Pollutant levels in discarded fish species by Spanish trawlers operating in the Great Sole Bank and the Atlantic coast of the Iberian Peninsula

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Abstract

Organic and inorganic pollutant levels were determined for the most discarded species from trawlers operating in Great Sole and Spanish coastal fishing grounds. Results for heavy metals indicated that Cd can reach values higher than legal limits for some species and tissues, while Hg and Pb concentrations are below established values. No significant variation was noticed with fishing grounds, but both season influence in the case of Pb and interspecies variation for Hg and Cd have been detected. Valorization recommendations could be therefore established according to the levels found in the different species.

Keywords Metals, persistent organic pollutants; discarded fish; tissues; valorization

Discards are among the best examples of shortcomings that the Common Fisheries Policy (CFP) reform will amend, since this biomass is considered as impossible to justify to fishermen or the public (EC, 2013). The discards reduction and/or by-catch utilization must be undertaken to the possible extent in a manner that is consistent with the Code of Conduct for Responsible Fisheries (FAO, 1995). These objectives of FAO were presented in a specific technical report for discards reduction (“International guidelines for by-catch management and reduction of discards”, in December 2010). The valorization of the inevitable unwanted by-catch is also the main motivation of the optimal and efficient discard management network that was and is being developed in FAROS and iSEAS projects, respectively. These projects were co-funded under the LIFE+ Environmental Program of the European Union (LIFE08 ENV/E/000119 – www.farosproject.eu and LIFE13ENV/ES/000131 – www.lifeiseas.eu). The objective of these initiatives was to demonstrate a real sustainable valorization, and therefore, aspects like environmental impacts and consumer’s safety issues associated with the valorization process must be addressed.

As a first step to attain sustainability, raw material quantification (percentage over the total discarded mass and quantity of each discarded specie in tones per year) and evaluation (status of stocks in their habitat as well as valorization potential) was carried out by the most discarded species in Great Sole Bank and Atlantic coast of the Iberian Peninsula (Ordóñez-del Pazo et al., 2014). Once the potential valorization strategy was established, the sustainable management of this biomass through its optimum recovery will depend on the quality of this new raw material, as well as of the products that may be obtained from it. Scientific studies, apart from fish product surveys on the markets of different countries and monitoring reports by the Public Administrations and the European Commission, reveal the presence of significant pollutant levels (particularly dioxins, PCBs, organochlorine pesticides and heavy

metals) in the commercial species of different fisheries (Licata et al., 2005; Nadal et al., 2008; Miniero et al., 2014; Cano-Sancho et al., 2015). Therefore, it is logical to consider the more than possible presence of these pollutants in the discarded non-commercial species even though their levels are not usually determined (Antelo et al., 2012). Furthermore, significant differences on pollutants concentration depending on the considered tissue have been reported by several studies in literature (Kojadinovic et al., 2007; Afonso et al., 2007; Lozano et al., 2009; Webster et al., 2009). Organs like liver are well-known for preferentially accumulate higher pollutant concentrations (both inorganic and organic) (Bustamante et al., 2003; Coelho et al., 2010; Storelli et al., 2011a).

With this aim, the pollutant content has been analyzed in the most discarded species of these two fisheries areas, including heavy metals (mercury, lead and cadmium) and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) and dioxin-like polychlorinated biphenyls (dl-PCB), and pesticides. A statistical analysis of the data was performed on heavy metal levels. Besides, different target tissues were analysed for cartilaginous fish (liver, skin, and cartilage). This task is a key step in the correct definition of recovery strategies that may overcome the drawbacks arising from certain fish by-products (such as fishmeal, oil, etc.), which may concentrate pollutants to non-appropriate levels. Comparison with current legislation for commercial species was developed, taking into account the variability due to the analysed tissues.

Samples of discards were collected by scientific observers on board Spanish fishing trawlers operating in Great Sole and Spanish coastal waters (ICES Areas VIIb, VIIc, VIIg, VIIh, VIIj, VIIk, VIIc and IXa) during 2011-2012 (Fig. 1). Samples for metals analysis were collected on a monthly temporal basis, while those for the determination of organic pollutants were collected seasonally (summer months in 2011 and winter months in 2012). A specific protocol for observers was created and

implemented on board in order to properly define target species, required sample amounts, collection methodology, conservation and labelling requirements.

The sampled species and their target tissues were selected based on discarded amounts and their valorisation potential, this being analysed for the considered métiers (Ordóñez-del Pazo et al., 2014). Therefore, nine discarded species were selected for Great Sole fisheries and ten for coastal waters, taking into account that some of them were common for both fishing grounds (Table 1). The number of species was not constant depending on the sampling month, due to variations in presence of the different species among total capture. Around 5-15 individuals of each species were collected by month, depending on the type of analysis to carry out (heavy metals, organic pollutants or both). The clean and entire specimens were preserved on polyethylene bags placed on ice within hermetic boxes. On board collected data by species include date, haul number and location (throw net and pick up net coordinates). Samples characterization was subsequently completed in land. Once at laboratory, samples were frozen (-25 °C) until the time of analysis (one week, approximately). Besides pollutant concentrations, species weight (g), size (cm) and gender were determined when possible, since some species were invertebrates and in other cases, immature individuals were collected.

Heavy metals determination was carried out in a laboratory that is homologated and accredited by the Spanish Entity of National Accreditation (ENAC) in accordance with regulation UNE-EN ISO/IEC 17025. Heavy metals (Hg, Cd and Pb) were determined on 100 g of homogenized sample for the target tissue, which was obtained from at least two individuals of each species. Acid digestion of the samples was carried out with nitric acid in a Berghoff microwave with several cycles of different duration and temperature until complete digestion was reached. The digestion products were carried to a volume of 100 mL with milliQ water. Pb and Cd were analyzed by electrothermal atomization, with a limit of quantification/detection (LOQ/LOD) ≥ 20

$\mu\text{g}\cdot\text{kg}^{-1}$ and $\geq 8 \mu\text{g}\cdot\text{kg}^{-1}$ while Hg was analyzed by atomic fluorescence with a limit of detection $\geq 2.5 \mu\text{g}\cdot\text{kg}^{-1}$ (wet weight basis). Regarding the precision of both methodologies, the relative standard deviation of obtained measurements is $<1\%$, as certified by the accredited laboratory.

An homogenized sample of 500 g for the target tissue was obtained from at least two individuals of each specie to perform organic pollutants analysis, according to requirements of EC Regulations (Regulation (EU) No. 589/2014 and Regulation (EU) No. 709/2015) in respect of methods and quality. For the determination of the 17 WHO congeners of polychlorinated dibenzo(p)dioxins and furans (PCDD/F) and the 12 WHO congeners of dioxin-like polychlorinated biphenyls (dl-PCB), internal $^{13}\text{C}_{12}$ marked standards were added to the homogenized sample (isotope dilution method). Fat extraction was carried out by Accelerated Solvent Extraction (Dionex ASE 300) after mixture with diatomaceous earth. The fat extract was separated from the solvent by a rotatory evaporator, and after being purified by chromatography, analysis on PCDD/F and dl-PCB were performed by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) (LOQ between $0.1 - 0.8 \text{ pg WHO-TEQ}\cdot\text{g fat}^{-1}$). Regarding organochlorine pesticides (OP) and pyrethroids, the sample material was extracted with acetone (acetone/water 2:1 v/v), accounting for the full natural water content of the sample. An organic extract was obtained through consecutive extractions with cyclohexane and ethyl acetate, being afterwards purified by chromatography. Finally, pollutants determination was performed by gas chromatography, using an electron capture detector (ECD) (LOQ between $0.001 - 0.01 \text{ mg}\cdot\text{kg}^{-1}$).

Statistical analysis of obtained pollutant data in discarded species was carried out using R software (v3.1). Differences between fishing grounds, seasons and species were studied by the test two-way analysis of variance (ANOVA) at 95% significance. For the specific case of the analysis of the relationship between season and metals (in

particular, Pb), a two-step, non-parametric method for multiple comparisons (Kruskal-Wallis followed by Steel-Dwass-Critchlow-Fligner analysis) was applied to identify possible significant interactions. These methods use functions of the response ranks, called rank scores. The relationships between fish size (length and weight) and tissue metal concentrations were investigated by linear regression analysis. Pearson and/or Spearman correlation analysis were used to establish the association between variables. Significance levels were set at $p < 0.05$ ($p < 0.001$ when specifically mentioned).

The results for Hg, Cd and Pb by species are shown in Table 2. Obtained values were compared with legal limits established by Regulation (EC) No 1881/2006 and its amendments. These levels were established in most cases for fish meat/muscle mainly in commercial species and for that reason, only pollutants concentration in muscle or in the entire specimen were shown in Table 2. Moreover, based on the valorization potential, pollutant levels on shells (crustaceans), skin, cartilage (small-spotted catshark) and viscera (small-spotted catshark and black-mouthed dogfish) were also analyzed (Table 1), being the levels in these tissues discussed further on next pages. It must be pointed out that legal limits for some species of non-commercial origin were not available. In these cases, maximum levels for species in the same phyla were used for comparison purposes. In general, it can be observed that Hg and Cd presented higher levels than Pb (Carvalho et al., 2005), except for echinoderms, anemones and argenterines. Different tendencies were also reported in literature regarding the levels of these three metals (Afonso et al., 2007; Kojadinovic et al., 2007; Storelli, 2008; Barone et al., 2013). Total Hg levels were below established limits for all sampled species in both fishing grounds, while Pb levels were higher than the legal limit only in one occasion in Great Sole Bank for sea anemone ($0.996 \text{ mg}\cdot\text{kg}^{-1} \text{ ww}$), although the concentration values for this specie were not directly comparable with legal limits (only for commercial species). However, Cd frequently exceeded regulation values, especially

in coastal waters. Hg values presented more variability within each specie and also showed a significant variation among the different species considered (Naccari et al., 2015). For instance, Hg concentrations between horse mackerel and sea anemone were very different. The differences between values in both species are expected since they have dissimilar food habits and different behaviors. Horse mackerel presented the highest Hg concentrations in muscle in practically all the sampled months. The results found in this study and for this specie for Hg and Pb (average of 0.13 and 0.024 mg·kg⁻¹ ww, respectively) were comparable to those reported in Vieira et al. (2011), with mean values of 0.15-0.18 and 0.016-0.014 mg·kg⁻¹ ww. However, average Cd concentrations were an order of magnitude higher in the present study (0.018 in front of 0.007 mg·kg⁻¹ ww in the study of Vieira et al., (2011)). On the contrary, Storelli (2008) reported very similar average values of Cd and Pb for horse mackerel (0.02 and 0.03 mg·kg⁻¹ ww, respectively), but significantly higher values for Hg (0.68 mg·kg⁻¹ ww). Naccari et al. (2015) also reported higher concentrations for Hg in horse mackerel and Atlantic mackerel (0.220 and 0.298 mg·kg⁻¹ ww), while Cd was only detected for Atlantic mackerel at a higher concentration than this study (0.120 mg·kg⁻¹ ww) and lead was not detected in these species. Highest Hg concentrations in muscle of horse mackerel were also found by Vieira et al. (2011), which associated this fact to the predatory nature of horse mackerel. Triglidae (red and grey gurnard) and cartilaginous species (small-spotted catshark and black mouthed dogfish) also presented important concentrations, due to same reason (predatory species), whereas lowest Hg levels were found in sea anemone. Values of Hg in muscle of *Scyliorhinus canicula* (0.108 mg·kg⁻¹ ww) were comparable to those obtained for youngest specimens of this specie in Coelho et al. (2007) (0.13 mg·kg⁻¹ ww). These results are then another indication of the importance of biomagnification as distribution route of this metal through the trophic chain (Cai et al., 2007; Naccari et al., 2015). However, in the case of cadmium (Cd), the highest

concentrations were found in sea anemone, crustaceans and small-spotted catshark. Boarfish (either in muscle and entire specimen) also presented high concentrations, as well as red gurnard, while the lowest cadmium concentrations were found in muscle tissues of black-mouthed dogfish and horse mackerel. The preferential accumulation of this metal in some species seems to be more related to physiological factors (like water and diet uptake, clearance rate) and environmental conditions (Franco-Uriá et al., 2010; Vieira et al., 2011). In the case of sea anemone, the absence of both internal organs and mobility could favor the accumulation in this specie. Again, the highest concentrations in the case of lead (Pb) were also found in sea anemone, followed by argentines, and with very similar results for the other species.

Levels of PCDD/Fs and dioxin-like PCBs as toxic equivalents (TEQ) in the analyzed species can be observed in Table 3. No statistical analysis was performed due to the low number of composite samples obtained. Of the screened OC pesticides, only DDT was detected in argentines ($0.005 \text{ mg}\cdot\text{kg}^{-1}$), horse mackerel ($0.004\text{-}0.005 \text{ mg}\cdot\text{kg}^{-1}$) and liver of small-spotted catshark ($0.037\text{-}0.051 \text{ mg}\cdot\text{kg}^{-1}$), but at low concentrations, well below legislation limits. The highest levels of PCDD/F and dl-PCB were observed in small-spotted catshark and boarfish, followed by triglidae, Atlantic mackerel and argentines. Levels of PCDD/F and dl-PBC in red gurnard were much lower than those reported in other studies ($31.2 \text{ ng}\cdot\text{g}^{-1}$ lipid in Storelli et al., 2011a). Regarding mackerels, higher levels were also detected for either the two pollutants. dl-PCB were detected in horse mackerel at higher values ($400 \text{ ng}\cdot\text{g}^{-1}$ lipid including other PCB congeners), but not detected in Mezzeta et al. (2011) in Atlantic mackerel, while values ranging from $0.004\text{-}0.0097 \text{ ng}\cdot\text{g}^{-1}$ lipid of PCDD/F were reported in this specie (Karl et al., 2002). Regarding tissues, liver of small-spotted catshark did not present the highest values of organic pollutants, as happened with metals (metal values in different tissues will be discussed later on the text). Levels of pesticides in liver were lower than those

obtained in other works (Mormede and Davis, 2003), as well PCDD/F content (Storelli et al., 2011b).

Associations between metals in the two fishing grounds were analyzed by correlation matrix, and can be summarized in Table 4. Cd and Pb are strongly correlated in both fishing grounds, thus indicating a common anthropogenic source for these pollutants and a similar bioaccumulation pattern. However, Hg was only partially and negatively correlated with Cd and Pb in Great Sole Bank, while in coastal waters no association was found. As mentioned before, Hg has a bioaccumulation behavior more related to the predatory habits of the species. Results of the two-way variance analysis ANOVA (Table 5) revealed that no statistically significant differences in heavy metal levels (Hg, Cd, Pb) were observed between fishing grounds. Selected species (those for which sufficient data is available - Table 5) showed statistical significant differences for Hg and Cd, while for Pb, no relevant differences were observed in the levels accumulated, as mentioned in the previous paragraph. Related with the influence of location on metal concentration, it is clear that anthropogenic pressure on marine areas will determine, among other factors, the concentration differences in marine species (Kress et al., 1999; Henry et al., 2004; Afonso et al., 2007; Antelo et al., 2012; Aoun et al., 2015; Naccari et al., 2015). However, it has to be considered the influence of the pollution level of a marine area in the trophic position of the specie. For example, high concentrations in benthic species are an indicator of bottom-contaminated sites, while the opposite behavior indicate clean bottom sediments (Henry et al., 2004; Naccari et al., 2015). More data would be needed to further study the influence of the fishing ground by each individual specie, considering those analyzed in this work.

Potential changes in heavy metal concentrations with the period/time of the year (seasonal study) were analyzed by two-way ANOVA test (Table S.1.), employing data of years 2011 and 2012. Winter season included January to March, spring comprises

April to June, summer season included July to September and, finally, autumn corresponds to the period between October and December. No statistically significant seasonal differences were found for Hg, Cd and Pb through the ANOVA analysis. However, obtained data from samples showed important differences in Pb levels along the year. Spring, summer and autumns months values were higher (range 0.0133-0.508 mg/kg wet weight) than those obtained in winter months (< 0.02 mg/kg wet weight). A non-parametric analysis was applied to the aggregated data for Pb in each season (without species differentiation) to analyze once again seasonality on the levels of this heavy metals (Table S.2., Supplementary Information). First, a Kruskal-Wallis analysis was developed to test the general hypothesis that all population medians (for each season) are equal. Since the null hypothesis was rejected for this case ($p = 0.00045$), the next step was to compare the individual groups. This was done by using the Steel-Dwass-Critchlow-Fligner pairwise ranking method. In this case, the seasonality variations were verified since statistical significance is established between winter and the rest of considered seasons (Table S.2.). This finding could be explained by an increase in the lipid concentration related with feeding habits in summer months (Pethybridge et al., 2014), since Pb was reported to be at higher concentrations in high-fat content species (Nadal et al., 2008; Vieira et al., 2011), and therefore, its bioaccumulation can be more related than other metals with the fat content of marine organisms. However, significant higher contents of Pb in species like horse mackerel or blue whiting were not observed in the present study.

Different tissues (liver, skin, cartilage, muscle and entire fish) were analyzed during sampling, although muscle and entire fish were the most frequently sampled, since the valorization potential of most species was the production of fishmeal or direct consumption (Table 1). Box plots of Hg, Cd and Pb concentrations in the different sampled tissues are shown in Figure S1. Hg presented higher levels in skin and muscle

than in liver of the two analyzed species, *Galeus Melastomus* and *Scyliorhinus canicula*. Hg was reported at higher levels in muscle than in liver (Zauke et al., 1999; Bustamante et al., 2003; Burger et al., 2007; Watanabe et al., 2012), even in demersal sharks (Pethybridge et al., 2010), including *Scyliorhinus canicula* (Coelho et al., 2010). However, higher concentrations are usually found in liver for high-size predators or benthic species (Bustamante et al., 2003; Afonso et al., 2007; Bebianno et al., 2007; Kojadinovic et al., 2007; Watanabe et al., 2012). Metal concentrations in the liver of the top predator small-spotted catshark and black-mouthed dogfish were those shown in Fig. S.1. In this case, Cd was accumulated at much higher levels in liver, while Hg and Pb were present at higher concentrations in skin. Cd is reported to be preferentially accumulated in liver for marine species belonging to different phyla, size or feeding habits (Henry et al., 2004; Kojadinovic et al., 2007; Bustamante et al., 2003; Burger et al., 2007; Ploetz et al., 2007; Barone et al., 2013) due to the metal-binding protein metallothionein, which has high affinity for Cd. Pb would not be captured by this protein so efficiently, which explains its lower levels in liver (Ploetz et al., 2007). However, Pb was found to be higher in livers of two species of sharks, being its content higher in the skin of one of the evaluated species (Lozano et al., 2009). Fig. S.2. shows the metal distribution in muscle and entire specimens of a small-size species (boarfish). The results indicated that no significant differences were found in these fractions as expected, this consequently indicating that no important preferential accumulation in other tissues was produced in this species, also caused by its low size. The pollutant level dependence in muscle with fish size (length and weight) was analyzed for only four species due to data availability (horse mackerel, sea anemone, blue whiting and triglidae). Only significant positive relationships ($P < 0.001$) with length (+0.921) and weight (+0.918) were found for Hg in horse mackerel and moderate positive relationship ($P < 0.05$) for blue whiting with length (+0.797). in the case of horse mackerel. Several authors have found positive

correlations for Hg with size, due to the bioaccumulation increase of this metal with age in predatory species (Bebianno et al., 2007; Burger et al., 2007; Coelho et al., 2010; Pethybridge et al., 2010).

When compared with legislation, Cd exceeded limit values in several occasions, while Hg and Pb remained below acceptable levels. Species most affected by Cd accumulation were sea anemone (all specimens but one), red gurnard, boar fish, and small-spotted catshark and black-mouthed dogfish, these latter cartilaginous species in liver. Other species like crustaceans or echinoderms presented punctual exceedance of legal limits. Globally, almost 30% of samples presented Cd values higher than current legislation. Regarding pesticides, although maximum residue levels (MRLs) are not already established for fish or fish-derived products, the screening results were lower than general maximum pesticide limits of food products for human consumption. Species like sea anemone, red gurnard, boarfish, small-spotted catshark and horse mackerel, that were at first considered of interest for valorization purposes (based on the high amounts of discards generated by year in the selected fleets), could not be suitable for this aim due to the excessive levels of some metals. This is the case of Cd in sea anemone (especially those specimens coming from coastal waters), considering the medical uses of extracted compounds with anticancer cytotoxic and antimicrobial properties (Cao et al., 2005; Fedorov et al., 2010). In the case of red gurnard and boar fish, levels of Cd in entire fish suggests that further monitoring of metal levels should be performed, thinking in the production of fish meal, since legal limits are set to $1 \text{ mg} \cdot \text{kg}^{-1}$ (EC, 2002). Dietary intake of this type of feeds in cultured fish could be a main exposure pathway (Douben, 1989; Szebedinszky et al., 2001; Campenhout et al., 2007). Regarding cartilaginous species (small-spotted catshark and black-mouthed dogfish), the most suitable tissue for valorization purposes should be cartilage. High Cd levels found in muscle and in liver would impede either direct consumption (EC, 2006) or the

extraction and concentration of valuable oil from these tissues, containing nutritional and valuable compounds (Blanco et al., 2007; Hareide et al., 2007). Besides, pollutants tend to be retained in the lipophilic phase, being oils in this case “natural” containers of these substances. This is the case for PCDD/F and dl-PCB, which were present in liver of small-spotted catshark, although at low values. In fact, several studies reported high levels of both inorganic and organic pollutants in oil produced from livers or entire organisms of marine species, either for fish feeds or direct human consumption (Eppe et al., 2005; Oterhals et al., 2007; Berntssen et al., 2010; Sprague et al., 2015). Skin also present important values of Hg and Pb in these species, and therefore, the production of gelatin or other compounds (Shyni et al., 2014; Limpisophon et al., 2015) must be subjected to precaution. Thus, although more than 4,000 tons of these species are discarded per year in these fisheries areas, the presence of heavy metals hampers their proper valorization. The recommendation would be to minimize the captures as much as possible. Horse mackerel presented the highest values for Hg, but always below legal limits. However, the extraction of proteins and oils from this specie (Eymard et al., 2009; Gehring et al., 2009) must be undertaken together with a pollutant monitoring of both the raw material and the final obtained products. The remaining species shown in Table 1 presented low values of pollutant content, being this biomass optimal from the point of view of future valorization strategies for Spanish fleet. The results of this sampling are available in the Management Geoportal Network developed in FAROS project (www.farosproject.eu).

More monitoring of these fisheries, involving a wider temporal horizon and, what is more important, the inclusion of additional compounds, would be needed to confirm the main conclusions of this work. As an example, the presence of arsenic (even considering a small fraction of toxic inorganic As) in crustacean species could be a problem thinking in their future valorization. Regarding to this latter, another key

factor would be to consider main species fractions in discarded marine organisms, where a wide scientific background exists in the literature, and the changes in metal speciation that the different valorization process might cause in the obtained products. On the other hand, the determination of total lipid content in discards is crucial to evaluate not only their nutritional value, but also to characterize bioaccumulation. Correlation empirical models should be established as a way to estimate concentration of several pollutants from this parameter. Finally, the distribution of pollutants within the intermediate products, effluents and final products of the different valorization processes must be also monitored. This would allow to know the safety of the process to consumers and to determine partition coefficients of the different contaminants within the process stages. These tasks are being currently developed in the framework of the iSEAS Project.

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Figure captions

Figure 1. Target ICES areas of the considered Spanish trawling fleet.

Table 1

Characteristics and location of selected species, and tissues analyzed

Species	Length (cm)	Weight (g)	Specie characteristics	Feeding habits ¹	Location ²	Tissue	Picture
Horse mackerel (<i>Trachurus trachurus</i>)	21.4 – 36.0	79.7 – 493.6	Pelagic, neritic	Feeds on fish, crustaceans and cephalopods	GS and SC	Muscle
Atlantic mackerel (<i>Scomber scombrus</i>)	19.8 – 38.0	50.5 – 512.0	Pelagic, neritic	Feeds on a variety of pelagic crustaceans and small fish	GS and SC	Muscle
Blue whiting (<i>Micromesistius poutassou</i>)	15.5 – 36.5	21.7 – 380.1	Bathypelagic	Feeds mostly on small crustaceans	GS and SC	Muscle
Argentines (<i>Argentina</i> spp.)	19.5 – 33.0	48.6 – 413.5	Bathydemersal	Feeds mostly on small crustaceans	GS	Muscle, entire fish
Boar fish (<i>Capros aper</i>)	10.1 – 15.6	26.5 – 77.0	Demersal	Feeds on crustaceans, worms and molluscs	GS and SC	Muscle, entire fish

Species	Length (cm)	Weight (g)	Specie characteristics	Feeding habits ¹	Location ²	Tissue	Picture
Grey gurnard (<i>Eutrigla gurnardus</i>)	21.9 – 27.3	103.2 – 165.3	Demersal	Feeds on crustaceans and fishes	SC	Muscle, entire fish
Red gurnard (<i>Aspitrigla cuculus</i>)	18.8 – 26.4	47.6 – 194.0	Demersal	Feeds on crustaceans and fishes	GS	Muscle, entire fish
Black-mouthed dogfish (<i>Galeus melastomus</i>)	39.2 – 48.0	187.0 – 383.3	Demersal	Feeds mainly on bottom invertebrates and small fishes (bony and elasmobranchs)	SC	Entire fish, liver
Small-spotted catshark (<i>Scyliorhinus canicula</i>)	29.9 – 63	80.4 – 970.2	Demersal	Feeds on a variety of benthic invertebrates and small bony fishes	GS and SC	Muscle, cartilage, skin, liver
Sea anemone (<i>Actinauge richardi</i>)	3.5 – 6.4	19.9 – 86.9	Benthic	Feeds on the available prey, usually benthic organisms	GS and SC	Muscle, entire specimen

Species	Length (cm)	Weight (g)	Species characteristics	Feeding habits ¹	Location ²	Tissue	Picture
Crustaceans (<i>Polybius henslowii</i> , <i>Munida</i> spp.)	6.28 – 21.6	7.92 – 15.6	Benthic	Non-selective feeding includes crustaceans, algae and polychaetes (as a predator) and particulate organic matter (as a deposit feeder)	SC	Muscle, entire specimen, shell
Echinoderms (<i>Stichopus</i> spp., <i>Holothuria</i> spp.)	17.5 – 26.0	169.6 – 302.8	Benthic	Detritivores that consume organic matter	GS and SC	Muscle, entire specimen

¹From <http://www.fishbase.org/>; Quesada et al., 2014; Romero et al., 2004 and Maxwell et al., 2009

²GS: Great Sole Bank; SC: Spanish Coastal Waters

Table 2

Heavy metal concentrations and legal maximum levels (ML) in discarded species (mg·kg⁻¹ wet weight) and number of composite samples (n)

Specie	Hg	ML	Cd	ML	Pb	ML
Horse mackerel (<i>Trachurus trachurus</i>) n= 10	0.132 ± 0.086 (0.02 – 0.26)	0.5	0.018 ± 0.017 (<0.008 – 0.06)	0.1	0.024 ± 0.016 (0.01 – 0.07)	0.3
Atlantic mackerel (<i>Scomber scombrus</i>) n= 9	0.046 ± 0.041 (0.01 – 0.15)	0.5	0.021 ± 0.010 (0.01 – 0.04)	0.1	0.024 ± 0.010 (<0.02 – 0.05)	0.3
Blue whiting (<i>Micromesistius poutassou</i>) n= 10	0.056 ± 0.044 (0.008 – 0.13)	0.5	0.032 ± 0.037 (<0.008 – 0.11)	0.05	0.022 ± 0.014 (0.01 – 0.06)	0.3
Argentines (<i>Argentina</i> spp.) n= 3	0.056 ± 0.016 (0.038 – 0.07)	0.5	0.022 ± 0.013 (0.01 – 0.036)	0.05	0.053 ± 0.035 (<0.02 – 0.09)	0.3
Boar fish (<i>Capros aper</i>) n= 11	0.080 ± 0.039 (0.022 – 0.146)	0.5	0.078 ± 0.087 (<0.008 – 0.259)	0.05	0.032 ± 0.017 (<0.02-0.070)	0.3
Grey gurnard (<i>Eutrigla gurnardus</i>) n= 5	0.098 ± 0.110 (0.006 – 0.28)	0.5	0.040 ± 0.057 (<0.008 – 0.14)	0.05	0.024 ± 0.009 (<0.02 – 0.04)	0.3
Red gurnard (<i>Aspitrigla cuculus</i>) n= 6	0.152 ± 0.162 (0.015 – 0.41)	0.5	0.009 ± 0.005 (<0.008 – 0.016)	0.05	0.020 ± 0.006 (0.01 – 0.03)	0.3
Black-mouthed dogfish (<i>Galeus melastomus</i>) n= 4	0.123 ± 0.119 (0.022 – 0.281)	1	0.192 ± 0.307 (<0.008 – 0.649)	0.05	0.045± 0.050 (<0.02 – 0.12)	0.3
Small-spotted catshark (<i>Scyliorhinus canicula</i>) n= 11	0.108 ± 0.118 (0.018 – 0.390)	1	0.194 ± 0.346 (<0.008 – 1.123)	0.05	0.052 ± 0.064 (<0.02 – 0.24)	0.3
Sea anemone (<i>Actinauge richardi</i>) n= 9	0.008 ± 0.012 (<0.002 – 0.04)	0.5	0.662 ± 0.782 (0.041 – 2.65)	0.05	0.155 ± 0.317 (<0.02 – 0.996)	0.3

[illegible]

Table 3

PCDD/F, dl-PCB's (pg/g fat) and pesticides (mg/kg) in discarded species, including information from Grand Sole and coastal waters fishing grounds, as well as different tissues analyzed

Species	Location	Tissue	PCDD/F and dl-PCB	Pesticides
Boar fish (<i>Capros aper</i>)	GS and SC	Entire fish	0.668 – 0,685	<LOQ
Small-spotted catshark (<i>Scyliorhinus canicula</i>)	GS and SC	Liver	10,809 – 10,830	<LOQ
Red gurnard (<i>Aspitrigla cuculus</i>)	GS	Muscle	0.396 - 0.399	<LOQ
Argentines (<i>Argentina</i> spp.)	GS	Entire fish	0.438 – 0.440	0.005
Horse mackerel (<i>Trachurus trachurus</i>)	GS and SC	Muscle	0.181 – 0.211	0.005
Atlantic mackerel (<i>Scomber scombrus</i>)	GS and SC	Entire fish	0.373 – 0.394	<LOQ
Grey gurnard (<i>Eutrigla gurnardus</i>)	SC	Entire fish	0.532 – 0.546	<LOQ

Table 4

Correlation coefficient matrix between metal concentrations in Grand Sole bank and Coastal Spanish waters

Great Sole Bank			
	Hg	Pb	Cd
Hg	1		
Pb	-0.715	1	
Cd	-0.699	0.945	1
Spanish Coastal Waters			
	Hg	Pb	Cd
Hg	1		
Pb	-0.289	1	
Cd	-0.186	0.973	1

Table 5

Two-way ANOVA results for analysing the influence of specie and fishing ground in metal concentrations

	df	Hg		Cd		Pb	
		F	p	F	p	F	p
Fishing ground ^a	1	0.69	0.43	0.86	0.38	2.06	0.189
Specie ^b	8	4.85	0.019	3.45	0.049	2.16	0.149
Error	8						
Total	17						

F_{critic} for Fishing ground = 5.32 ($\alpha = 0.05$).F_{critic} for Specie = 3.44 ($\alpha = 0.05$).^aGrand Sole Bank and Spanish Coastal waters^bSpecies: sea anemone, boarfish, gurnards, horse mackerel, Atlantic mackerel, small-spotted catshark, blue whiting, equinoderms, crustaceans

Table 6

Two-way ANOVA results for analysing the influence of specie and season in metal concentrations

	df	Hg		Cd		Pb	
		F	p	F	p	F	p
Season	3	1.53	0.24	0.47	0.71	2.0	0.15
Specie ^a	6	2.83	0.04	12.16	0.000017	1.44	0.25
Error	18						
Total	27						

 F_{critic} for Season = 3.16 ($\alpha = 0.05$). F_{critic} for Specie = 2.66 ($\alpha = 0.05$).^aSpecies: sea anemone, boarfish, gurnards, horse mackerel, Atlantic mackerel, small-spotted catshark, blue whiting

Table 7

Non-parametric multiple comparison test for the Pb levels obtained for the different seasons

Kruskal-Wallis test:

K (Observed value)	17.947
K (Critical value)	7.815
DF	3
p-value (bilateral)	0.00045
α	0.05

Steel-Dwass-Critchlow-Fligner pairwise comparison method (p-values; $\alpha = 0.05$):

	Winter	Spring	Summer	Autumn
Winter	1	0.033	< 0.0001	0.010
Spring	0.033	1	0.795	0.829
Summer	< 0.0001	0.795	1	0.109
Autumn	0.010	0.829	0.109	1