Marine Pollution Bulletin 123 (2017) 387–394

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Baseline

Concentrations of mercury and other trace elements in two offshore skates: sandy ray *Leucoraja circularis* and shagreen ray *L. fullonica*

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ARTICLE INFO

Keywords:
Trace metals
Sandy ray
Shagreen ray
Bay of Biscay
Celtic Sea

ABSTRACT

Trace metal concentrations in muscle and liver tissues from two offshore species of skate were examined. Concentrations of mercury in muscle of *Leucoraja circularis* (n = 20; 23–110.5 cm total length, 157–490 m water depth) and L. *fullonica* (n = 24; 28.5–100 cm total length, 130–426 m water depth) were 0.02–1.8 and 0.04–0.61 mg kg^{-1}, respectively. Concentrations of both As and Hg increased with total length. Only the largest specimen had a concentration of Hg in muscle > 1.0 mg kg^{-1}. Data were limited for specimens > 90 cm long, and further studies on contaminants in larger-bodied skates could usefully be undertaken.

Skates (Rajformes) are demersal elasmobranchs that are widespread in shelf seas and deep-water habitats. This speciose order includes approximately 290 species (Last et al., 2016), that range up to ca. 250 cm total length. In terms of their trophic position, the order contains many species that are benthic predators, whilst some are more piscivorous, including some species that predate on other elasmobranchs. The estimated trophic levels of skates range from 3.48–4.22 (Ebert and Bizzarro, 2007). As some species are of low market value, skates are an important group of commercial fish in many parts of the world, including Europe. Given their demersal habitat and potential longevity, they may bioaccumulate various contaminants, such as mercury and persistent organic contaminants (Nicolaus et al., 2016a; Lyons and Adams, 2017). Trace elements reach the marine environment via anthropogenic and natural inputs (Nicolaus et al., 2016b) and ultimately bind to sediments due to their strong affiliation with particulate matter (Zhang et al., 2007). Consequently, demersal fish that may forage and bury in upper surficial sediments, such as skates, may be exposed to trace metals in sediments.

Various studies have examined the contaminants of skates from the inner continental shelf of European seas (Dixon and Jones, 1994; De Gieter et al., 2002; Storelli and Barone, 2013) and elsewhere, but data are limited for those skates living in deeper water (Mormede and Davies, 2001), despite there being some evidence that Hg concentrations in marine fish can increase with water depth (Choy et al., 2009).

Sandy ray *Leucoraja circularis* and shagreen ray L. *fullonica* are two offshore skates that are widespread along the edge of the continental shelf from Iceland and northern Norway to north-west Africa, including the Mediterranean Sea (Ebert and Stehmann, 2013). Despite their broad distribution range, the offshore nature of these two species means there are very few published biological investigations (Du Buit, 1972; Consalvo et al., 2009; Mnsari et al., 2009). Currently, the life histories of these two species are poorly known, they are both relatively large-bodied species, reportedly attaining maximum lengths of 120 cm. Both species predate on crustaceans and fish (Du Buit, 1972), with L. *fullonica* (trophic level = 4.6) also predating on other elasmobranchs (Ebert and Bizzarro, 2007).

The United Kingdom's Clean Seas Environmental Monitoring Programme (CSEMP) samples flatfish species, mostly at sites within 22 km (12 nautical mile) of shore, to assess spatial and temporal concentrations of contaminants in fish liver and muscle (Nicolaus et al., 2016b). The dab *Limanda limanda* is a useful indicator species for inshore waters, but it does not occur along the edge of the continental shelf. To better understand the concentrations of trace elements in offshore fish, samples of muscle and liver from L. *circularis* and L. *fullonica* caught in the Bay of Biscay and Celtic Sea were analysed.

Specimens of L. *circularis* (n = 20) and L. *fullonica* (n = 24) were caught during annual trawl surveys of the Bay of Biscay and Celtic Sea (EVHOE survey: ÉValuation Halieutique de l'Ouest de l'Europe; see Mahé and Poulard, 2005 and ICES, 2010) in 2014–2016. These specimens were collected from the deeper parts of the survey area (Fig. 1) in waters of 130–490 m depth. Specimens were frozen whole for subsequent collection of biological data (total length (L_T), disc width, total

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http://dx.doi.org/10.1016/j.marpolbul.2017.08.054
Received 27 June 2017; Received in revised form 9 August 2017; Accepted 23 August 2017
Available online 30 August 2017

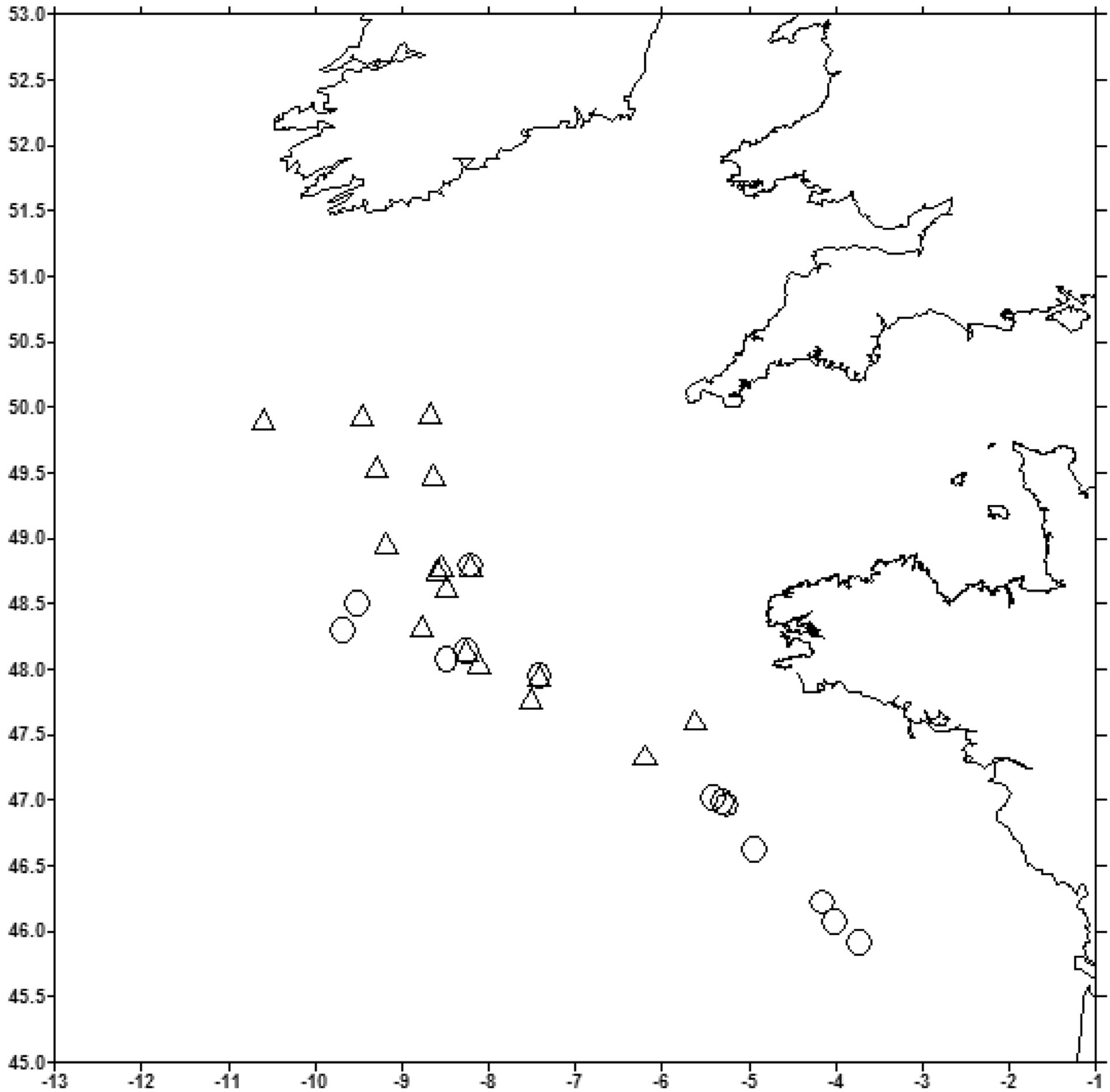

Fig. 1. Sampling locations of *Leucoraja circularis* (open circles) and L. *fullonica* (open triangles) in the Bay of Biscay and Celtic Sea.

weight, sex and maturity) and tissue sampling. Sections of dorsal muscle, excluding skin and ceratotrichia, were excised, and either the whole liver (small specimens) or sub-samples from the three main lobes taken. These samples were then re-frozen until analysed for trace elements.

Trace metal analysis followed standard procedures (Jones and Laslett, 1994). Tissue samples underwent an acid digestion using an enclosed vessel microwave (Multiwave 3000, Anton Paar, Hertford, UK). Typically, approximately 1 g of homogenised sample was weighed out and pre-digested overnight in 6 mL of nitric acid (Aristar grade 69%, VWR, Leicestershire, UK). The digestion was performed using a temperature-controlled microwave programme specific for the sample matrix. The digest was diluted further prior to analysis by inductively coupled plasma-mass spectrometry (ICP-MS) using an Agilent 7500ce (Agilent Technologies, Waldbronn, Germany). Quantification of the trace elements was performed by external calibration and deploying eight levels (0, 0.5, 1, 5, 10, 20, 100 and 500 μg L^{-1}) of working standard solutions which were prepared from a customised mixed metal standard solution of 100 mg L^{-1} (SPEX Certiprep Ltd., Middlesex, UK).

To ensure a high level of quality assurance, a reagent blank and a certified reference material (CRM TORT-2-Lobster hepatopancreas, National Research Council Canada, Halifax, Nova Scotia, Canada) was analysed within-batch to monitor method performance on a day-to-day basis. Concentration data derived from the analysis of the CRM were then added to existing quality control Shewhart charts (using North West Analytical Quality Analyst™, Northwest Analytical Inc., USA) for the assessment of the on-going method performance from the batch analysis of real samples. The validity of results was established using the warning and control limits of the Shewhart chart, which are defined as 2σ and 3σ of the mean, respectively.

In addition to internal quality control, the analytical laboratory biannually participates in the proficiency testing scheme Quasimeme (Quality Assurance of Information for Marine Environmental Monitoring in Europe) as external quality assurance. A summary of the accuracy of the analytical methods is provided in the Supplementary Material (Table S1).

To carry out an effect-based assessment, the approach of Nicolaus et al. (2015) and Nicolaus et al. (2017) was used, which compares the measured environmental concentrations (MEC) for set determinants to derived assessment criteria (Table 1). A risk characterisation ratio

Table 1
Assessment criteria used to analyse the contaminant status of *Leucoraja circularis* and L. *fullonica* for concentrations (mg kg^{-1}) of Cd, Pb and Hg in muscle (based on European regulations on the maximum levels in foodstuffs (CEC, 2006)) and Cd and Pb in liver (based on the preliminary OSPAR, 2009 indicators of environmental quality, whereby it was suggested using the statutory dietary limits of Cd and Pb in bivalves as proxy thresholds for concentrations in fish liver). Note: CEC (2006) lists Hg limits for "rays (*Raja* species)" as 1.0, and this is the assumed level used in the current analysis, as both species were formerly in the genus *Raja*.

Metal	Threshold concentration (mg kg^{-1})		Percentage of *Leucoraja circularis* exceeding limits		Percentage of L. *fullonica* exceeding limits	
	Muscle	Liver	Muscle	Liver	Muscle	Liver
Cd	0.05	1	0%	5%	0%	0%
Pb	0.3	1.5	0%	0%	0%	0%
Hg	1.0	–	5%	–	0%	–

(RCR) was then calculated by dividing the MEC by either the limits defined in European Commission Regulations (proxy Environmental Assessment Criteria - EAC) that cite the safe maximum levels of contaminants in seafood (above which the concentration may be harmful to human health (CEC, 2006)) or the thresholds suggested by OSPAR (2009) for indicators of environmental quality.

It was also assessed whether there were differences in contaminant concentrations at length (L_T) between the two species for three contaminants: As, Se and Hg. The models used for As and Se were linear models (contaminant concentration = $a + b\ L_T$ + error), with a non-linear model used for Hg (contaminant concentration = $a\ {L_T}^{b}$ + error), where a and b are parameters estimated by least squares and the error is assumed to be Normally distributed with mean 0 and constant variance. To compare the concentration for the species across all lengths, the fit to the data was compared (as defined by the residual sum of squares RSS1) when a model was fitted to each species separately against the fit when a single model was fitted to all data (RSS0). An F-test was used to evaluate a p-value based on the differences between RSS0 and RSS1 (Mead and Curnow, 1984).

Concentrations of 11 trace elements (As, Cd, Cr, Cu, Fe, Hg, Mn, Pb, Ni, Se and Zn) were analysed in the muscle and liver of L. *circularis* (n = 20; 23–110.5 cm total length, 157–490 m water depth) and L. *fullonica* (n = 24; 28.5–100 cm total length, 130–426 m water depth). Data (Table 2, and Tables S2–S3 for raw data) indicate that both Pb and Cr occurred in low concentrations in the muscle, with 52.3% and 54.5% of samples (species combined) below the detection limits for Pb and Cr, respectively. For those specimens with detectable limits, the mean concentrations of Cr and Pb were 0.010 and 0.011 mg kg^{-1}, respectively (species combined).

Concentrations of As in the muscle ranged from 4.9–95 and 22–141 mg kg^{-1} in L. *circularis* and L. *fullonica*, respectively, and there was a significant difference in the concentration of As between the two species with length (F = 161.8, df = 4,40, $p < 0.001$). Whilst concentrations of As increased significantly with length (Fig. 2), data were limited for skates > 90 cm L_T.

Concentrations of Hg in muscle were 0.02–1.8 and 0.04–0.61 mg kg^{-1} in L. *circularis* and L. *fullonica*, respectively (Table 2), and increased with L_T (Fig. 2). Only one specimen (a 110.5 cm L_T L. *circularis*) had a concentration of Hg in muscle > 1.0 mg kg^{-1} (and had a Hg concentration of 4.1 mg kg^{-1} in the liver). There is a need to examine more samples of skates > 90 cm L_T to better understand the proportion of large skate specimens that might exceed EC regulations on safe maximum levels of contaminants. For Hg, there was also a significant difference between the two species at different lengths (F = 452.7, df = 4,40, $p < 0.001$; Fig. 2).

Concentrations of Se in muscle ranged from 0.36–0.64 and 0.29–0.4 mg kg^{-1} in L. *circularis* and L. *fullonica*, respectively, and concentrations were significantly different between the two species at different lengths (F = 348.2, df = 4,40, $p < 0.001$; Fig. 2).

Whilst sample sizes were relatively limited, there was the indication that concentrations (at length) of both Hg and Se were higher in L. *circularis*, and As higher in L. *fullonica*, although the reasons for this are unclear.

Comparing the results to the available assessment criteria, one specimen of L. *circularis* failed the proxy EAC for Cd in liver, no samples failed the proxy EAC for Hg or Pb in liver. For Hg in the muscle tissue, only one specimen of L. *circularis* failed the proxy EAC (1.0 mg kg^{-1}).

By analysing the Hg:Se ratio, it became apparent that only one sample for *Leucoraja circularis* showed a ratio above one, indicating that the organism itself could suffer from physiological impacts (Nicolaus et al., 2016a). If the ratio is above one, it means that there are more Hg

Table 2
Mean concentrations (mg kg^{-1}; ± SD and range) of 11 trace elements in muscle and liver tissues of *Leucoraja circularis* (n = 20) and L. *fullonica* (n = 24) [samples below detection limit concentrations were omitted from the summary data, which explains the lower sample sizes for some trace elements].

Species	Trace element	Liver			Muscle		
		Mean ± SD	Range	N	Mean ± SD	Range	N
L. circularis	As	16.77 ± 6.41	6.4–34	20	46.65 ± 19.38	4.9–95	20
	Cd	0.39 ± 0.56	0.09–2.7	20	0.01 ± 0.00	0.01–0.01	7
	Cr	0.03 ± 0.04	0.01–0.17	20	0.06 ± 0.05	0.01–0.19	20
	Cu	7.34 ± 4.15	2.3–20	20	0.26 ± 0.11	0.13–0.57	20
	Fe	89.15 ± 60.24	39–301	20	2.01 ± 0.99	0.96–5.4	20
	Pb	0.02 ± 0.01	0.01–0.05	9	0.01 ± 0.00	0.01–0.02	10
	Mn	0.44 ± 0.17	0.26–1	20	0.29 ± 0.24	0.08–1.0	20
	Hg	0.33 ± 0.89	0.01–4.1	20	0.43 ± 0.38	0.02–1.8	20
	Ni	0.03 ± 0.03	0.01–0.1	18	0.04 ± 0.03	0.01–0.14	17
	Se	1.13 ± 0.63	0.65–3.7	20	0.45 ± 0.08	0.36–0.64	20
	Zn	10.65 ± 2.60	7.9–19	20	4.73 ± 1.17	3.4–7.8	20
L. fullonica	As	19.33 ± 5.77	4.9–35	24	47.88 ± 24.15	22–141	24
	Cd	0.17 ± 0.05	0.08–0.26	24	0.01 ± 0.00	0.01–0.01	13
	Cr	0.04 ± 0.03	0.01–0.16	22	0.11 ± 0.08	0.01–0.37	24
	Cu	3.00 ± 1.72	1.4–8.4	24	0.32 ± 0.09	0.17–0.48	24
	Fe	40.63 ± 24.46	15–113	24	2.38 ± 0.96	1.2–5	24
	Pb	0.01 ± 0.00	0.01–0.02	8	0.01 ± 0.00	0.01–0.02	11
	Mn	0.59 ± 0.13	0.35–0.84	24	0.30 ± 0.16	0.11–0.61	24
	Hg	0.04 ± 0.03	0.01–0.12	23	0.13 ± 0.14	0.04–0.61	24
	Ni	0.04 ± 0.03	0.01–0.16	22	0.08 ± 0.07	0.02–0.36	23
	Se	0.96 ± 0.25	0.16–1.3	24	0.35 ± 0.03	0.29–0.4	24
	Zn	10.71 ± 5.33	4.5–34	24	4.91 ± 1.06	3.4–6.7	24

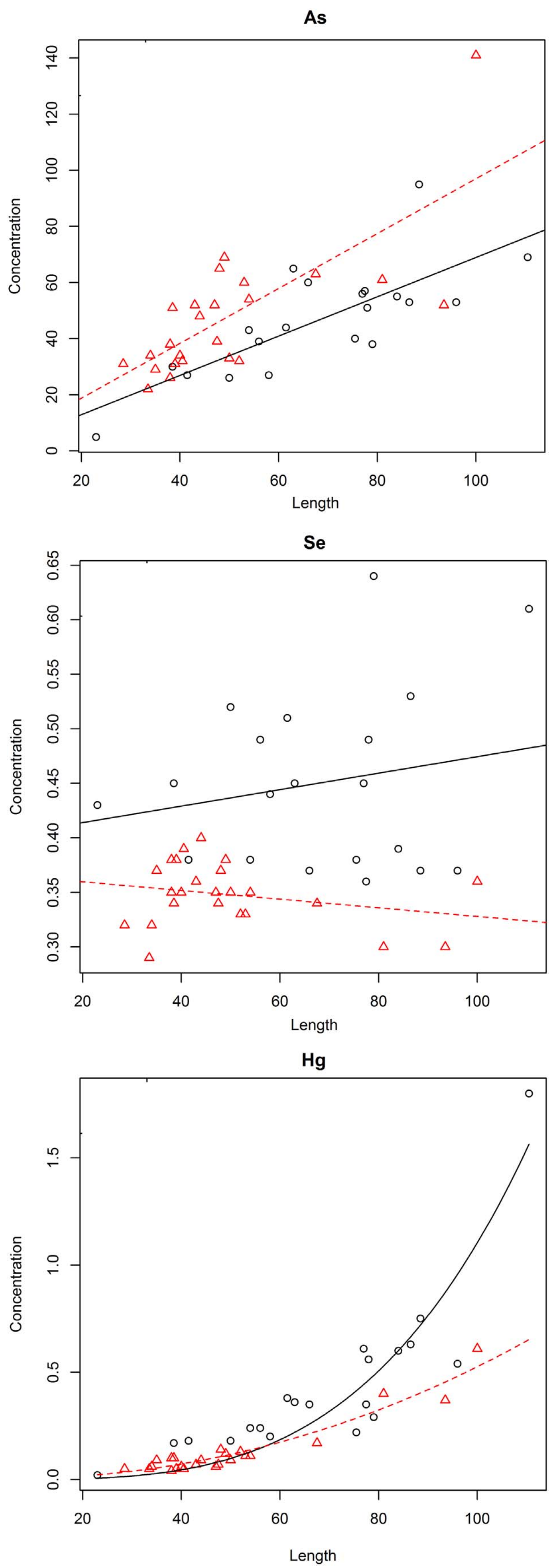

Fig. 2. Concentrations of As, Hg and Se (mg kg^{-1}) in muscle in relation to total length in *Leucoraja circularis* (SAR, circles) and L. *fullonica* (SHR, triangles) from the Bay of Biscay and Celtic Sea. The relationship between concentrations and length in L. *circularis* were $As = -1.167 + 0.701L_T$, $Se = 0.399 + 0.000755L_T$, and $Hg = 1.156 * 10^{-7} L_T^{3.49}$. The relationship between concentrations and length in L. *fullonica* were $As = -0.846 + 0.979L_T$, $Se = 0.386–0.000396L_T$, and $Hg = 2.462 * 10^{-5} L_T^{2.165}$.

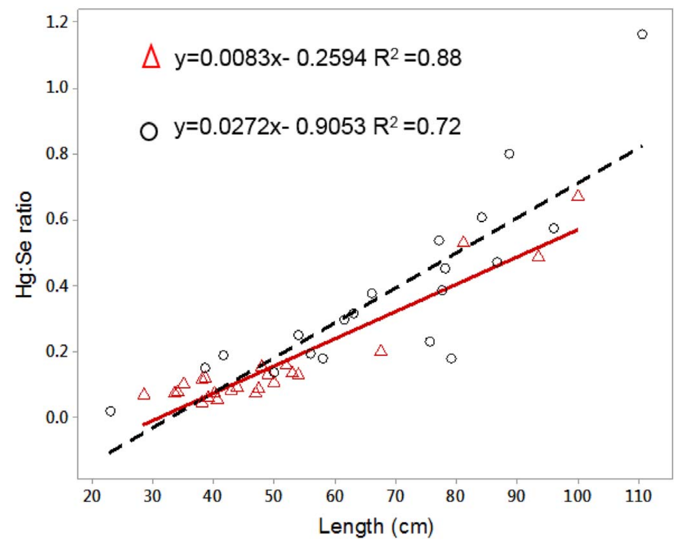

Fig. 3. Relationship between the Hg:Se ratio and total length in the muscle of *Leucoraja circularis* (circles; dashed trendline) and L. *fullonica* (triangles; solid trendline).

moles than Se moles present in the individual, which suggests that methylmercury exposure could limit selenoprotein synthesis, which is part in the routine functioning of enzymes in multiple biological processes (EERC, 2011). There was a positive and similar relationship between the Hg:Se ratio in both species (Fig. 3).

Mean concentrations of other trace elements (Ni, Cu, Zn, Mn and Fe) were low (Table 2). The concentrations of Cu in muscle were all at detectable limits, the highest concentrations were only 0.57 and 0.48 mg kg^{-1} for L. *circularis* and L. *fullonica*, respectively. Furthermore, the mean Cu concentrations in the muscle were only 0.26 (*L. circularis*) and 0.32 (*L. fullonica*) mg kg^{-1}, which are generally lower than the concentrations reported in shallower-water fish species (Collings et al., 1996; Mormede and Davies, 2001).

Monitoring programmes examining metals and other contaminants in fish and shellfish around the UK provided data for a range of skate species during the 1970s (Murray, 1979, 1981; Murray and Portmann, 1984), with thornback ray *Raja clavata* also examined regularly into the 1990s (Franklin, 1987; Franklin and Jones, 1995). Since then, however, monitoring efforts have focused on a smaller groups of core species, with dab presently the main indicator species studied (Nicolaus et al., 2016b).

Other previous studies on contaminants in skates from European seas have generally focused on the more coastal and more widespread *R. clavata* (Dixon and Jones, 1994; Chouvelon et al., 2012; Türkmen et al., 2013; Torres et al., 2016), a species that has also been used in experimental studies of metal accumulation (Pentreath, 1973, 1976, 1977a, 1977b). In contrast, the levels of contaminants in other skate species have been subject to more limited study (Table 3).

This is the first published study on the trace element concentrations in L. *fullonica*, and in L. *circularis* from Atlantic waters, and results indicated that concentrations of Hg could exceed 1.0 mg kg^{-1} in larger specimens. Storelli et al. (1998) reported a mean Hg concentration of 1.47 mg kg^{-1} in muscle of L. *circularis* from the southern Adriatic Sea, with this based on two pooled samples from 10 specimens. The case-study species are both members of the outer shelf and upper slope fish assemblage, and neither species are frequent in shallow waters.

Table 3
Published studies on mercury in the muscle of skates (Rajiformes). Scientific names updated as per Last et al. (2016) and trophic levels (TL) as reported by Ebert and Bizzarro (2007). Fish size refers to total length, unless specified otherwise (D = disc width) with values in parentheses indicating mean size. Hg concentrations (mg kg^{-1}) refer to total mercury concentrations by wet weight, unless specified otherwise. Notes: [1] Concentration of Hg related to dry weight; [2] Concentration of Hg refers to methylmercury; [3] Concentration of Hg refers to whole animal, not specific tissues; [4] Concentration of Hg reported to increase with length; [5] Study provided data for other tissues; [6] Study provided data for other trace elements or contaminants; [7] Species identification might be questionable, given location of capture and/or maximum size; [8] Concentrations refer to mean and 95% CI.

Scientific name	TL	Geographic area	Year	Depth range (m)	Sample size (number of pools)	Size range (cm)	Hg concentration mean ± SD (range)	Notes	Source
Amblyraja radiata	3.82	Nova Scotia	1970	–	10	–	0.03–0.24	[2]	Zitko et al. (1971)
		Halifax	1972	–	10	–	0.12–0.41	–	Freeman et al. (1974)
		Georges Bank	1971	27–113	– (2)	60–93	0.24 (0.21–0.26)	[5]	Greig et al. (1975)
		North Sea	1975	–	10	–	0.09 (0.06–0.17)	[6]	Murray (1981)
		Barents Sea	1994	–	5	–	0.72 ± 0.70	[1,5,6,8]	Zauke et al. (1999)
Dentiraja cerva	–	Tasmania	1976–1977	–	32	42.2–59.7 (D)	0.31 ± 0.04 (0.03–0.85)	–	Thomson (1985)
Dipturus batis	4.06	Irish Sea	1992–1993	–	8	(72.8)	1.2 ± 1.1 (0.27–3.14)	[5,6,7]	Collings et al. (1996)
Dipturus kwangtungensis	–	East China Sea	2001–2002	131–133	3	(35)	< 0.05	[1,3,6]	Asante et al. (2008)
Dipturus oxyrinchus	–	Southern Adriatic Sea	1995	–	12 (3)	–	1.56 ± 0.95 (1.00–2.65)	–	Storelli et al. (1998)
Leucoraja circularis	–	Southern Adriatic Sea	1995	–	10 (3)	–	1.47 ± 1.12 0.68–2.27	–	Storelli et al. (1998)
		Bay of Biscay and Celtic Sea	2014–2016	157–490	20	23–110.5	0.43 ± 0.38 (0.02–1.8)	[4,5,6]	This study
Leucoraja erinacea	3.70	George's Bank	1971	24–113	–	45–50	0.15 (0.13–0.16)	[5]	Greig et al. (1975)
		Southern New England	2009–2012	–	173	21.5–29.5 (D)	0.4 ± 0.3	[1,4]	Taylor et al. (2014)
Leucoraja fullonica	4.06	Bay of Biscay and Celtic Sea	2014–2016	130–426	24	28.5–100	0.13 ± 0.14 (0.04–0.61)	[4,5,6]	This study
Leucoraja naevus	3.91	North Sea (Aberdeen)		–	10	–	0.054 ± 0.029 (0.02–0.11)	[6]	Davies (1981)
		Irish Sea	1976	–	2	–	0.35 (0.28–0.41)	[6]	Murray and Portmann (1984)
		Bay of Biscay	2001–2010	120–199	10	60.4 ± 2.8	0.569 ± 0.239 (0.396–1.205)	[1]	Chouvelon et al. (2012)
Leucoraja ocellata	4.04	George's Bank	1971	18–62	(1)	–	0.15	[5]	Greig et al. (1975)
		Southern New England	2009–2012	–	148	20.5–54.7 (D)	0.3 ± 0.2	[1,4]	Taylor et al. (2014)
Okamejei kenojei	–	East China Sea	2001–2002	147–149	2	(39)	0.17	[1,3,6]	Asante et al. (2008)
Raja asterias	–	Southern Adriatic	1995	–	100 (20)	–	0.73 ± 0.47 (0.05–1.50)	–	Storelli et al. (1998)
Raja brachyura	3.82	Irish Sea	1976	–	7	–	0.18 (0.07–0.66)	[6]	Murray and Portmann (1984)
		Bristol Channel	1976	–	5	–	0.32 (0.17–0.71)	[6]	Murray and Portmann (1984)
Raja clavata	3.69	Sagres (Portugal)	1972	–	2	–	0.53	[1,5,6]	Stenner and Nickless (1975)
		North Sea	1975	–	7	–	0.16 (0.06–0.28)	[6]	Murray (1981)
		North Sea (coastal)	1975	–	10	–	0.16 (0.07–0.24)	[6]	Murray (1981)
		North Sea	1976	–	50	–	(0.03–2.0)	[5, 6]	Murray and Portmann (1984)
		Bristol Channel	1976	–	10	–	(0.06–0.38)	[5, 6]	Murray and Portmann (1984)
		Irish Sea	1976	–	18	–	(0.14–1.0)	[5, 6]	Murray and Portmann (1984)
		English Channel	1977	–	10	(41)	0.11 (0.09–0.13)	[6]	Franklin (1987)
		Morecombe Bay	1979	–	9	(55)	0.22 (0.12–0.37)	[6]	Franklin (1987)
		Liverpool Bay	1981	–	10	(38)	0.29	[6]	Franklin (1987)
		Bristol Channel	1983	–	9	(68)	0.39	[6]	Franklin (1987)
		Swansea Bay	1983	–	24	(53)	0.21	[6]	Franklin (1987)
		Southern North Sea	1983	–	14	(48)	0.15	[6]	Franklin (1987)
		English Channel	1990	–	18	–	0.07–0.10	[6]	Franklin and Jones (1995)
		Southern North Sea	1990–1992	–	8	–	0.05–0.11	[6]	Franklin and Jones (1995)
		North Sea	1992	–	22	41–50.6	0.097 (0.007–0.270)	[4]	Dixon and Jones (1994)
		Southern Adriatic	1995	–	84 (14)	–	1.24 ± 0.23 (0.86–1.60)	–	Storelli et al. (1998)

(continued on next page)

Table 3 *(continued)*

Scientific name	TL	Geographic area	Year	Depth range (m)	Sample size (number of pools)	Size range (cm)	Hg concentration mean ± SD (range)	Notes	Source
		North Sea and eastern Channel	1997–1999	–	19	–	0.039 ± 0.021 0.037 ± 0.019	[5] [2,5]	Baeyens et al. (2003)
		Bay of Biscay	2001–2010	120–199	11	73.5 ± 11.1		1.02-1 ± 0.-816 (0.524–-3.147)	[1]
		Chouvelon et al. (2012)							
Azores	20-13	–	30	40.5–79.5	0.14–1.47*	[1,4,5,6]	Torres et al. (2016)		
Raja eglanteria	3.68	S. Carolina-Florida	–	(inshore)	1	–	1.3	[1,5,6]	Windom et al. (1973)
		Delaware Bay	1975		3	25–27 (D)	0.214 ± 0.098 (0.119–0.321)		Gerhart (1977)
Raja microocellata	3.88	Bristol Channel	1976	–	5 5	–	0.15 (0.06–0.20) 0.07 (0.04–0.10)	[6]	Murray and Portmann (1984)
		Irish Sea	1976	–	3	–	0.37 (0.23–0.49)	[6]	Murray and Portmann (1984)
		Bay of Biscay	2001–2010	< 30	5	69.4 ± 9.9	0.169 ± 0.04 (0.128–0.217)	[1]	Chouvelon et al. (2012)
Raja miraletus	3.67	Southern Adriatic Sea	1995	–	40 (10)	–	1.10 ± 0.38 (0.60–1.78)	–	Storelli et al. (1998)
		Adriatic Sea	2010	–	127 (10 pooled samples)	33.5–48.5	1.09 ± 0.39 (0.40–1.78)	[4,6]	Storelli and Barone (2013)
		Adriatic Sea	2011	–	35 (pooled into 11 samples)	35.0–85.7	0.98 ± 0.33 (0.28–1.54)	[6,7]	Storelli et al. (2013)
Raja montagui	3.59	English Channel	1975	–	17	–	0.12 (0.06–0.19)	[6]	Murray (1981)
		Bristol Channel	1976	–	2	–	0.06–0.07	[6]	Murray and Portmann (1984)
		Irish Sea	1992–1993	–	6	(39.3)	0.3 ± 0.1	[5,6]	Collings et al. (1996)
Rajella fyllae	3.78	Rockall Trough	1998	850–950		38–54	0.044–0.410	[5,6]	Mormede and Davies (2001)
		Barents Sea	1994	–	5	–	1.35 ± 0.19	[1,5,6,8]	Zauke et al. (1999)
Rostroraja velezi	–	Mexico (Pacific)	2012	–	1	83 (D)	1.127	[1,5]	Ruelas-Inzunza et al. (2013)
		Costa Rica (Pacific)	2010–2011	< 100	19	17.5–55.4 (D)	0.25 ± 0.16 (0.01–0.50)	[1,4]	Sandoval-Herrera et al. (2016)
Sympterygia bonapartei	–	Bahia Blanca, Argentina	1985–1986	–	–	29–50.5	0.18 ± 0.06	[6]	Marcovecchio et al. (1988)
Zearaja chilensis	–	Chile	2009–2012	–	102	–	0.088 ± 0.05	[6]	Lopez et al. (2014)
Bathyraja spinicauda	4.02	Barents Sea	1994	–	3	–	0.58–1.21	[1,5,6,8]	Zauke et al. (1999)
Beringraja binoculata	–	Gulf of Alaska	2012–2013	–	20	92–175	0.09 ± 0.06 (up to 0.61)	[4,5,6]	Farrugia et al. (2015)
Beringraja rhina	–	Gulf of Alaska	2013	–	20	109–133	0.34 ± 0.18 (up to 0.61)	[4,5,6]	Farrugia et al. (2015)

Consequently, the concentrations of contaminants are not expected to be related to recent anthropogenic inputs. Observed contaminants levels, in particular Hg, are therefore likely to reflect ocean basin scale burden (from natural processes and longer-term, historical inputs over a continental scale), rather than local/regional input.

Most published studies on metals in skate muscle have indicated that Hg concentrations are often < 1.0 mg kg^{-1} (Table 3), Hg concentrations > 1.0 mg kg^{-1} wet weight have been observed in a range of skate species, including *R. clavata* (Storelli et al., 1998), long-nosed skate *Dipturus oxyrinchus* (Storelli et al., 1998), common skate *Dipturus batis* (Collings et al., 1996; although the timing and location of sampling infers a degree of doubt to the species, and it may refer to *R. clavata*), and brown ray *Raja miraletus* (Storelli et al., 1998, 2013). The latter study reported that 35% of samples of *R. miraletus* from the Adriatic Sea exceeded 1.0 mg g^{-1} (wet weight). Other studies have reported Hg concentrations > 1.0 mg kg^{-1} dry weight, including for round skate *Rajella fyllae* and spinytail skate *Bathyraja spinicauda* (Zauke et al., 1999) and *R. clavata* (Chouvelon et al., 2012). Skates can also have high concentrations of other toxic contaminants, such as As (Collings et al., 1996; De Gieter et al., 2002). Consequently, further studies could usefully ascertain levels of contaminants of skates, focusing on areas of known contamination, and also on longer-lived (or larger-bodied) species.

Whilst some studies (e.g. Storelli and Barone, 2013; Lopez et al., 2014; Taylor et al., 2014) have used robust sample sizes, many previous studies examining the concentrations of metals in skates have been based on low sample sizes (see Table 3). Studies indicating high concentrations of contaminants but based on small sample sizes, should be used with a degree of caution, given the potential variability in metal concentrations.

Within Europe, EC Regulation 1881/2006 established a maximum level of 1.0 mg kg^{-1} of Hg in *Raja* spp., presumably with the intent to refer to all members of the family, with the maximum concentrations of Pb and Cd being 0.30 and 0.05 mg kg^{-1}, respectively (CEC, 2006). Whilst the concentrations of Pb and Cd observed in the present study were all below these limits, concentrations of Hg can exceed maximum levels, and further studies of skates in either area of known historical contamination and of larger-bodied species could usefully be undertaken to better ascertain spatial and ontogenetic influences on Hg concentrations.

Skates in European seas are not aged routinely by national fisheries laboratories, and age estimates for the species studied here are not currently available. The increasing concentrations of contaminants in larger specimens may be related to both biomagnification (e.g. through eating higher trophic level prey) and/or bioaccumulation (Lyons and Adams, 2017). Older fish typically exhibit reduced growth rates, and so there can be more variability in the length at age for older fish. Given this, and that bioaccumulation of contaminants can be related more closely to age than length (Braune, 1987), there is a need to have appropriate sample sizes of fish in larger size classes.

Skates are large-bodied demersal species, often of commercial value, and the order Rajiformes has both a broad bathymetric range and an extensive biogeographic range, especially in temperate and subtropical seas. Hence, they could potentially be a useful taxon for examining broader scale patterns in contaminant levels. It would be useful if published studies on contaminants in skates also included depth information, clarification of whether the 'size' reported is disc width or total length, and covered as broad a size (age) range as possible.

Acknowledgements

We thank the scientists and crew of RV *Thalassa* for collecting samples of the two species, and David James and Lee Warford for assisting with laboratory analyses of metals. The collection of biological material was supported by Defra contracts MB5201 and MF6001, and the analysis of trace elements by SLA22. We also thank Robin Law for his editorial advice given during the compilation of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marpolbul.2017.08.054.

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