ 1.50	0.08 $\pm$ 0.02	0.05 $\pm$ 0.03	3.09 $\pm$ 1.83	55.6 $\pm$ 22.6	0.326 $\pm$ 0.277	1.95 $\pm$ 0.69	0.45 $\pm$ 0.63	0.05 $\pm$ 0.05	1.72 $\pm$ 0.09	0.07 $\pm$ 0.02	24.5 $\pm$ 6.4
<i>Arctozenus risso</i>	0.01 $\pm$ 0.00	10.20 $\pm$ 3.73	0.03 $\pm$ 0.01	0.11 $\pm$ 0.07	3.89 $\pm$ 1.32	181.6 $\pm$ 144.6	0.090 $\pm$ 0.039	4.22 $\pm$ 2.27	1.64 $\pm$ 1.40	0.05 $\pm$ 0.03	2.21 $\pm$ 0.22	0.11 $\pm$ 0.06	56.4 $\pm$ 17.1
<i>Searsia koefoedi</i>	0.02	38.91	0.36	0.22	1.45	148.9	0.285	3.62	3.58	0.24	2.50	0.45	34.5
<i>Serrivomer beanii</i>	0.01 $\pm$ 0.00	15.71 $\pm$ 5.34	1.79 $\pm$ 1.08	0.15 $\pm$ 0.03	1.62 $\pm$ 0.13	185.3 $\pm$ 28.1	0.332 $\pm$ 0.135	3.90 $\pm$ 0.56	2.63 $\pm$ 0.87	0.06 $\pm$ 0.02	1.68 $\pm$ 0.21	0.22 $\pm$ 0.02	51.6 $\pm$ 5.4
<i>Argyroleucus olfersii</i>	0.01 $\pm$ 0.00	53.60 $\pm$ 0.55	0.33 $\pm$ 0.08	0.23 $\pm$ 0.07	4.83 $\pm$ 1.03	166.9 $\pm$ 72.1	0.152 $\pm$ 0.071	6.30 $\pm$ 2.00	3.49 $\pm$ 2.01	0.07 $\pm$ 0.03	2.77 $\pm$ 0.18	0.17 $\pm$ 0.07	60.9 $\pm$ 1.3
<i>Chauliodus sloani</i>	0.01 $\pm$ 0.00	5.53 $\pm$ 1.41	0.09 $\pm$ 0.01	0.26 $\pm$ 0.19	2.07 $\pm$ 1.00	322.4 $\pm$ 291.9	0.462 $\pm$ 0.274	5.94 $\pm$ 3.47	4.75 $\pm$ 3.83	0.07 $\pm$ 0.02	1.99 $\pm$ 0.18	0.28 $\pm$ 0.18	41.0 $\pm$ 17.6
<i>Stomias boa</i>	0.01 $\pm$ 0.00	4.61 $\pm$ 1.49	2.43 $\pm$ 2.31	0.11 $\pm$ 0.02	1.60 $\pm$ 0.50	333.3 $\pm$ 184.5	1.365 $\pm$ 0.659	7.67 $\pm$ 3.82	1.57 $\pm$ 0.04	0.08 $\pm$ 0.01	2.01 $\pm$ 0.21	0.21 $\pm$ 0.02	103.7 $\pm$ 45.4
<i>Aphanopus carbo</i> (juveniles)	0.02 $\pm$ 0.01	14.88 $\pm$ 1.58	0.45 $\pm$ 0.32	0.03 $\pm$ 0.01	2.47 $\pm$ 0.92	50.9 $\pm$ 8.9	0.586 $\pm$ 0.057	2.19 $\pm$ 0.30	0.18 $\pm$ 0.21	0.03 $\pm$ 0.00	2.72 $\pm$ 0.32	0.05 $\pm$ 0.01	38.2 $\pm$ 4.7

crustacean groups (groups 1 and 2) and 4 fish groups (groups 3–6) (Fig. 4; Table S3). Moreover, for species with several samples analysed, these samples were grouped into a same group (with the only exception of the fish *Arctozenus risso*, whose samples were divided into the groups 4 and 6). The groups 1 and 2 including only crustaceans had the highest values in several elements: As, Cu, Mg and Cd for the group 1 (composed of the crustacean species *Pasiphaea sivado* only); Ag, Co, Mn, Mo and Sr for the group 2 (including the 3 other crustacean species sampled). Alternatively, the crustacean group 1 had the lowest values for several parameters or elements (namely ED, Co, Fe, Mn, Pb), while the group 2 only had the lowest P concentrations (Fig. 4, Table S3). The fish group 4, containing 2 of the 3 sampled myctophid species (*N. kroeyeri* and *M. punctatum*) and most of *A. risso* samples was characterized by the highest ED values, but the lowest concentrations in several elements including non-essential ones (namely As, Cd, K, Hg, Na, Mg, Mo, Sr, V and Zn). The fish group 5 containing samples of *A. olfersii*, *L. crocodilus* (the third myctophid species) and juveniles of *Aphanopus carbo* was characterized by the highest Ca et P concentrations, while the fish group 3 including the Alepocephalidae *X. copei* and the Platytroctidae *S. koefedi* had the lowest Ca values. Alternatively, this group 3 had the highest concentrations in K, Pb, Se, V and Zn. Finally, the fish group 6 containing the two Stomiidae species *S. boa* and *C. sloani* as well as samples of *Serrivomer beanii* was characterized by the highest Fe, Hg and Na concentrations, but the lowest Ag, Cu and Se concentrations (Fig. 4; Table S3).

### 3.5. Relationships between trace elements and $\delta^{15}\text{N}$ values

Among trace elements, only Hg concentrations showed a significant positive correlation with  $\delta^{15}\text{N}$  values (Spearman correlation coefficient test,  $p = 0.006$ ). There was a significant negative correlation between elemental concentrations and  $\delta^{15}\text{N}$  values for Ag, As, Cd, Co, Cu and Mo (Spearman correlation coefficient tests, all  $p < 0.05$ ). Concentrations of Fe, Mn, Se, Pb, V, Zn showed no significant correlation with  $\delta^{15}\text{N}$  values (Spearman correlation coefficient tests, all  $p > 0.05$ ).

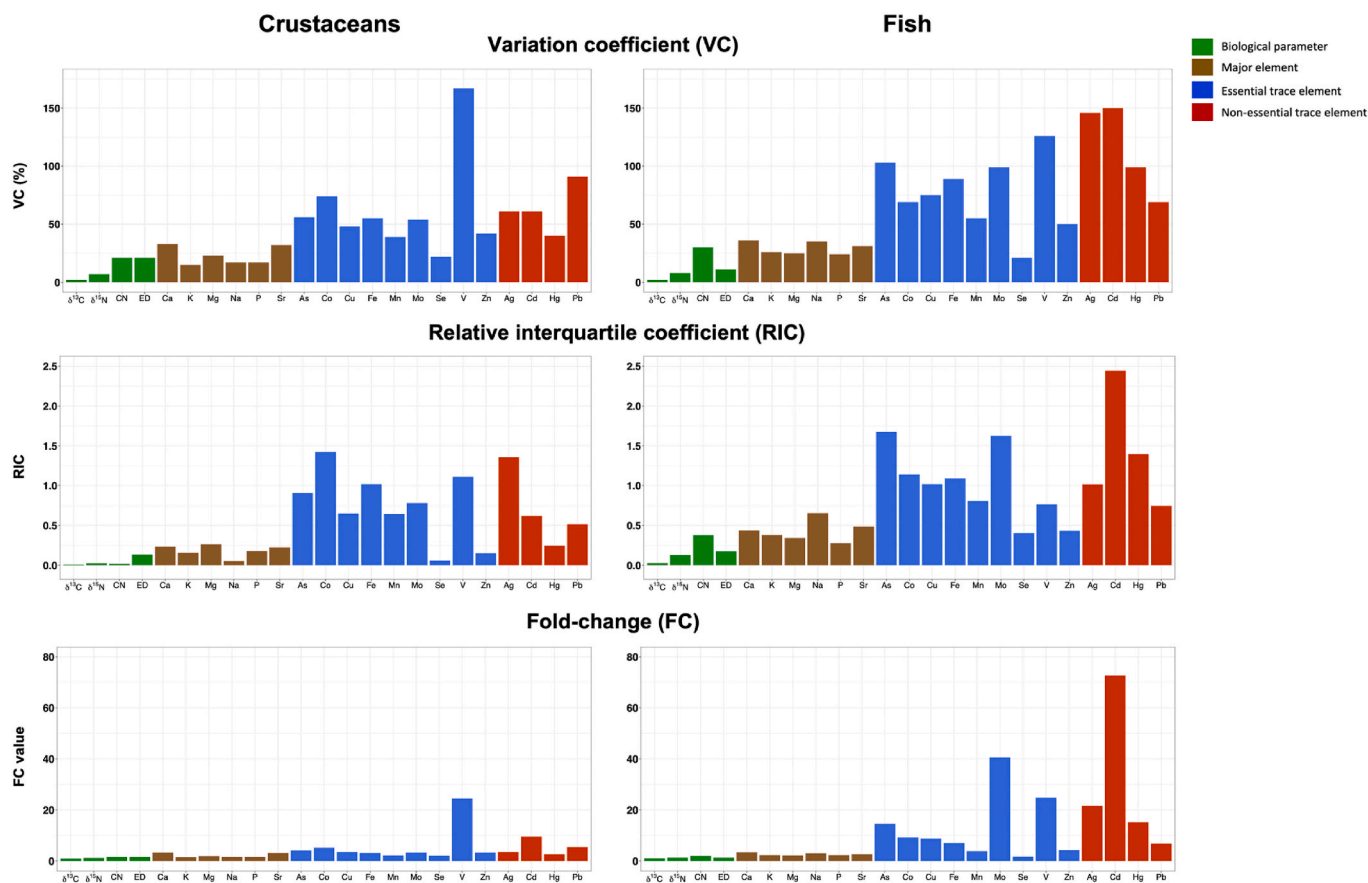
### 3.6. Confrontation to European guidelines and thresholds

After conversion of our data on a wm basis using the moisture percentages given in Table 1, 76% of fish samples exceeded the BAC for Cd, 67% the BAC for Hg, and 15% the BAC for Pb. In addition, 83% of the crustacean samples and 58% of the fish samples exceeded the respective health thresholds for Cd in marine crustaceans and fish, while health thresholds were not exceeded for Hg and Pb (Fig. 5).

## 4. Discussion

For the first time in the Bay of Biscay, NE Atlantic, our results showed a large diversity of intrinsic composition of a micronektonic mesopelagic community, including both biological parameters and chemical elements, although the number of samples per species was relatively low.





**Fig. 2.** Variation coefficients (in %), relative interquartile coefficients and fold-change values calculated for the different parameters and elements on samples of the micronektonic species collected in the twilight zone of the Bay of Biscay, NE Atlantic, in October 2017 (N = 6 for crustaceans and N = 33 for fish; see 2.5 and Table 2 for explanation on calculations). Variables are first listed according to the type of parameters and distinguished by different colours (i.e. biological parameters vs. major elements vs. trace essential elements vs. trace non-essential elements), then by alphabetical order within each type of parameter. This enables keeping the same order for easier comparison between crustaceans and fish. The same scale is also applied for crustaceans and fish. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

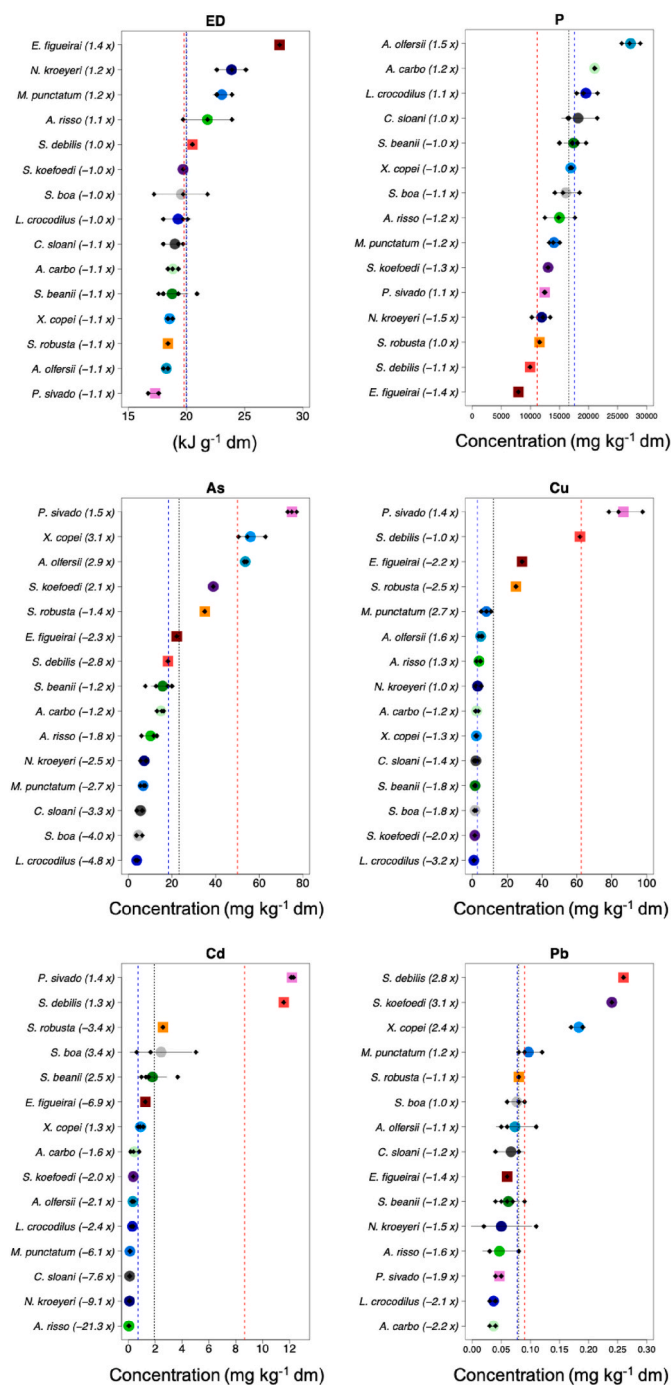
Certain parameters or elements appeared much more variable than others (Ag, As, Cd, Co, V) in both taxons. Crustaceans and fish were significantly different regarding their concentrations in some chemical elements (Ag, As, Cd, Cu, P, Sr), and certain species also presented peculiar compositions. Finally, species could be grouped following their intrinsic composition, and this composition largely differed among species groups. All these results suggest that changes in species relative abundance (within the oceanic micronekton community) can affect the quality of matter transfers towards deep-sea areas and predators, and ultimately the functioning of some biogeochemical cycles.

#### 4.1. Food web considerations and biomagnification or biodilution of trace elements

The  $\delta^{13}\text{C}$  values measured within the sampled community (15 species) were consistent with those described previously in the Bay of Biscay for species from oceanic or deep-sea areas (Chauvelon et al., 2012b). The low variability observed for these values (i.e. less than 2‰) also suggested that the sampled species are supported by similar sources of organic matter. Most of the deep-sea ecosystems are perceived as heterotrophic, depending ultimately on the flux on organic matter produced in the overlying surface ocean through photosynthesis (Ramirez-Llodra et al., 2010). Conversely, a larger variability was observed for  $\delta^{15}\text{N}$  values within the sampled community (i.e. more than 4‰ between the minimum and maximum species values), corresponding to more than one theoretical trophic level if we refer to the average

difference of 3.4‰ reported between two trophic levels (Post, 2002). This variability confirms that the considered species occupy different trophic positions, with fish species generally presenting higher trophic positions than crustaceans. In fact, three major feeding guilds have been reported in mesopelagic fishes: 1) zooplanktivores such as lanternfishes (Myctophidae) or hatchetfishes (e.g. *A. olfersii*); 2) pelagic micronektonivores such as dragonfishes (Stomiidae) or serrivomerid eels (e.g. *S. beanii*); and 3) pelagic generalists (Drazen and Sutton, 2017). Besides,  $\delta^{15}\text{N}$  values were recently shown to not only reflect the trophic position of species but also their dwelling depth. Shallower dwelling species feeding within epipelagic food webs generally have lower  $\delta^{15}\text{N}$  values than deeper dwelling (non-migratory) species, the latter being supported by  $^{15}\text{N}$ -enriched particulate organic matter (Richards et al., 2020). This phenomenon may therefore explain, at least in part, the highest  $\delta^{15}\text{N}$  values observed for the Platytroctidae species *S. koefedi* among the 15 species studied. Fish from this family would be indeed more bathypelagic than mesopelagic, being mostly caught below 700 m depth (Novotny, 2018). Although all individuals of this study were collected simultaneously at the same depth and at night within the deep scattering layer, this species may thus feed deeper than the other species during the day.

As comparison, the same ranges of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were reported in similar pelagic crustacean and fish species off the Catalan slope in the NW Mediterranean Sea (Fanelli et al., 2011; Papiol et al., 2013). Alternatively, in deep pelagic fishes from the Sulu, Celebes and Philippine seas in the Pacific, a similar range of variation for  $\delta^{15}\text{N}$  values but a



**Fig. 3.** Spectra of values for selected elements (one or two elements per type of parameters considered), with species classified in the order of increasing mean per species ( $\pm$ standard deviation) and then in the order of the Species Relative difference with the Taxon (Species RDT, in brackets (see 2.5)). The dotted black line corresponds to the mean value including both crustaceans and fish (individual sample data, N = 39), and dashed red and blue lines to mean values for crustaceans (N = 6) and fish (N = 33), respectively. Selected elements are energy density (ED) among biological parameters, phosphorus (P) among major chemical elements, arsenic (As) and copper (Cu) among essential trace elements, and cadmium (Cd) and lead (Pb) among non-essential trace elements. See Figs. S1–S4 for spectra considering all parameters and elements measured. Crustaceans are represented by squares and fish by circles, as on Fig. 1. Species full names are listed in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

higher range of variation for  $\delta^{13}\text{C}$  values was reported (Asante et al., 2010). With regard to the isotopic values of the three myctophid fish species sampled, our results were in accordance with the relatively narrow range of values (and consequently trophic positions) reported for several species of this family in the Atlantic (Olivar et al., 2019). Despite possible trophic overlap between closely related species (e.g. zooplanktivorous myctophids), the relative heterogeneity of nitrogen isotopic compositions measured within the whole community thus suggests perhaps more diverse diets and more complex trophic relationships than expected, as well as different dwelling depths for the species here sampled (see above; Richards et al., 2020). Diet data for bathypelagic fishes, for instance, remain very scarce (Drazen and Sutton, 2017) and because of its unique features, the deep sea would in fact supports a specialized fauna (Ramirez-Llodra et al., 2010).

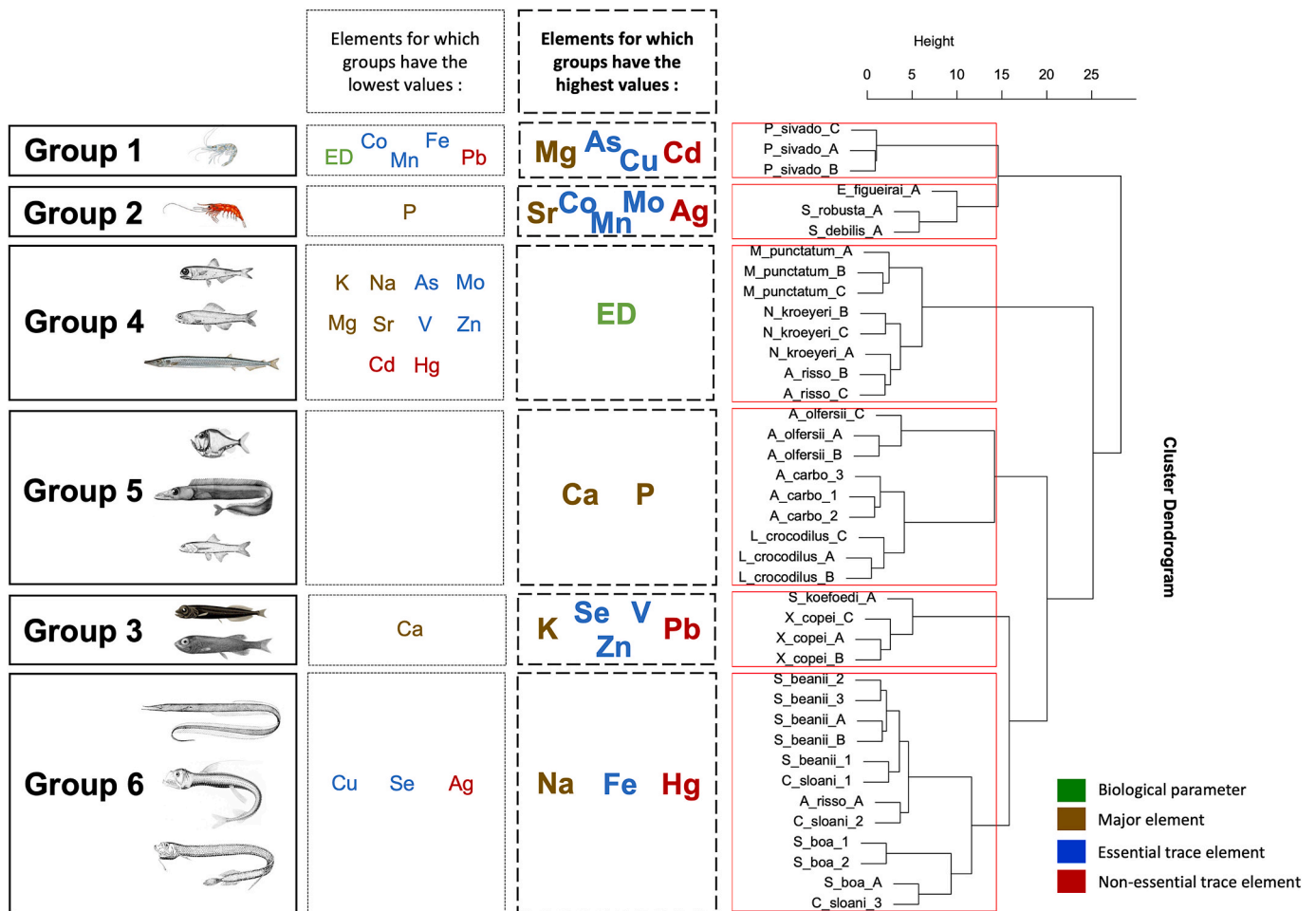
Finally, the only significant positive correlation between  $\delta^{15}\text{N}$  values and trace elements was found for Hg concentrations and was consistent with the well-documented biomagnification of this metal in aquatic food webs (e.g. Lavoie et al., 2013; Sun et al., 2020). Conversely, the significant negative correlations found between  $\delta^{15}\text{N}$  values and the other trace elements Ag, As, Cd, Co, Cu and Mo suggested a biodilution of these elements in the considered food web. However, these last results are to be moderated due to the strong difference in the concentrations observed for some elements between crustaceans and fish (i.e. for Ag, As, Cd and Cu in particular, see above). Crustaceans indeed presented the lowest  $\delta^{15}\text{N}$  values and the highest concentrations of these elements, orienting the observed relationship. When crustaceans were retired, only the significant negative correlation between  $\delta^{15}\text{N}$  values and Co was maintained (i.e. in fish), as well as the significant positive correlation between  $\delta^{15}\text{N}$  values and Hg. Finally, no significant correlations with  $\delta^{15}\text{N}$  values were observed for Fe, Mn, Se, Pb, V and Zn. While Zn is sometimes thought to bioaccumulate in (fish) food chains (Mathews and Fisher, 2008; Wang, 2002), conclusions regarding other elements and especially Cd, Pb and Ag (all of these being non-essentials elements) often differ (Cheung and Wang, 2008; Luoma and Rainbow, 2005; Reinfelder et al., 1998; Sun et al., 2020).

#### 4.2. Heterogeneity of oceanic micronekton's composition

Very few studies have investigated the concentrations of such a diversity of chemical elements in various oceanic and/or deep pelagic species, including both crustaceans and fish, as in the present study. Studies reporting metal contamination of oceanic and deep pelagic species, for instance, generally focused on Hg only (e.g. Chauvelon et al., 2012a; Choy et al., 2009; Monteiro et al., 1996; Seco et al., 2020), and/or on a particular taxon or group of species (e.g. on crustaceans: Fowler, 1986; Ridout et al., 1989, 1985; on fish: Asante et al., 2010; Cipro et al., 2018; on lanternfish: Figueiredo et al., 2020; Olsen et al., 2019).

The concentrations we measured for the different elements were globally in the same order of magnitude than the concentrations previously reported for micronektonic specimens from deep pelagic waters (Fowler, 1986; Ridout et al., 1989, 1985 for crustaceans; Asante et al., 2010; Cipro et al., 2018; Figueiredo et al., 2020; Olsen et al., 2019; Wiech et al., 2020 for fish). Silver (Ag), Cd, Co, Hg, Mn, Mo, Pb, Se and V concentrations were thus mostly below 1 mg kg<sup>-1</sup> or of a few mg kg<sup>-1</sup> dm (<10 mg kg<sup>-1</sup> dm), the order of magnitude depending on the taxon considered for Ag and Cd (i.e. crustaceans > fish, see below). Arsenic (As) and Cu concentrations were generally between a few mg kg<sup>-1</sup> (<10 mg kg<sup>-1</sup> dm) and a few tens of mg kg<sup>-1</sup> (<100 mg kg<sup>-1</sup> dm), while Sr, Fe and Zn were between a few tens (<100 mg kg<sup>-1</sup> dm) and a few hundred of mg kg<sup>-1</sup> (<1000 mg kg<sup>-1</sup> dm). Finally, as expected, Ca, K, Mg, Na and P concentrations were in the range of a thousand (1000 mg kg<sup>-1</sup> dm) to a few tens of thousands of mg kg<sup>-1</sup> (being all <100 000 mg kg<sup>-1</sup> dm).

Overall, considering both crustaceans and fish and the various indexes of data dispersion calculated, the elements that presented the greater variability were the trace elements Ag, As, Cd, Co and V, and to a



**Fig. 4.** Hierarchical clustering analysis using Ward's minimum variance method (see 2.5) and including 20 variables corresponding to a concentration per unit mass measured on whole organisms (i.e. energy density (ED) among biological parameters, 6 major chemical elements and 13 trace elements including both essential and non-essential elements). Species full names are listed in Table 1. Within a species, letters are used for samples corresponding to a pool of individuals (for small fish and crustaceans), while numbers are used for samples including one individual.

lesser extent Pb. They were closely followed by Fe, Hg and Mo in fish. However, the higher variability observed in fish compared to crustaceans for most elements and most of the indexes calculated (whether they are individual sample data- or species-based) may be explain, at least in part, by the higher number of samples analysed for fish. Among the most variable elements cited above, Ag, Cd, Hg and Pb are all considered non-essential elements for biological organisms, with no known biological role, while As, Co, Fe, Mo and V are bioactive. Species-specific trace metal regulation and/or detoxification mechanisms have been documented in aquatic animals, leading to species differences in metal bioaccumulation that can be highly significant for both essential and non-essential elements (e.g. Le Croizier et al., 2018; Luoma and Rainbow, 2005; Pan and Wang, 2009). These metal regulation and detoxification mechanisms generally involve metallothioneins or metallothionein-like proteins (Wang and Rainbow, 2010). It may also involve some essential metals (e.g. Se, Zn) in the case of the detoxification of non-essential metals such as Hg (Gerson et al., 2020; Siscar et al., 2014). Additionally, the assimilation efficiency of both essential and non-essential trace elements, which may be defined as “the fraction of ingested element or compound that is incorporated into biological tissue” (Wang and Fisher, 1999), was experimentally shown to vary considerably for a given element in both crustaceans and fish, according to i) food characteristics (quantity ingested, quality, partitioning of elements in the food, etc.), ii) physiology and feeding behavior of the animals (frequency of ingestion, ingestion rate, prey selectivity, age,

etc.) or even iii) abiotic factors (temperature, salinity, etc.) (Luoma and Rainbow, 2005; Pouil et al., 2018; Wang and Fisher, 1999). As a consequence, the bioaccumulation of the different elements and hence the elemental composition of different species within a community can greatly differ. In the case of Hg, for instance, which is a typically bioaccumulating and biomagnifying element, individuals' age, growth rate, trophic level as well as their dwelling and foraging depths were thus described as significant factors that lead to a wide range of Hg concentrations measured in marine organisms (Chauvelon et al., 2012a; Choy et al., 2009; Cossa et al., 2012). Similarly, in the case of Cd, species differences in the accumulation of this element by mesopelagic crustaceans may be such that the use of Cd as a potential dietary marker in food web studies has even been suggested (Ridout et al., 1986).

None of the biological parameters (C, N, ED) or major elements (Ca, K, Mg, Na, P, Sr) considered in the present study had a variation of several orders of magnitude among species of the community sampled, unlike the trace elements mentioned above. Several essential trace elements (namely Se, Cu, Mn, Zn when considering both crustaceans and fish) also presented a relatively low variability following the different indexes of data dispersion calculated. As major constitutive elements or trace elements with a biological role for organisms, they were indeed expected to be potentially less variable than non-essential elements, for which species may have more or less developed effective detoxification mechanisms. However, if these elements that are less variable did not present “extreme” values and hence did not induce high values of the

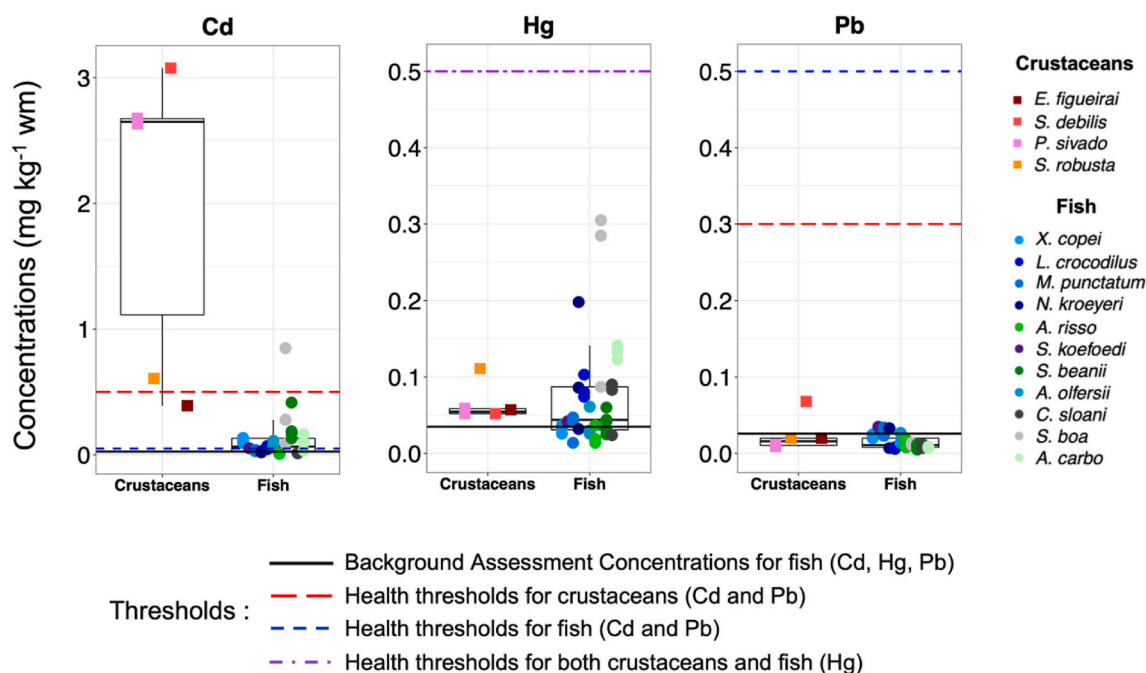


Fig. 5. Confrontation to existing European guidelines and thresholds for cadmium (Cd), mercury (Hg) and lead (Pb): Background Assessment Concentrations defined for fish by the OSPAR Commission (2009a, 2009b), and health thresholds for foodstuff defined by the European Commission (2006) See 2.5 for details on the thresholds used. Species full names are listed in Table 1.

relative dispersion indices calculated within each taxon, they presented an interesting gradient of concentrations within the community (e.g. ED, Na, Sr, P, Cu, Mg, Se). Moreover, some of these elements with a relatively low variability could be significantly different between taxa in terms of levels of concentrations (namely Sr, P, Cu), and most of them also largely contributed to the definition of groups in species clustering. For instance, copper (Cu) is one constituent of crustaceans' respiratory pigment (hemocyanin), and crustaceans therefore naturally accumulate Cu in relatively high proportions in their tissues (White and Rainbow, 1985). Besides, high concentrations of some elements such as Na can be attributed to (residual) seawater in samples (Olsen et al., 2019). Na concentrations were indeed the highest for the five species (all fish) with a body moisture percentage >80%, namely *S. beani*, the two Stomiidae *S. boa* and *C. sloani*, *S. koefedi* and *X. copei*.

#### 4.3. Implication for matter transfers towards deep-sea areas and predators

Almost all the species of the community considered in the present study are diel vertical migrators (Table 1). The only exceptions are *A. risso* and *S. koefedi*, for which no diel vertical migrations are documented with certainty, at least towards epipelagic waters at night for feeding (as documented for the other species; Table 1). Platytroctidae species such as *S. koefedi* are indeed possible migrators, but towards deeper waters in the bathypelagic zone (Novotny, 2018). Migrating communities can contribute to modify the elemental composition of surface and deep pelagic waters by active downward transport of organic matter and associated elements, as already shown for phosphorus by migrating zooplankton, for instance (Hannides et al., 2009). We may then expect that migrant nekton including fish also contribute to this modification of waters' elemental composition and/or downward active transport of matter (Turner, 2015), particularly through the faecal matter (and potentially associated elements; Fisher et al., 1991) they release during the day in the different depth layers, while they acquire this matter and/or elements through feeding in epipelagic waters at night. In this sense, diel migrating species likely play an undeniable role in the biogeochemical cycles of matter and of various

elements (both major and trace), and not just those of carbon (Davison et al., 2013; Hidaka et al., 2001). Moreover, as the intrinsic composition of the different species was here shown to differ strongly in terms of ED, major and trace element concentrations, the species composition of micronektonic communities will undeniably affect the stock of elements (whether essential/micronutrients or non-essential/toxic) available for predators feeding on these communities. Oceanic micronekton supports a number of high trophic level species including large teleost fishes such as tunas, sharks, but also marine mammals and seabirds (Choy et al., 2013; Danielsen et al., 2010; Ochoa-Acuña et al., 2002; Pusineri et al., 2008; Robinson et al., 2012; Spitz et al., 2011). In the Bay of Biscay, the continental slope – where the micronektonic community considered was collected – was thus shown to be a much more important area of prey removals by the cetacean community in comparison with the continental shelf (Spitz et al., 2018). The heterogeneity we showed in terms of micronekton's elemental composition further suggests that (prey) species (or group of species) are not interchangeable, because they are all likely to bring different chemical elements (both in terms of levels and patterns) to the predators feeding on them. Among fish, for instance, FC values were higher than 5 for most trace elements, even reaching the values of 14.6, 40.6 and 24.8 for the essential elements As, Mo and V respectively, and values of 15.2, 21.6 and 72.7 for the non-essential elements Hg, Ag and Cd respectively (Table S2). This will obviously not have the same implication in terms of contaminant transfer to a predator if one or other of these oceanic micronektonic species is consumed. However, the speciation of these elements may be different depending on the species, implying a greater or lesser bioavailability for the predator. On a global perspective, it is then anticipated that changes in prey availability could affect the quality of ingested food by predators, especially if converted in wet mass (i.e. lower quantities of energy or essential element per ingested mass unit, and possibly deficiency), and ultimately the predator fitness due to dietary deficiencies (Rosen and Trites, 2000). More broadly, an industrial exploitation of mesopelagic communities could constitute an issue for predators, in terms of potential changes in the abundance and biodiversity of these communities that support their nutritional needs and/or conversely contribute to transfer them some contaminants (i.e. non-essential elements),



especially if this exploitation is species-specific.

#### 4.4. Exceedance of some thresholds and implications for the exploitation of deep-sea resources by humans

With regards to the BACs (environmental thresholds), they are specifically defined in the fish muscle for Hg and in the fish liver for Cd and Pb (OSPAR Commission, 2009b, 2009a). Here, the whole organisms were considered, as non-commercial species so far and potential prey for oceanic/deep-sea predators (consumed whole). However, in small pelagic fish, Hg concentrations were shown to be similar between the muscle and whole organisms, while Cd and Pb concentrations in the liver are generally higher than concentrations in whole organisms (Chauvelon et al., 2019). For Cd and Pb, this would thus indicate an even greater "environmental contamination" when the concentrations measured in whole fish organisms exceed the BACs defined in the liver. With regards to health thresholds, they are specifically defined for the edible parts of seafood products (generally including muscle flesh only, although depending on species; European Commission, 2006), and whole organisms were thus considered here (both for crustaceans and fish). As mentioned above, Hg concentrations in the muscle and whole organisms were shown to be similar in small pelagic fish, but Cd and Pb concentrations in the muscle are conversely far lower to those measured in whole organisms (Chauvelon et al., 2019), which could induce a bias (i.e. higher probability of exceeding health thresholds (defined in the muscle) for Cd and Pb when analysing whole organisms instead of the muscle tissue). However, in the perspective of the potential use of whole oceanic micronekton to feed farmed animals, for instance, and thus indirectly for human consumption, we still estimated interesting to compare our data with these guidelines, as done in recent similar studies (Grimaldo et al., 2020; Olsen et al., 2019; Wiech et al., 2020).

The high levels of Cd we measured in the meso-to bathypelagic organisms analysed here (i.e. exceedance of the Cd BAC (environmental threshold) for 76% of fish samples, exceedance of Cd health thresholds for 83% of crustacean samples and 58% of fish samples) can be partly explained by the fact that Cd presents a nutrient-like behavior in the marine environment. This behavior induces a depletion of dissolved Cd concentrations in the euphotic zone followed by an increase in concentrations with depth, in areas of organic matter remineralization (sinking from the surface to the bottom). Concentrations of Cd in seawater (dissolved and/or labile Cd) are thus documented to naturally reach a maximum in the mesopelagic zone, around 800–1000 m depth (Mason, 2013 and associated references), especially in oceanic waters of the Bay of Biscay (Le Gall et al., 1999; Waelles et al., 2004, 2009), and the dissolved (ionic) or labile forms of Cd are considered to be the most bioavailable physico-chemical forms for organisms (Neff, 2002). Moreover, crustaceans may accumulate relatively high quantities of Cd in their exoskeleton in particular (Sarkar et al., 2016; White and Rainbow, 1986). In Norwegian waters, recent studies also reported Cd concentrations in mesopelagic composite samples exceeding European health thresholds (i.e. Cd concentrations above the  $0.05 \text{ mg kg}^{-1} \text{ wm}$  threshold in particular), especially when these composite samples included crustaceans (Grimaldo et al., 2020; Olsen et al., 2019; Wiech et al., 2020). Similarly to Cd, mesopelagic species and notably fish were already shown to present relatively high Hg concentrations compared to epipelagic species (Chauvelon et al., 2012a; Choy et al., 2009), due to a probably higher exposition of mesopelagic organisms to strongly bioavailable methyl-Hg in deep pelagic layers of organic matter remineralization (Cossa et al., 2009; Heimbürger et al., 2010). This could thus explain the relatively high levels of Hg we also measured in a significant part of our samples (exceedance of the Hg BAC for 67% of fish samples).

Overall, this comparison to existing European environmental and health thresholds suggests that deep-sea resources are not totally free of certain undesirables such as Cd (Grimaldo et al., 2020; Olsen et al., 2019; Wiech et al., 2020), Hg or even Pb (exceedance of Pb BAC for 15%

for fish samples), although the contamination sources of these undesirables may be at least partly of natural origin in the mesopelagic waters (see above). In the context of increasing fishing pressure on deep pelagic resources, generally considered remote from anthropogenic disturbances, analysing chemical substances of almost exclusive anthropogenic origin (e.g. polychlorinated biphenyls) on these meso-to bathypelagic communities may be then of great interest. Some studies indeed showed that organic synthetic compounds from anthropogenic origin can reach deep-sea fauna, but these studies generally focused on demersal deep-sea organisms (Koenig et al., 2013; Takahashi et al., 2010; Webster et al., 2014) and/or fauna from abyssal plains and hadal trenches (Jamieson et al., 2017; Reid et al., 2018) rather than on meso-to bathypelagic communities.

## 5. Conclusions

Although very little is known about deep-sea pelagic ecosystems, anthropogenic pressures (especially fishing) are expected to increase in the future, with the risk of altering their functioning and their role in providing ecosystem services (e.g. provisioning and regulation services). Our results emphasized the high diversity of oceanic micronekton in terms of elemental composition, especially for trace elements. Consequently, all species have not the same benefit for predators, and some changes in species abundance within this community (due to fishing pressure for instance) could significantly affect some nutrient fluxes to higher trophic levels, and ultimately nutrient cycles. Moreover, measured Cd and Hg concentrations exceeded both the BACs and the human health limits fixed by European policies in several samples, highlighting another risk for (human) consumers. Here, a limited number of samples encompassing key species of an oceanic micronekton community was sampled at once by pelagic trawl in the deep scattering layer of the Bay of Biscay, in order to reduce potential bias (e.g. spatial or temporal variations) in comparing species, but further studies should investigate the intra-specific variability of species elemental composition (e.g., temporal, spatial, ontogenetic variations). More broadly, our study has set the scene for the variability in elemental composition of the meso-to bathypelagic fauna, but it would be necessary to conduct a benefit-risk assessment for the various consumers of these deep-sea resources in the future, including humans.

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## Declaration of competing interest

The authors declare that they have no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2021.112379>.

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