
Variability of PCB burden in 5 fish and sharks species of the French Mediterranean continental slope ★

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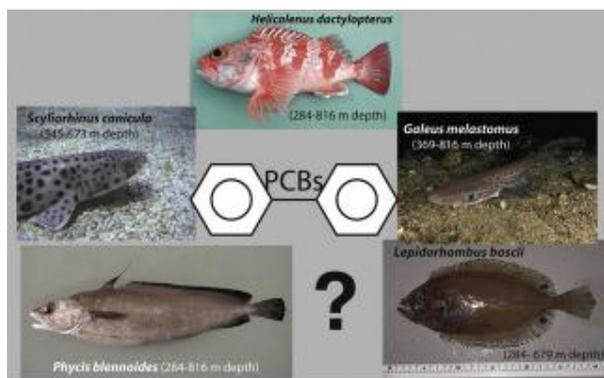
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Abstract :

Despite being generally located far from contamination sources, deep marine ecosystems are impacted by chemicals like PCB. The PCB contamination in five fish and shark species collected in the continental slope of the Gulf of Lions (NW Mediterranean Sea) was measured, with a special focus on intra- and interspecific variability and on the driving factors. Significant differences occurred between species. Higher values were measured in *Scyliorhinus canicula*, *Galeus melastomus* and *Helicolenus dactylopterus* and lower values in *Phycis blennoides* and *Lepidorhombus boschii*. These differences might be explained by specific abilities to accumulate and eliminate contaminant, mostly through cytochrome P450 pathway. Interindividual variation was also high and no correlation was observed between contamination and length, age or trophic level. Despite its major importance, actual bioaccumulation of PCB in deep fish is not as documented as in other marine ecosystems, calling for a better assessment of the factors driving individual bioaccumulation mechanisms and originating high variability in PCB contamination.

Graphical abstract



Highlights

► PCB concentrations were measured in 5 deep Mediterranean fish and shark species. ► Sharks and blackbelly rosefish had higher PCB values than megrim and forkbeard. ► These differences are consistent with specific contaminant absorption and excretion. ► Age, length and trophic position poorly explained PCB contamination. ► Actual effect of these factors should be better assessed for deep species.

Keywords : Bioaccumulation, Continental slope, Gulf of Lions, PCB

1. Introduction

Release of chemicals is one of the major threats for marine environment and alteration of ecosystem functioning due to chemicals are detected at all organization levels, from individual to ecosystem (Halpern et al., 2008; Tartu et al., 2013). This concern is high in the Mediterranean Sea, a sea rounded by industrialized and highly-populated countries (Durrieu de Madron et al., 2011).

Since they are far from anthropogenic source of contaminant, deep marine ecosystems were initially considered as lightly concerned by contamination, but several studies documented high contamination in deep species, raising concern about these ecosystems as well (Solé et al., 2001; García et al., 2000; Storelli and Perrone, 2010; Koenig et al., 2013a, 2013b; Cresson et al., 2014). In addition, some deep species have a high commercial interest, and high contamination level increase the associated risk for human health. Thus, most studies documented a potential human-health hazard, but did not look for the biological factors originating the contamination levels. The concern is notably high for polychlorobiphenyls (PCB).

PCB is a group of synthetic chemicals which were used until the 1990s before their complete ban. The chemical structure of PCB, two phenol rings with 2 to 10 chlorine substitution, made them highly resistant to degradation and highly persistent in the environment. When bound on particles, PCBs can remain for a long time, allowing their dispersal to remote zones such as deep sea, persistence in sediment after deposal (Tolosa et al., 1995) and potential integration in trophic webs. In addition, PCB have a high affinity for lipids and are considered highly-bioaccumulable, meaning that for a given individual, contamination increases with increasing length, age or trophic position (Fisk et al., 2001).

In the Gulf of Lions (NW Mediterranean Sea), the continental shelf is thin, and the continental slope is indented by several submarine canyons, meaning that deep ecosystems occur really close to the coast. Understanding the general functioning of these peculiar ecosystems has been the core of several papers, highlighting notably the pivotal role of the canyons in energy and matter transfer (Canals et al., 2013) and their vulnerability to anthropic pressures such as fisheries or contamination (Koenig et al., 2013b; Fabri et al., 2014). The biological and trophic organizations of these ecosystems were also largely studied, to understand how deep species cope with resource scarcity and interspecific competition for food. Sharks and teleost (“ray-fined fish”) received most attention due to their key role in the ecosystem functioning (Tecchio et al., 2013). Earlier analyses of stomach contents revealed that carnivory is the prevalent feeding behavior in this deep ecosystem, even if a wide range of prey can be consumed, from benthic invertebrates to fish (e.g. Macpherson, 1981; Carrassón and Cartes, 2002; Morte et al., 2002). The recent generalized use of C and N stable isotopes allow an integrated view of the food web organization, tracking organic matter fluxes all along the whole trophic webs (e. g. Polunin et al., 2001; Fanelli et al., 2013). Phytoplanktonic production falling from the euphotic zone was demonstrated as the main trophic resource supporting the trophic webs, but also as the main carrier of contaminants (Cresson et al., 2014).

Chemical contamination in fish is a complex process resulting from a balance between inputs of contaminants, mostly through diet for fish, and their further elimination (García et al., 2000; Solé et al., 2001; Trudel and Rasmussen, 2001; Cresson et al., 2014). Investigating contamination levels in fish require first understanding what organic matter sources (and associated contamination) fuel the trophic webs, and what metabolic processes are involved in detoxification. Several biological, environmental and physiological factors can affect dietary input and excretion of contaminant, potentially originating high inter- and intraspecific variability.

Thus, this study is dedicated to document the PCB levels measured in muscle of three fish (blackbelly rosefish *Helicolenus dactylopterus*, four-spot megrim *Lepidorhombus boscii*, and greater forkbeard *Phycis blennoides*) and two shark species (black-mouthed dogfish *Galeus melastomus* and lesser spotted dogfish *Scyliorhinus canicula*) collected in the continental slope of the Gulf of Lions (NW Mediterranean Sea), to compare the variability at species and individual levels, and to investigate the effect of some biological parameters (length, age and trophic position) on the ability of these species to bioaccumulate PCB.

2. Material and methods

2.1. Species selection and sampling

Sampling occurred during the MEDITS survey (International Bottom Trawl Survey in the Mediterranean Sea) performed by Ifremer (French Research Institute for Exploitation of the Sea) in June 2013 (R/V L'Europe). MEDITS survey is operated annually since 1994, following a standardized protocol designed to produce information on demersal species of the continental shelf and the upper slope of the whole Mediterranean (Bertrand et al., 2002). Stations considered in the present study are located on the upper slope of the Gulf of Lions continental shelf, at depths ranging between 284 and 816 m, and in the vicinity of the submarine canyons (Fig. 1).

Three fish (blackbelly rosefish *Helicolenus dactylopterus*, four-spot megrim *Lepidorhombus boscii*, and greater forkbeard *Phycis blennoides*) and two shark species (black-mouthed dogfish *Galeus melastomus* and lesser spotted dogfish *Scyliorhinus canicula*) were targeted on the basis of their similar high trophic position, their abundance and their importance in slope and canyons community functioning (Carrassón and Cartes, 2002; Morte et al., 2002; Fanelli et al., 2013; Goujard et al., 2013; Papiol et al., 2013; Tecchio et al., 2013; Fabri et al., 2014; Cresson et al., 2014). In addition, there is a great concern for the chemical contamination in deep-living fish species, the biological and ecological specificities of which, e.g. longer lifespan or reduced growth rate, made them at risk for chemical contamination, as confirmed by high values recorded in those species previously (García et al., 2000; Storelli et al., 2003b, 2006; Cresson et al., 2014). A high contamination level in these species may also represent a human hazard, since they are targeted by fisheries and enter human diet locally. The total sampling comprised 72 individuals (Tab. 1)

2.2. Samples preparation

Individuals were stored frozen on board and thawed at laboratory. Sex was determined based on external morphology for sharks (presence of claspers for male individuals) and on macroscopic observation of the gonads for teleosts. Individuals were measured (total length, to the nearest mm) prior to the dissection of two samples of muscle without skin. Dorsal muscle (~1g wet mass) was sampled for isotopic analyses, following international accepted procedures and previous laboratory experience (Pinnegar and Polunin, 1999; Cresson et al., 2014, 2015). A whole filet (~ 15 g wet mass) was dedicated to PCB analyses since concentration in muscle is considered to reflect a more long-term exposure to contaminant than in liver or gonads (Albaigés et al., 1987; García et al., 2000; Koenig et al., 2013b). Muscle samples were stored frozen before freeze-drying and grinding. Samples dedicated to PCB analyses were stored in pre-burned (450°C, 6h) aluminum foil, to prevent any organic contamination. Sagittal otoliths were extracted from cranial cavity to determine age of teleosts. Otoliths (literally „ear-stones“) are paired calcified parts of the inner ear of teleosts only (not of sharks) that grow continuously during fish life forming annual increments (Panfili et al., 2002). Annual increments were counted under a binocular microscope (50x

magnification, transmitted light) connected to a digital camera and using a dedicated image-analysis software (TNPC, <http://www.tnpc.fr/en/tnpc.html>).

2.3. Stable isotope and PCB analyses

Individuals' position in the trophic web was assessed by measuring carbon and nitrogen stable isotope ratios (hereafter $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ respectively) with a continuous flow mass spectrometer (Delta V Advantage, Thermo Scientific, Bremen, Germany) coupled with an elemental analyzer (FlashEA 1112 Thermo Scientific, Milan, Italy). Results are expressed following the standard δ notation: $\delta X = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 10^3$ where X is ^{13}C or ^{15}N , and R the ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ respectively. One measurement of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was performed for each individual. Experimental precision was < 0.1 ‰ (based on repeated measurement of acetanilide standard). C:N ratios were calculated from the %C and %N measured with the elemental analyzer. High C:N ratios (> 4) is considered as a threshold for high lipid content, a potential bias of $\delta^{13}\text{C}$ measurement (Post et al., 2007; Hoffman and Sutton, 2010). Carbon isotopic ratios measured in three individuals (2 *S. canicula* and 1 *G. melastomus*) were thus normalized to correct for the effect of high lipid content, following the protocol specifically developed by Hoffman and Sutton (2010) for deep-sea fishes and resulting in 0.6, 1.6 and 2 ‰ $\delta^{13}\text{C}$ increase respectively.

PCBs burdens were determined following analytical procedures in accordance with standard NF ISO 10382 and XP X33-012 and previously described in Cresson et al. (2015). A precisely weighed amount of the powdered muscle, 2 g dry mass, was extracted with acetone, pentane and iso-octane in acid medium with liquid-solid extraction ultrasonic extraction method. GC-MS/MS determination was carried out on a GC 7890A (Agilent) with mass spectrometry detection (MS/MS, 7000GCTQD, Agilent), equipped with capillary column HP MS 30 m x 0.25 mm i.d. x 0.25 μm film thickness (19091S-433). Nine PCB congeners were analyzed in this work, the 7 PCB indicators defined by ICES (CB 28, 52, 101, 118 138, 153, 180 – the sum of their concentrations is referred hereafter as $\Sigma 7$) in addition to two "dioxin-like" congeners (CB 105 and 156), describing compounds with 3 to 7 chlorine molecules. One analysis was performed for all individual. Concentrations were expressed in ng g^{-1} dry mass. Measurement accuracy was checked by the analysis of certified samples, with standard deviations of ~ 20 % for all congeners. During the analysis of the real samples, analytical blanks were systematically measured every 10 samples. The blank concentrations were always below the concentrations of the lowest standards of all congeners. Finally, a reference material (SRM 2974a, mussel tissue) was repeatedly analyzed. PCB recoveries varied between 67 and 111 %.

Two univariate PERMANOVA (Anderson, 2001) were run to test for inter-specific differences in CB153 and $\Sigma 7$ concentration in muscle. CB153 concentrations and $\Sigma 7$ were $\log(X+1)$ transformed prior to the generation of Euclidean distance similarity matrices. P-values were calculated by 9999 random unrestricted permutations of raw data (Anderson, 2001). Significance was set at $\alpha = 0.05$. All of these analyses were performed using the PRIMER 6 software and the PERMANOVA add-on (Clarke and Warwick, 2001). In addition, a clustering analysis, based on Ward grouping method and Euclidean distance, was performed to group species with similar contamination profile, using average concentration for each congener and each species as input data. Analysis was run with "cluster" package and R statistical software (Maechler et al., 2013; R Core Team, 2015).

3. Results and discussion

PCB contamination profiles were largely dominated by highly chlorinated congeners. Hexa- and hepta chlorinated congeners always represented more than 90 % of the total PCB

burden (Tab. 2) and were mostly represented by CB 153, CB 180 and CB 138 (Fig. 2). CB 153 concentration was strongly correlated with $\Sigma 7$ ($R^2 > 0.98$, p-value $< 10^{-5}$ for all species), a classical result in marine fishes (e.g. Harmelin-Vivien et al., 2012) that confirms CB 153 as an efficient proxy of the PCB burden. On the contrary, all CB 28 (3 Cl congener) concentrations were below detection limits, and CB 52 (4 Cl congener) was detected in *H. dactylopterus* only. Predominance of highly chlorinated compounds is consistently observed in Mediterranean marine fish (García et al., 2000; Storelli et al., 2003a, 2007, 2008; Bodiguel et al., 2008; Storelli, 2008; Koenig et al., 2013a, 2013b; Cresson et al., 2015) and is explained by the faster degradation of lowly-chlorinated compounds, whereas highly-chlorinated compounds are hard to degrade and remain for a long time in the environment (Tolosa et al., 1995). Nevertheless, the predominance of congeners CB 153, 138 and 180 is a specificity of deep species, explained by the even higher resistance of these compounds to degradation processes, in environment or in biological tissues (Solé et al., 2001; Storelli et al., 2007; Koenig et al., 2013a). This contamination profile might also track the importance of resuspended sedimentary organic matter as a source of organic matter and of contaminants for deep trophic webs, since deep sediment profile is also dominated by of highly-chlorinated congeners (Tolosa et al., 1995; Koenig et al., 2013b).

Whatever the species, average CB 153 concentrations ranged between 3.83 and 12.69 ng g⁻¹ dry mass (Tab.2). Direct comparison of those values with others found in literature is complex or even impossible as methods used differ between studies. For example, it is impossible to compare values measured in lipid-rich tissues, like liver or female gonads, with values from lipid-poor tissues like muscle, since PCB has a high affinity for lipids. In addition, fast turnover of liver tissues tracks a recent contamination, whereas contamination in muscle is thought to reflect more long-term exposure (Albaigés et al., 1987; García et al., 2000; Koenig et al., 2013b). Similarly, PCB burden is described in literature with different variables (CB 153, $\Sigma 7$ or the sum of 20 to 40 congener's concentrations) or units (dry mass, wet mass or lipid mass). Nevertheless, values presently measured in *P. blennoides* and *L. boscii* appeared consistent with previous measurements in individuals of these species sampled in deep zones off Spanish Mediterranean coast (García et al., 2000; Solé et al., 2009a), even if this comparison requires assuming an empirical conversion factor between concentrations expressed relatively to dry mass or wet mass (*i.e* $dm = 5 \times wm$)

The clustering analysis separated species in two groups, based on significantly different PCB burdens between species (PERMANOVAs $F = 7.10$, p-value < 0.001 for CB 153; $F = 9.19$, p-value < 0.001 for $\Sigma 7$) and consistently with the expected contamination pattern (Fig. 3). The first group included three species with high average concentrations, the teleost *H. dactylopterus* and the two shark species *G. melastomus* and *S. canicula*. *H. dactylopterus* was subsequently separated from shark species in a sub-cluster, as it exhibited significantly higher concentrations. *L. boscii* and *P. blennoides* belonged to the other group, due to their lower CB 153 concentrations. High PCB concentrations are classical in *G. melastomus* and *S. canicula* in the Mediterranean Sea (Storelli et al., 2003b, 2006). Experimental studies demonstrated that the rate of contaminant accumulation is higher for sharks than for teleosts, notably for *S. canicula* (Jeffree et al., 2006, 2010). Unfortunately, this was tested for metals and radionuclides only and has to be assumed for organic pollutants. Similarly, in all studies investigating contamination in fish communities, blackbelly rosefish (*H. dactylopterus*) is always the species with the higher or one of the highest contaminant burdens, whether organic or metallic, and notably when compared with *P. blennoides* or *L. boscii* (Storelli, 2008; Storelli et al., 2003a; Cresson et al., 2014). Despite being commonly observed, factors causing high concentrations in *H. dactylopterus* were not specifically investigated but might be linked with specific differences in enzymatic detoxification metabolism. These works, and others, demonstrated that bioaccumulation may also be taxonomically driven, and that species with taxonomic similarities may share similar contaminant bioaccumulation and excretion abilities. Future work, with a larger sample size, in laboratory or in the field may

nevertheless be needed to support further this hypothesis, notably when comparing teleost species.

Level of contaminants in an organism result from a balance between contaminant exposure, mostly through diet for fish (García et al., 2000; Cresson et al., 2014), and excretion. Accumulation occurs when inputs of contaminants exceeds elimination (Trudel and Rasmussen, 2001). PCB is a class of hydrophobic compounds and their excretion requires some chemical transformations, like the addition of functional groups (Koenig et al., 2012). This process is mediated by the super-family of cytochrome P450 enzymes (classically abbreviated as CYP) in fish. Level of activity of CYP pathway reflects the contamination pressure but several biological, physiological and environmental parameters affect this activity (Sarasquete and Segner, 2000; Amato et al., 2006; Solé et al., 2010; Koenig et al., 2012). It has been previously demonstrated that CYP activity is lower for sharks than for teleosts (Gorbi et al., 2004; Solé et al., 2009b, 2010), another factor that may explain high values measured for *S. canicula* and *G. melastomus*. In addition, the lower CYP activity measured for *S. canicula* than for *G. melastomus* may originate the difference in PCB contamination between these two species (Solé et al., 2009b). Although not measured in the present study, it may also be hypothesized that a lower CYP-mediated oxidation activity in *H. dactylopterus* may cause higher contaminants burdens than in other species. In a study comparing fish sampled in polluted and reference sites, Amato et al (2006) observed a 2 times higher CYP activity for *H. dactylopterus* in polluted sites whereas it was 3.5 times higher for co-occurring European conger *Conger conger*, demonstrating a potentially lower contaminant oxidation and excretion in *H. dactylopterus*. In addition, facing exposure to several PAH, another class of organic pollutants the detoxication of which is mediated by CYP enzymes, *H. dactylopterus* demonstrated a lower level of biliary PAH-type metabolites. This lower value may thus potentially confirm the lower metabolic detoxication activity for this species, and explain the higher accumulation of PCB (Insausti et al., 2009).

Similarly, the specific-detoxification activity might explain why *P. blennoides* and *L. boscii* exhibit the lower PCB burdens of the dataset whereas being particularly exposed. This is notably true for *L. boscii*, a flatfish exposed to contamination through its diet but also through its skin in direct contact with sediment (García et al., 2000). Regarding, *P. blennoides*, this species is sometimes nicknamed „sea-rat“ since it is classically observed in highly-polluted environments and is commonly included in contamination studies (García et al., 2000; Solé et al., 2006, 2009a, 2009b, 2010; Storelli et al., 2008; Insausti et al., 2009; Cresson et al., 2014). Strong detoxifying activity was previously measured in *P. blennoides* and was proposed as an adaptive mechanism developed to cope with repeated exposure to contaminant (García et al., 2000; Solé et al., 2006). Similarly, Insausti et al (2009) observed higher level of FAC - Fluorescent Aromatic Compounds, considered as a proxy of PAH detoxifying activity - for species living just above the sediment, among those *P. blennoides*. Such a mechanism was not specifically investigated in *L. boscii* but might also be proposed as a response to the repeated exposure to contaminant in sediment. It can explain why *L. boscii* exhibit higher CYP activity than co-occurring *Callionymus lyra*, whereas both species were sampled in same sites and thus exposed to the same contamination levels (Martínez-Gómez et al., 2006).

This study was also an opportunity to investigate factors driving individual bioaccumulation pattern and potentially explaining the large interindividual variability. Three biological descriptors were considered, length, trophic level (inferred from $\delta^{15}\text{N}$) and age. PCBs are classically considered highly bioaccumulable contaminants, meaning that contamination increases within a given organism with increasing age, length or trophic position. Surprisingly, an extensive review of the literature demonstrated that this paradigm has been poorly confirmed by dedicated analyses for species collected in deep marine environments (*i.e.* lower than 300 m depth, Table S1). In the 28 papers dedicated to this subject we could find, actual relationship between PCB concentration and length, age or trophic level is assessed in only five and not in the Mediterranean. Results of those papers are contrasted,

and bioaccumulation of PCB is not always observed. In addition, most studies analyzed pools of similar individuals, as required by the amount of matter needed for chemical analyses. Considering pools of individuals and/or determining average age or trophic position for one species is sufficient to compare PCB levels in species at different level of the trophic web, and to confirm bioaccumulation from the base to the top of this web. Nevertheless, this methodology does not allow investigating individual variation of the accumulation mechanisms. In the present paper, length, age or trophic level were not sufficient to explain this variability (Fig. 4). Even if the present sample size is rather low (but consistent with most other papers, Table S1) and that a further sampling effort might be needed to reach a formal conclusion, some particular features of deep sea environments and species might drive the blurred bioaccumulation pattern observed in the present paper and others and might represent important parameters to consider when investigating variability of PCB burden (Tab. S1).

As demonstrated earlier, PCB excretion is mediated by the activation of CYP pathway, the intensity of which differs between species but also at individual level. Biological parameters like sex, reproductive status, food availability or diet were notably demonstrated to significantly affect the enzymatic activity, and consequently the PCB excretion (Porte et al., 2000; Solé et al., 2009b, 2010; Koenig and Solé, 2012). Combining PCB measurement in muscle and liver could provide insight into specific and individual variation in detoxification activity (García et al., 2000). Similarly, lipid content was not investigated in the present study despite being a major driving factor that may differ between tissues and between individuals and drive contamination discrepancies (García et al., 2000; Koenig et al., 2013b). Sex was also demonstrated to significantly affect PCB bioaccumulation. Lower bioaccumulation is commonly observed in females and is explained notably by the transfer of PCB to gonads during oogenesis and further excretion by spawning (Bodiguel et al., 2009; Cresson et al., 2015). In the present, no clear sexual pattern could be observed (Fig. 4), but the unbalanced sex-ratio and the differences in mean length and age between males and females (*e.g.* for *G. melastomus* females, more contaminated but notably larger than males) may represent confounding factors that should be further investigated, considering a larger dataset with individuals of same length and/or age of both sexes. Finally, another blurring factor might be the importance of canyons as feeding areas for some individuals. Submarine Mediterranean canyons were demonstrated to act as channels for particles-bound contaminants, resulting in higher contamination for individuals feeding in the canyons (Koenig et al., 2013a). The 5 species considered here were abundantly observed in canyons during underwater surveys (Fabri et al., 2014) but were collected outside, meaning that they dwell in both environments. Migration between slope and canyon was already proposed as an hypothesis to explain the PCB profile in species living in deep environments (Solé et al., 2009a; Koenig et al., 2013a).

Despite marked interspecific differences in PCB burdens potentially resulting from species-specific accumulation and excretion abilities, large intraspecific differences were observed, precluding from a formal conclusion on bioaccumulation patterns. So far, studies on PCB burden in deep Mediterranean species were focus on human-health (*i.e.* documenting a potential risk of human contamination while consuming fish) or on metabolic detoxification activity, but poorly focusing on biological mechanisms underlying individual variability in contamination. Results of this preliminary study are consistent with the importance of detoxification mechanisms at specific level, and proposed some biological and ecological factors that may drive PCB burden in species dwelling in deep marine zones. Investigating the actual role of each of these parameters on fish contamination is a prerequisite to decipher the large variability in PCB burdens, and a potential use of PCB as tracers of ecological mechanisms.

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Figures

Figure 1: Sampling locations and depths. Names refer to the submarine canyons close to the trawling stations

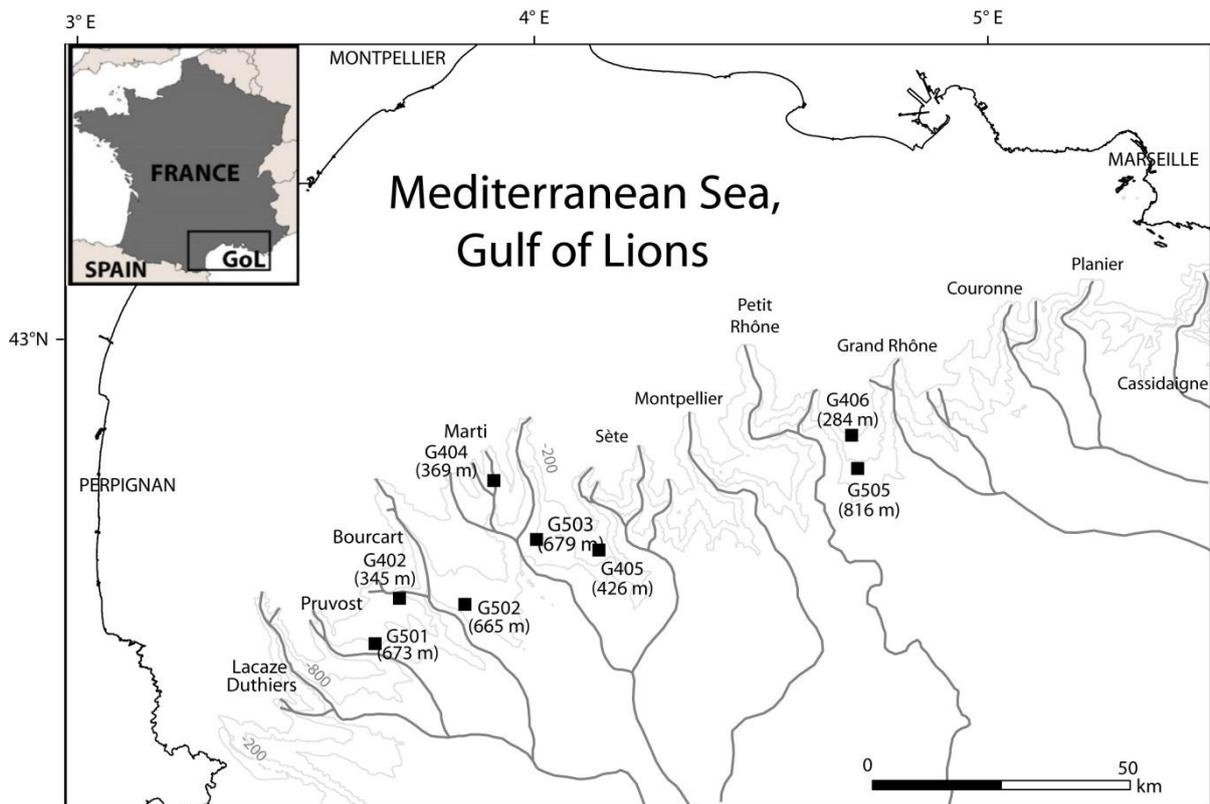


Fig.2: Relative proportion of each congener in the total PCB burden for all species. Number of Cl substitution is provided for each congener.

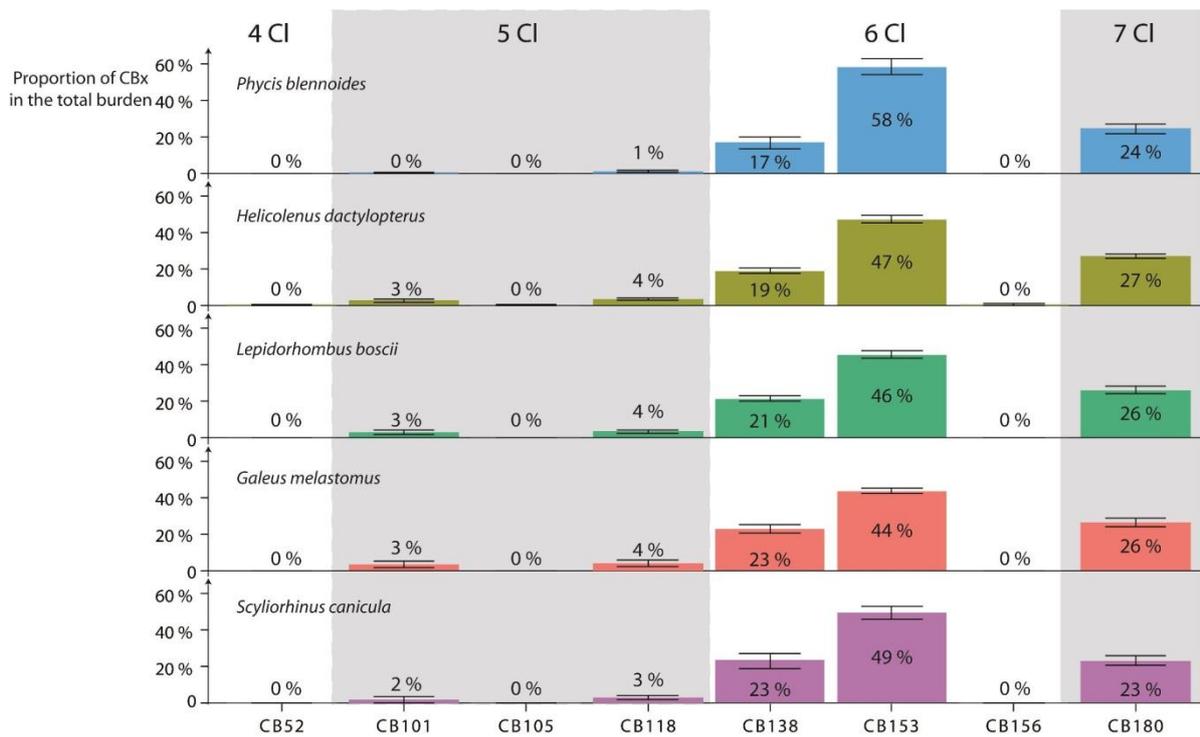


Fig.3: Hierarchical clustering tree based on the average concentrations of the 9 PCB congeners for each species, using Euclidean distance and Ward method

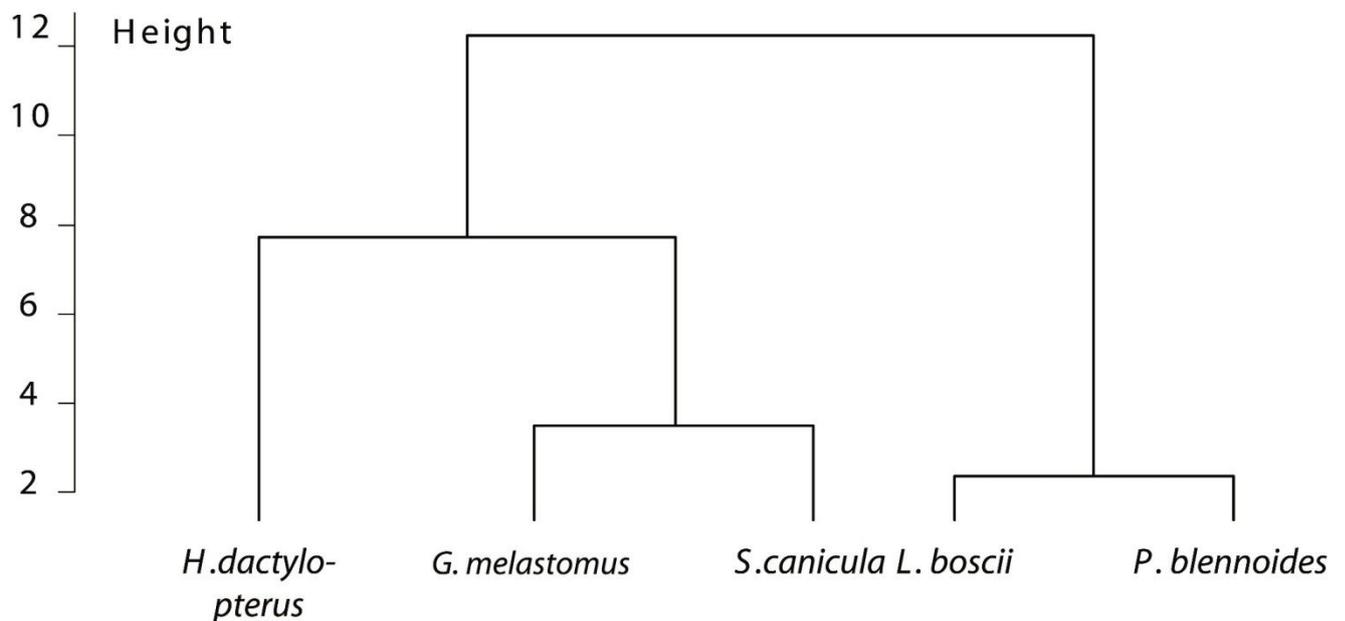


Fig. 4: Relationships between CB153 concentration (ng g⁻¹ dry mass) and biological descriptors: age (in years old, a) total length (mm), and trophic level ($\delta^{15}\text{N}$, ‰) depending on the sex of the individuals (shape of the symbols: circle: female; triangle: males; squares: unidentified) Parameters of the linear regression between CB 153 concentration and descriptor are detailed in each plot. Age could not be determined for *G. melastomus* and *S. canicula*.

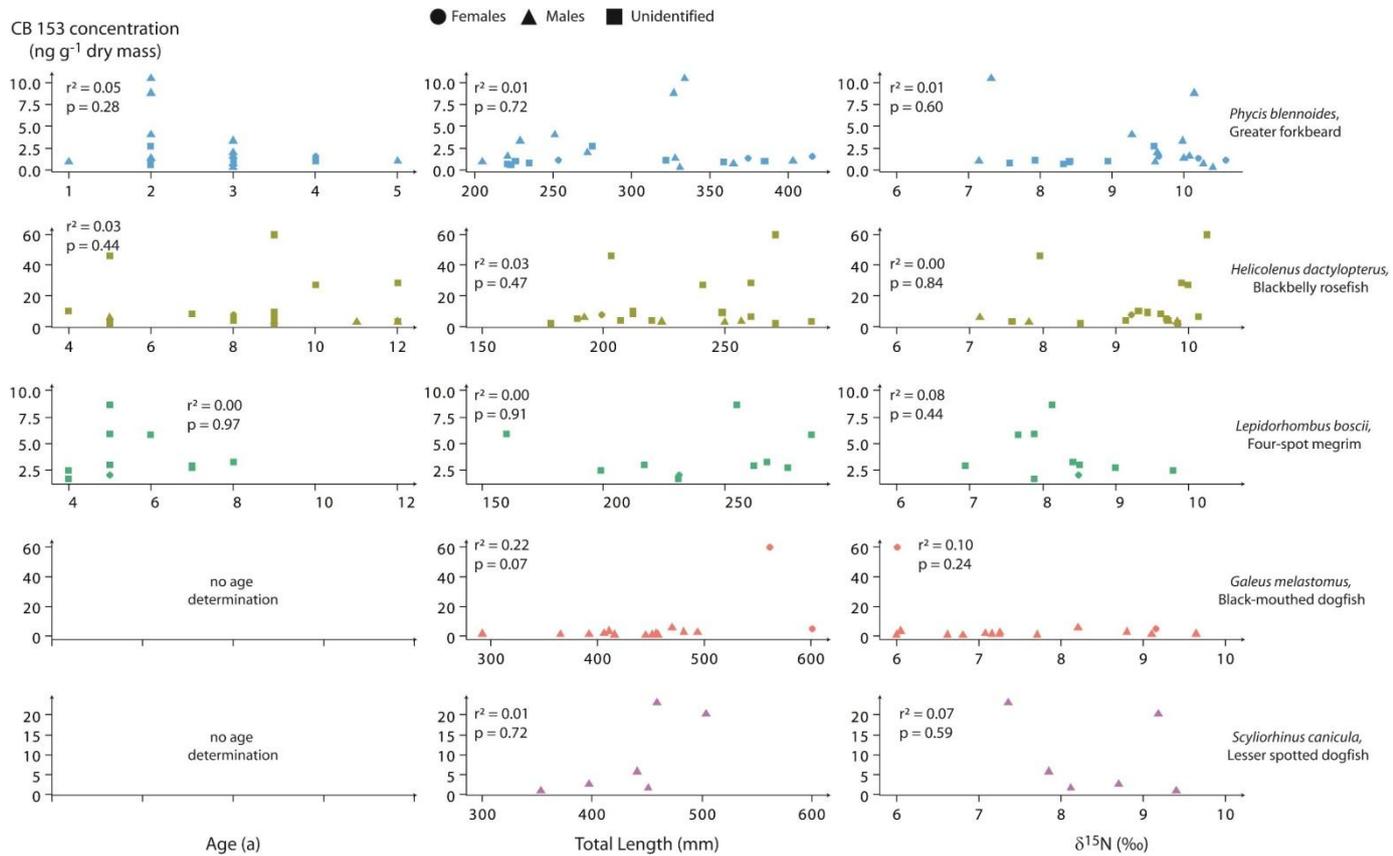


Table 1: Number of individuals, sex-ratio (females, males and non-identified), total length (mean, standard deviation, minimum and maximum values), age range (in years, a), stable isotope ratios (mean and standard deviation), mean C/N ratio (mean and standard deviation). Species were sorted based on taxonomical criterion, the three teleosts (“ray-fined fish”) first and the two shark species then (grey background). Species authority, taxonomic information and common name are from the World Register of Marine Species database (www.marinespecies.org).

Order, family	Scientific name	Common name	n	Sex-ratio (F/M/NI)	Depth range (m)	Total length (mm)		Age (a) Min – Max	$\delta^{13}\text{C}$ (‰) Mean \pm sd	$\delta^{15}\text{N}$ (‰) Mean \pm sd	C/N Mean \pm sd
						Mean \pm sd	Min – Max				
Gadiformes, Phycidae	<i>Phycis blennoides</i> , (Brünnich, 1768)	Greater forkbeard	22	3/11/8	284 – 816	298 \pm 68	205 – 415	1 – 5	-18.77 \pm 0.54	9.21 \pm 1.08	3.4 \pm 0.2
Scorpaeniformes, Sebastidae	<i>Helicolenus dactylopterus</i> , Delaroche, 1809	Blackbelly rosefish	19	1/4/14	284 – 816	231 \pm 32	178 – 286	4 – 12	-18.39 \pm 0.49	9.19 \pm 0.93	3.4 \pm 0.2
Pleuronectiformes, Scophthalmidae	<i>Lepidorhombus boscii</i> , Risso, 1810	Four-spot megrim	10	1/0/9	284 – 679	238 \pm 39	160 – 286	4 – 8	-18.70 \pm 0.60	8.27 \pm 0.78	3.3 \pm 0.2
Carcharhiniformes, Pentanchidae	<i>Galeus melastomus</i> , Rafinesque, 1810	Black-mouthed dogfish	15	2/13/0	369 – 816	447 \pm 75	292 – 601	nd	-18.18 \pm 0.51	7.52 \pm 1.20	3.5 \pm 0.4
Carcharhiniformes, Scyliorhinidae	<i>Scyliorhinus canicula</i> , (Linnaeus, 1758)	Lesser spotted dogfish	6	0/6/0	345 – 673	434 \pm 53	353 – 504	nd	-19.65 \pm 0.63	8.43 \pm 0.80	4.1 \pm 0.9

Table 2: PCB contamination pattern. Mean, standard deviation, minimal and maximal values for CB 153 and $\Sigma 7$ (sum of congeners 28, 52, 101, 118, 138, 153, and 180, ICES indicator PCBs), and mean and sd values for each PCB class, depending on the number of Cl substitutions. All concentrations are expressed in ng g⁻¹ dry mass. Mean CB153 and $\Sigma 7$ values with different letters are significantly different. <DL: below detection limits. Species were sorted based on taxonomical order, teleost first and shark then (See Tab. 1).

Species	CB 153			$\Sigma 7$			3Cl (CB 28)	4 Cl (CB 52)		5 Cl (CB 101, 105, 118)		6 Cl (CB 138, 153, 156)		7 Cl (CB180)	
	Mean	sd	Min-Max	Mean	sd	Min-Max		Mean	sd	Mean	sd	Mean	sd	Mean	sd
Teleosts															
<i>Phycis blennoides</i>	2.14 ^a	2.59	0.30 – 10.43	3.94 ^a	4.80	0.30 – 18.96	<DL	<DL	0.12	0.27	2.86	3.42	0.96	1.12	
<i>Helicolenus dactylopterus</i>	12.69 ^d	16.14	2.32 – 59.79	25.28 ^d	31.06	5.07 – 115.04	<DL	0.04	0.07	1.20	1.22	16.94	20.51	7.31	10.05
<i>Lepidorhombus boscii</i>	3.83 ^b	2.22	1.61 – 8.62	8.24 ^b	4.28	3.82 – 16.83	<DL	<DL	0.53	0.32	5.57	3.02	2.14	1.08	
Sharks															
<i>Galeus melastomus</i>	5.96 ^{ab}	15.14	0.62 – 60.38	12.76 ^{ab}	31.14	1.43 – 124.65	<DL	<DL	0.61	0.92	8.19	19.59	4.03	10.93	
<i>Scyliorhinus canicula</i>	9.01 ^{bd}	9.97	0.88 - 23.08	17.00 ^{bd}	18.11	1.88 -44.83	<DL	<DL	0.60	0.49	12.17	12.76	4.23	5.13	

Supplementary table 1

Bibliographical review of PCB contamination in deep sea teleosts and chondrychtian, whatever the tissue analyzed (liver, gonads, gill, digestive tract etc.). The number between brackets after each species name is the sample size (nd: no data available in the paper). Papers were searched using “fish deep PCB” in Web of Science and Google Scholar. Papers were conserved if data about PCB contamination and biological factor (length, mass, age or trophic level) were provided. In addition, papers were conserved if sampling occurred below 300 m depth or if species were explicitly described as “deep”.

Papers were then divided in three categories, depending on how the contamination vs. biological factor is considered. Studies performing coupled analyses of contamination and biological parameters and then statistically assessing the relationship for all individuals were classified in the first group (“yes”). Second group (“partial”) gathers studies where a potential effect of biological parameters was tested but from bibliographical data about biological parameter and/or at species level (eg. attributing a mean age or trophic level to each species, based on previous studies about species’ diet or expected age-at-length). Finally, studies in the third group (“no”) did not explicitly measure relationship between contamination and biological parameter, even if length, age or trophic level is discussed as a potential driver of the patterns observed. Papers were sorted based on the group they belong to and then by chronological order.

Sea/Ocean	Species (n)	Sampling location	Sampling depth (m)	Sampling date	Biological factor measured	Relationship assessment (Yes/Partial/No) and major conclusions	Reference
Arctic Ocean	<i>Centroscyllium fabricii</i> , <i>Hydrolagus afinis</i> , <i>Brosme brosme</i> , <i>Antimora rostrata</i> , <i>Macrourus berglax</i> , <i>Sebastes</i> spp, <i>Reinhardtius hippoglossoides</i> , <i>Anarhichas denticulatus</i> (a)	Davis Strait, off Greenland (~61 to 63°N, 50 to 52°W)	200 - 2100	May-June 1992	Length	Yes. Linear correlation between length and PCB concentration, with r^2 ranging between 0.26 and 0.50 depending on the species	(Berg et al., 1997)
Atlantic Ocean	<i>Lophius piscatorius</i> (38 ^b), <i>Aphanopus carbo</i> (54 ^b)	<i>Rockall Trough</i> , off West Scotland (56 to 60°N - ~10°W)	400 - 1150	Sept-Oct 1998	Length and mass	Yes. Significant correlation between length and Σ PCB in <i>L. piscatorius</i> but not in <i>A. carbo</i>	(Mormede and Davies, 2001)
Arctic Ocean	<i>Somniosus microcephalus</i> (15), <i>Reinhardtius hippoglossoides</i> (4)	Cumberland Sound (65°58'N, 66°41'W)	~554	Apr 1999	Length, mass, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$	Yes. PCB concentration was significantly influenced by length, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$	(Fisk et al., 2002)
Pacific Ocean	20 species, with sample size ranging between 1 and 23 individual by species ^c	East China Sea,	89 - 512	Oct-Nov 2001, Nov 2002, Nov 2003	Length, mass, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$	Yes. Good correlation between species trophic level (inferred from $\delta^{15}\text{N}$) and PCB contamination after lipid correction	(Tanabe et al., 2005)

Sea/Ocean	Species (n)	Sampling location	Sampling depth (m)	Sampling date	Biological factor measured	Relationship assessment (Yes/Partial/No) and major conclusions	Reference
Atlantic Ocean	<i>Coryphaenoides rupestris</i> (51)	Porcupine Sea Bight (49°N to 52°N - ~12°W)	1000 - 1900	Sept 2000 to Sept 2002	Mass, age inferred from age- mass relationships	Yes. Weigh do not explain much of the PCB variability	(Lemaire et al., 2010)
Mediterranean Sea	<i>Mora moro</i> (nd)	Gulf of Lions, NW Mediterranean Sea (4 stations)	986 - 1136	Spring 1996	Length and mass, with individuals of similar length grouped for analyses	Partial : comparison of PCB burden between length-based groups, but no calculation of regression between parameters	(Solé et al., 2001)
Pacific Ocean	18 species, with sample size ranging between 1 and 10 individual by species ^d	Tokohu, Off Eastern Japan coast	150 - 1300	Dec 1995	Length and mass	Partial , discussion about the effect of trophic level, based on bibliographical knowledge about species diet	(de Brito et al., 2002)
Pacific Ocean	<i>Zenion hololepis</i> (3), <i>Rouleina</i> sp. (6), <i>Bathygadus</i> sp. (2), <i>Lamprogrammus niger</i> (1), <i>Synaphobranchus brevidorsalis</i> (1) <i>Malakichthys elegans</i> (3), <i>Glyptopidium japonicum</i> (3), <i>Lioscorpius longiceps</i> (3)	Sulu Sea, West Philippines	292 - 1015	Nov-Dec 2002	Length, mass, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$	Partial. The relationship between $\delta^{15}\text{N}$ values and contamination is slightly discussed but no regression parameters are provided	(Ramu et al., 2006)
Mediterranean Sea	<i>Trachyrinchus trachyrinchus</i> (307 ^b), <i>Coelorhynchus coelorhynchus</i> (203 ^b)	Adriatic Sea	nd ^e	June - Sept 2006	Length and mass	Partial. Bibliographical data about diet was used to discuss higher values in <i>T. trachyrinchus</i>	(Storelli et al., 2009)
Mediterranean Sea	<i>Alepocephalus rostratus</i> (30), <i>Lepidion lepidion</i> (20), <i>Aristeus antennatus</i> (3 pools), <i>Coelorinchus mediterraneus</i> (25)	Blanes Canyon, NW Mediterranean (41°15N, 2°504E)	900 - 1500	Nov 2008 and Feb 2009	Length and mass - $\delta^{15}\text{N}$ and age-at-length from previous studies	Partial: PCB pattern is consistent with bibliographical data about species' age and trophic level	(Koenig et al., 2013b)

Sea/Ocean	Species (n)	Sampling location	Sampling depth (m)	Sampling date	Biological factor measured	Relationship assessment (Yes/Partial/No) and major conclusions	Reference
Atlantic Ocean	<i>Lopholatilus chamaeleonticeps</i> (16)	Atlantic Bight, Lyndonia (40°21'N - 67°31'W) and Hudson (39°03'N-72°37'W) canyons	150 - 200	Summer 1981 and 1982	Length	No. Length (and consequently inferred age) was proposed as an explanation of markedly different contamination between both sites, but the actual relationship was not assessed	(Steimle et al., 1990)
North Sea	<i>Etmopterus spinax</i> (10), <i>Molva molva</i> (2), <i>Brosme brosme</i> (6), <i>Coryphaenoides rupestris</i> (1)	Nordfjord, Norway ~62°N / 6°W	~400	June 1993	Length	No	(Berg et al., 1997)
Pacific Ocean	<i>Hoplobrotula armata</i> (2), <i>Helicolenus hilgendorfi</i> (2), <i>Zenopsis nebulosa</i> (1), <i>Deania calcea</i> (2), <i>Chlorophthalmus albatrossis</i> (15), <i>Glossanodon semifasciatus</i> (7)	Suruga Bay, Japan	200 - 740	Oct-Nov 1993, Oct 1994	Length and mass	No	(Lee et al., 1997)
Pacific Ocean	<i>Zenopsis nebulosa</i> (2), <i>Deania calcea</i> (2), <i>Helicolenus hilgendorfi</i> (2), <i>Pterothrissus gissu</i> (3), <i>Coelorinchus</i> sp. (2), <i>Hoplobrotula armata</i> (2), <i>Chlorophthalmus albatrossis</i> (15), <i>Glossanodon semifasciatus</i> (7)	Suruga Bay, Japan	250 - 740	Oct-Nov 1993, Oct 1994	Length and mass	No	(Takahashi et al., 1998)
Mediterranean Sea	<i>Lepidion lepidion</i> (16), <i>Coryphaenoides guentheri</i> (24), <i>Bathypterois mediterraneus</i> (36)	NW Mediterranean Sea, 3 stations off Spanish coast	1500 - 1800	nd	Length, mass, condition factor and liver somatic index	No	(Porte et al., 2000)
Mediterranean Sea	<i>Lepidorhombus boschii</i> (nd), <i>Phycis blennoides</i> (nd)	NW Mediterranean Sea, off Barcelona	350 - 450	May - Sept 1993	Length	No	(García et al., 2000)
Atlantic Ocean	<i>Hoplostethus atlanticus</i> (29), <i>Molva dyptergia</i> (15), <i>Coryphaenoides rupestris</i> (33), <i>Bathysaurus ferox</i> (16), <i>Aphanopus carbo</i> (66)	Mid Atlantic ridge: Rockall Trough, Meriadzec, Azores, Sesimbra, Madeira	1000 - 2000	Apr- Oct 1999	Length and mass	No	(Mormede and Davies, 2003)

Sea/Ocean	Species (n)	Sampling location	Sampling depth (m)	Sampling date	Biological factor measured	Relationship assessment (Yes/Partial/No) and major conclusions	Reference
Mediterranean Sea	<i>Galeus melastomus</i> (450 ^b)	Adriatic and Ionian Sea	nd ^e	June – Sept 1999	Length and mass	No	Storelli et al. 2003
Mediterranean Sea	<i>Chimaera monstrosa</i> (170 ^b), <i>Raja asterias</i> (95 ^b), <i>Raja clavata</i> (131 ^b), <i>Raja miraletus</i> (107 ^b)	Adriatic Sea	nd ^e	June - Sept 2000	Length and mass	No	(Storelli et al., 2004)
Mediterranean Sea	<i>Scyliorhinus canicula</i> (156 ^b)	Adriatic Sea	nd ^e	June 200 - Aug 2002	Length and mass	No	(Storelli et al., 2006)
Mediterranean Sea	<i>Coelorhynchus coelorhynchus</i> (352 ^b), <i>Nezumia sclerorhynchus</i> (1054 ^b)	Adriatic Sea	nd ^e	May- June 2003	Length and mass	No	(Storelli et al., 2007)
Mediterranean Sea	<i>Phycis blennoides</i> (180 ^b)	SE Mediterranean Sea, Adriatic Sea, Off Italian coasts	nd ^e	June-Sept 2006	Length and mass	No	(Storelli et al., 2008)
Mediterranean Sea	<i>Hoplostethus mediterraneus</i> (250 ^b), <i>Nettastoma melanurum</i> (140 ^b)	SE Mediterranean Sea, Adriatic Sea, Off Italian coasts	nd ^e	June - Sept 2006	Length and mass	No	(Storelli and Perrone, 2010)
Atlantic Ocean	<i>Coryphaenoides rupestris</i> (53), <i>Aphanopus carbo</i> (32), <i>Centroscyllium fabricii</i> (4 pools of 5)	Rockall Trough, off West Scotland	600 -1700	2006 - 2008	Length and mass	No. Length and age differences proposed as an explanation of interannual differences in PCB concentrations in <i>C. rupestris</i> but not explicitly tested	(Webster et al., 2011)
Mediterranean Sea	<i>Alepocephalus rostratus</i> (6), <i>Lepidion lepidion</i> (6), <i>Coelorinchus mediterraneus</i> (6), <i>Aristeus antennatus</i> (20 ^f)	NW Mediterranean Sea, Spain (41°15'N - 2°50'E)	nd ^g	Feb 2009	Length	No	(Koenig et al., 2012)
Mediterranean Sea	<i>Alepocephalus rostratus</i> (20), <i>Lepidion lepidion</i> (20), <i>Aristeus antennatus</i> (nd)	Blanes Canyon, NW Mediterranean (4 stations)	900, 1200 and 1500	Feb, Sept and Nov 2009	Length, mass and sex	No	(Koenig et al., 2013a)
Atlantic Ocean	<i>Coryphaenoides rupestris</i> (43), <i>Aphanopus carbo</i> (33), <i>Centroscyllium fabricii</i> (20)	<i>Rockall Trough</i> , off Scotland (56 to 60°N - ~10°W)	600 - 1800	Sep 2009- Aug and Sept 2011 and 2012	Length, mass and trophic level ($\delta^{15}\text{N}$)	No	(Webster et al., 2014)

a: Sample size is 9-10 individuals for all species except for *M. berglax*, where 20 individuals were analyzed.

b: individuals of similar size subsequently pooled for analyses

c: Actual list of species and sample size for the work of Tanabe et al (2005): *Malakichtys wakiyae* (7), *Diaphus chrysochrychus* (20), *Diaphus suborbitalis* (16), *Glossanodon semifasciatus* (5), *Diaphus watasei* (26), *Polypus spinifer* (20), *Caolorinchus jordani* (17), *Synagrops japonicus* (17), *Caolorinchus productus* (2), *Etmopterus lucifer* (4), *Squalus japonicus* (1), *Heptranchias perlo* (4), *Chascanopsetta lugubris* (3), *Conger myriaster* (4), *Argentina kagoshimae* (5), *Helicolenus hilgendorffii* (14), *Chaunax abei* (3), *Peristedion cataphractum* (5), *Chlorophthalmus acutifrons* (3), *Bathysphyraenops* sp. (23)

d: Actual list of species and sample size for the work of de Brito et al (2002): *Ereunias grillator* (4), *Etmopterus lucifer* (4), *Sebastolobus macrochir* (3), *Sebastolobus macrochir* (3), *Lampanyctus jordani* (4), *Lampanyctus jordani* (4), *Scomber japonicas* (1), *Malacocottus zonurus* (3), *Coelorinchus gilbert* (2), *Lycodes hubbsi* (3), *Albatrossia pectoralis* (1), *Cottiusculus schmidtii* (10), *Careproctus cypselurus* (1), *Pterothirissus gissu* (1), *Synaphobranchus kaupii* (2), *Lumpenella longirostris* (4), *Gadus macrocephalus* (4), *Coryphaenoides acrolepis* (3)

e: no detailed data on sampling depth, but species are described as “deep sea fish” in the title of the paper

f: 4 pools of 5 individuals

g: no detailed data on sampling depth, but species are described as deep species, and same species (potentially same individuals) were sampled at deep stations in papers from same authors

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