Effect of pyrethroid treatment against sea lice in salmon farming regarding consumers' health

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Abstract

Pyrethroids are the most popular drug against sea lice in salmon farming. Although they are more toxic to insects, they have toxic effects in mammals. Pyrethroids were detected in 100 % of farmed salmon with a mean concentration of 1.31 ± 1.39 ng g^{-1} ww and in 50 % of wild salmon with a mean of 0.02 ± 0.03 ng g^{-1} ww. Cypermethrin and deltamethrin, the active ingredients of anti-sea lice formulations, represented 77 ± 27 % of the total contamination of farmed salmon. Although farmed salmon had higher concentrations than wild salmon, the daily intake of pyrethroids through salmon consumption was several orders of magnitude below the accepted daily intake (ADI). Thus, the pyrethroids treatment on salmon does not pose a threat on the health of the consumers.

Keywords: pesticide; risk assessment; enantiomer; fish farming; seafood

1. Introduction

Sea lice (Copepoda: Caligidae) have been the most widespread pathogenic marine parasite in the three decades of salmon farming industry. In the second part of this period, pathogenic infestations on other farmed fish and wild salmonids have increased notoriously [Ragias *et al*., 2004; Costello, 2006]. The impact of sea lice on the host ranges from mild skin damage to mortality induced by stress, including epidemics in wild fish populations in Europe and British Columbia [Costello, 2006]. A non-comprehensive list of other effects would include epithelium loss, increased mucus discharge, bleeding, tissue necrosis and consequent exposure to secondary infections; reduced appetite, growth and food-conversion efficiency; anaemia and reduced lymphocytes [Tully and Nolan,2002; Johnson *et al.*,2004; Costello, 2006].

Pyrethroids became the most popular drug against sea lice around 1995, substituting organophosphates, which had previously been the preferred compounds [Grave *et al.*, 2004]. The anti-sea lice pesticide formulations AlphaMax® and Excis® are emulsifiable concentrates containing 1 % of the synthetic pyrethroids deltamethrin or cypermethrin as the active ingredient, respectively. Both pesticides are effective against all attached stages of sea lice including adults [Haya *et al.*, 2005; Burridge *et al.*, 2010]. Treatment of salmon is either a 40-minute bath with AlphaMax® at a target concentration of 2.0 µg/L deltamethrin [SEPA, 2008] or a 1-h bath with Excis® at a target concentration of 5.0 µg/L cypermethrin [Van Geest *et al.*, 2014].

Pyrethroid insecticides are applied for household, commercial and farming purposes, in medicine against lice and scabies and to control malaria in tropical countries by impregnating mosquito nets with them [Bradberry *et al.*, 2005]. In salmon farming pyrethroids are used against the sea lice, which parasite the fish [Haya *et al.*, 2005]. Pyrethroids were considered ideal insecticides because they were thought not to be persistent in the environment and to be metabolised by mammals instead of accumulated [Casida *et al.*, 1975; Leng *et al.*, 1997]. Therefore, their popularity grew during the 1970s and pyrethroids substituted other banned pesticides [Ridgway *et al.*, 1978]. Nowadays, they account for 25 % of the insecticides used worldwide, which

equals about 100 tons of pyrethroids a year [Casida and Quistad, 1998; Shafer *et al.*, 2005].

Their toxic effects include disruption of the function of the neurons' sodium channels as they provoke repetitive after-discharges in neurons and muscle cells that produce repeated stimulation [Narahashi *et al.*, 1998; Pollack *et al.*, 1999]. At high concentrations of pyrethroids, the sodium intake may block conduction, causing paralysis. Lethal concentration 50 (LC_{50}) of pyrethroids have been reported for some fish. LC_{50} values cover a wide range, e.g. 0.06 $\mu g\, l^{-1}$ for tefluthrin on trout, 19 $\mu g\, l^{-1}$ for allethrin on trout and 150 $\mu g\, l^{-1}$ for bifenthrin on trout [Lewis *et al*. 2016]. Toxicity of pyrethroids is 2,250 times higher to insects than mammals, since insects have more sensitive sodium channels, smaller bodies and lower body temperatures. On the other hand, several acute and chronic effects on humans have been reported [IARC, 1991; Muller-Mohnssen and Hahn, 1995; Kolaczinski and Curtis, 2004; Bradberry *et al.*, 2005; EPA, 2015].

Because of their toxicity, exposure of aquatic organisms to pyrethroids has always caused concern [Mauck and Olson, 1976]. Used on the land or for domestic purposes as vector control, pyrethroids can enter the aquatic environment through atmospheric deposition, river runoff or municipal treatment discharges. They associate with sediments and then benthic organism become exposed to pyrethroids by ingestion or contact of sediment particles or from interstitial water. Fish are exposed to pyrethroids through diet or gill absorption due to the lipophilicity of these compounds [Edwards *et al.*, 1987].

Although pyrethroids are believed to be converted to non-toxic metabolites in mammals by hydrolysis and oxidation [Abernath *et al.*, 1973; Casida *et al.*, 1975], our research group found evidence that they bioaccumulate in marine mammals from Brazil [Alonso *et al.*, 2012] and Spain [Aznar-Alemany *et al.,* 2017]. These insecticides have also been detected in human breast milk [Zehringer and Herrmann, 2001; Corcellas *et al.*, 2012]. Due to their aforementioned toxic effects and the evidence of their accumulation in mammals, the World Health Organisation (WHO) has reported a no-observed-adverse-effect level (NOAEL) and acceptable daily intake (ADI) for the

individual pyrethroids expressed in quantity of the compound per kilogram of a person's body weight (bw) per day [WHO, 2005]. NOAELs for the pyrethroids in this work are between 1 and 5 $mg\,(kg\,bw)^{-1}\,day^{-1}$ and maximum ADIs are between 0.02 and 0.05 $mg\,(kg\,bw)^{-1}\,day^{-1}$.

Pyrethroids derive from allethrin, type I pyrethroids contain a carboxylic ester and type II pyrethroids have an additional cyano group; most of the pyrethroids contain a cyclopropane, too [Bradberry *et al.*, 2005]. Because of these groups, type I pyrethroids possess 2 chiral centres and type II possess 3 of them. This means that type I and type II pyrethroids have 2 and 4 diastereoisomers (therefore enantiomer pairs), respectively, which could show different toxicity and accumulation [Jin *et al.*, 2012]. This is relevant as isomeric composition is an important toxicological parameter for a number of compounds [Zhao *et al.*, 2010; Sun *et al.*, 2016; Wang *et al.*, 2016].

The aim of this work was to compare the occurrence of 10 pyrethroid compounds in farmed salmon to wild salmon and assess the effect of the pyrethroids baths against sea lice. Additionally, comparisons of farmed salmon's pyrethroid levels were performed, first between places of origin of the samples and second between processed (i.e. marinated or smoked) and non-processed samples. The risk on consumer's health was assessed comparing an estimated daily intake of pyrethroids through consumption of salmon to the ADI. Finally, enantiomer selective accumulation for different species was studied.

2. Materials and method

2.1. Sampling

Samples of salmon (51), including farmed (39) and wild (12) salmon, were purchased at retail stores and fishmongers from Barcelona (Spain) between February 2014 and February 2016. Salmon from 6 different species, farmed in 8 different countries and sold in 5 different presentations were collected. The farming locations of the samples included Alaska, Chile, Denmark, France, Norway, the Pacific Ocean, Scotland and Spain. The presentations available were fresh, frozen, marinated, refrigerated and smoked. The species of salmon included *Oncorhynchus gorbuscha*, *Oncorhynchus keta*,

Oncorhynchus kisutch, *Oncorhynchus mykiss*, *Oncorhynchus nerka* and *Salmo salar*. See Table 1 for more details. Previous to freeze-drying, the skin was removed. Muscle of all samples was freeze-dried, homogenised and stored frozen.

Table 1. Samples information

	processing	origin	species	purchase date	water (%)	fat (% ww)
farmed	frozen	Norway	*Salmo salar*	Apr 2014	73.7	3.73
		Chile	*Salmo salar*	Feb 2014	69.5	6.06
		Chile	*Salmo salar*	Feb 2015	74.5	3.27
	refrigerated	Spain	*Oncorhynchus mykiss*	June 2014	77.2	3.11
		Spain	*Oncorhynchus mykiss*	Feb 2015	78.4	1.80
		Spain	*Oncorhynchus mykiss*	Apr 2014	74.6	5.25
		Norway	*Salmo salar*	Apr 2014	68.5	6.01
		Norway	*Salmo salar*	Apr 2014	65.5	10.6
		Norway	*Salmo salar*	Apr 2014	65.9	9.79
	smoked	Denmark	*Oncorhynchus mykiss*	Mar 2014	67.1	4.09
		Spain	*Salmo salar*	Apr 2014	62.2	12.7
		Spain	*Salmo salar*	Feb 2015	60.2	11.6
		France	*Oncorhynchus mykiss*	Apr 2014	68.0	6.12
		France	*Oncorhynchus mykiss*	Apr 2014	63.8	5.34
		Norway	*Salmo salar*	Feb 2014	60.0	8.85
		Norway	*Salmo salar*	Apr 2014	65.2	4.64
		Norway	*Salmo salar*	Apr 2014	64.0	7.82
		Norway	*Salmo salar*	Apr 2014	62.8	12.2
		Norway	*Salmo salar*	Apr 2014	41.1	17.2
		Norway	*Salmo salar*	Apr 2014	68.1	7.43
		Norway	*Salmo salar*	Apr 2014	63.6	10.3
		Norway	*Salmo salar*	Apr 2014	51.5	23.6
		Norway	*Salmo salar*	Apr 2014	57.7	13.0
		Norway	*Salmo salar*	Apr 2014	62.7	5.97
		Norway	*Salmo salar*	Apr 2014	54.2	17.9
		Norway	*Salmo salar*	Apr 2014	55.7	15.0
		Norway	*Salmo salar*	Apr 2014	57.9	12.3
		Scotland	*Oncorhynchus mykiss*	Apr 2014	65.2	8.37
		Scotland	*Salmo salar*	Mar 2014	51.9	26.4
		Scotland	*Salmo salar*	Apr 2014	59.7	7.19
		Scotland	*Salmo salar*	Apr 2014	60.6	11.6
		Scotland	*Salmo salar*	Feb 2015	60.4	7.53
	marinated	Norway	*Salmo salar*	Feb 2014	61.6	6.67
		Norway	*Salmo salar*	Apr 2014	62.1	14.1
		Norway	*Salmo salar*	Apr 2014	60.2	14.6
		Norway	*Salmo salar*	Feb 2015	65.2	9.07
	fresh	Spain	*Oncorhynchus mykiss*	Apr 2014	76.6	2.03

	processing	origin	species	purchase date	water (%)	fat (% ww)
		Norway	*Salmo salar*	Feb 2014	72.1	4.23
		Norway	*?*	Nov 2014	67.8	9.32
wild	marinated	Alaska	*Oncorhynchus nerka*	Feb 2015	73.2	0.71
		Alaska	*Oncorhynchus nerka*	Feb 2015	64.6	5.78
		Alaska	*Oncorhynchus nerka*	Feb 2015	70.3	1.39
	frozen	Alaska	*Oncorhynchus nerka*	Feb 2015	74.0	2.41
		Alaska	*Oncorhynchus kisutch*	Feb 2015	75.5	0.73
		Pacific Ocean	*Oncorhynchus gorbuscha*	Apr 2014	80.8	0.56
		Pacific Ocean	*Oncorhynchus gorbuscha*	June 2014	74.9	0.81
		Pacific Ocean	*Oncorhynchus gorbuscha*	Oct 2014	76.7	0.95
		Pacific Ocean	*Oncorhynchus gorbuscha*	Oct 2014	76.9	1.14
		Pacific Ocean	*Oncorhynchus keta*	Apr 2014	77.5	0.51
		Pacific Ocean	*Oncorhynchus keta*	Oct 2014	76.0	0.59
		Pacific Ocean	*Oncorhynchus keta*	Feb 2015	76.7	0.38

2.2. Standards and reagents

Bifenthrin, *λ*-cyhalothrin, fluvalinate, resmethrin and a mixture of pyrethroids containing cyfluthrin, cypermethrin, deltamethrin, fenvalerate, permethrin and teramethrin were used as analytical standards. Internal standards were d_6-*trans*-permethrin and d_6-*trans*-cypermethrin. All of them were certified pyrethroids standards purchased from Dr. Ehrenstorfer (Augsburg, Germany). Pesticide grade organic solvents were obtained from Sigma-Aldrich (St. Louis, MO, USA). Standard solutions were prepared in ethyl acetate. Solid phase extraction (SPE) C18 (2 g/15 ml) and basic alumina (5 g/25 ml) cartridges were obtained from Isolute Biotage and Interchim, respectively.

2.3. Sample preparation

Sample preparation was carried out according to Feo *et al*. 2012. Salmon meat (0.1 g dry weight (dw)) was spiked with deuterated internal standards (d_6-*trans*-permethrin and d_6-*trans*-cypermethrin). The sample was stirred and extracted by sonication with 20 ml of hexane:dichloromethane (2 : 1) and centrifuged twice. Both organic phases were transferred to one vial and evaporated. The remaining fat was re-dissolved with 20 ml of acetonitrile and underwent a clean-up by filtering the extract through basic

alumina and C18 SPE cartridges in tandem. The eluate was evaporated and re-dissolved with 100 µl of ethyl acetate.

To determine the lipid content of the samples, 1 g dw of sample was also extracted with 20 ml of hexane:dichloromethane (2 : 1) twice and evaporated. Then the lipid content was determined gravimetrically.

2.4. Instrumental analysis

The pyrethroid analysis was performed with an Agilent 7890A gas chromatograph coupled to an Agilent 7000B triple quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) according to Feo *et al.* 2011. Chromatographic conditions were as follows: injection volume was 3 µl; inlet temperature was 270 °C; DB-5ms capillary column (15 m × 0.25 mm, 0.1 µm film thickness) containing 5 % methyl phenyl siloxane; carrier gas was He at 1 ml/min, and temperature was 100 °C for the first minute, then raised from 100 to 230 °C for 8 min, then from 230 to 310 °C for 8 min and, finally, was constant for 2 min. Transfer line temperature was 275 °C, the ion source temperature for negative ion chemical ionisation in tandem mass spectroscopy (NICI-MS/MS) was 250 °C and the reagent gas was ammonia at 2×10^{-4} torr. Run time for each sample was 17 min.

Selective reaction monitoring (SRM) mode was used with two transitions monitored for each compound. The most intense transition was used for quantification and the second transition provided a confirmation comparing the SRM_1/SRM_2 ratio calculated for the samples with the ratio found in the standards (supplementary Table S1).

The enantiomeric analysis was performed with the same mass spectrometer conditions according to Corcellas *et al.* 2014. Chromatographic conditions were as follows: inlet temperature was 270 °C; BGB-172 (20 % *tert*-butyldimethylsilyl-β-cyclodextrin dissolved in 15 % phenyl-, 85 % methylpolysiloxane; 30 m × 0.25 mm, 0.25 µm film thickness) (BGB Analytik, Switzerland); carrier gas was He at 1.5 ml min^{-1}, and temperature was 180 °C for the first 2 min, then raised to 220 °C and held for 30 min, then it was increased to 230 °C and held for 25 min and, finally, it was ramped to 240 °C for 5 min, all ramps were at 5 °C min^{-1} and total analysis time was 74 min.

2.5. Analytical parameters

The analytical method was selected to monitor 10 different pyrethroids: bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, detamethrin, fenvalerate, fluvalinate, permethrin, resmethrin and tetramethrin. Method recoveries ranged from 53 to 116 % and relative standard deviations (RSD) ranged from 2 to 20 %. Method detection limits (MDLs) and method quantification limits (MQLs) ranged from 0.02 to 0.46 ng g^{-1} lipid weight (lw) the former and from 0.08 to 1.54 ng g^{-1} lw the latter [Alonso *et al*., 2012].

2.6. Enantiomeric study

The enantiomeric factor (EF) is defined as the chromatographic peak area of a selected enantiomer divided by the total area of both enantiomers. In this study, the first enantiomer to be eluted was selected. An EF could be calculated for each enantiomeric pair. The exceptions were the *trans* enantiomeric pairs of cypermethrin since they co-elute. EF = 0.5 corresponds to a racemic mixture of enantiomers, that is, equal amounts of both. EF > 0.5 means dominance of the selected enantiomer, while EF > 0.5 shows that the other enantiomer is present in a higher quantity. The diastereoisomeric ratios between different *cis* and *trans* diastereoisomers ($R_{cis/trans}$, $R_{cis1/cis2}$, $R_{trans1/trans2}$) was also assessed.

2.7. Statistical analysis

The statistical analysis was performed using t-test taking $p < 0.01$ as the criterion for statistical difference. In box plots figures, outliers (×) were calculated as values above Q3 + 1.5 IQR and below Q1 − 1.5 IQR (Q3 = third quartile, IQR = interquartile range, Q1 = first quartile).

3. Results and discussion

3.1. Levels and profiles

Insecticide pyrethroids were detected in 100 % of the farmed salmon and 50 % of the wild salmon (Table 2). In farmed salmon, bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, fenvalerate, permethrin and tetramethrin were found. In wild salmon, the list would be the same, except for deltamethrin and fenvalerate. The

Table 2. Mean pyrethroids concentration (ng/g wet weight), range and frequency of detection for farmed salmon, according to farming location, and wild salmon

salmon		tetramethrin[a]	bifenthrin	cyhalothrin	permethrin	cyfluthrin	cypermethrin	deltamethrin	fenvalerate	total[b]
Norway	mean[b]	0.02	0.08	0.02	0.10	0.02	0.75	0.76	n. q.	1.78
n = 23	range[c]	n. d.-0.31	n. d.-0.25	n. d.-0.18	n. d.-1.86	n. d.-0.32	0.03-4.42	n. d.-2.19	n. d.-0.45	0.18-5.78
	freq. of det. (%)	39	96	83	44	13	100	91	48	100
Scotland	mean	n. d.	0.09	0.01	0.05	n. d.	0.18	1.03	n. d.	1.37
n = 5	range	n. d.-n. q.	0.04-0.17	n. d.-0.04	n. d.-0.21	n. d.-n. q.	0.09-0.39	n. d.-1.83	n. d.-n. q.	0.25-2.18
	freq. of det. (%)	20	100	80	40	20	100	80	40	100
Denmark	*n* = 1	n. d.	0.44	n. d.	0.05	n. d.	n. q.	n. d.	n. d.	0.51
France	mean	n. d.	0.03	n. d.	0.11	n. d.	0.08	0.12	n. d.	0.36
n = 2	range	n. d.-n. q.	0.03-0.03	-	0.08-0.13	n. d.-n. q.	0.07-0.10	n. d.-0.24	n. d.-n. q.	0.20-0.52
	freq. of det. (%)	50	100	0	100	50	100	50	50	100
Spain	mean	n. q.	n. q.	n. d.	n. q.	n. d.	0.07	0.25	n. d.	0.35
n = 5	range	n. d.-0.01	n. d.-0.01	n. d.-n. q.	n. d.-0.04	-	n. q.-0.21	n. d.-0.56	n. d.-n. q.	0.04-0.61
	freq. of det. (%)	50	67	17	50	0	100	83	17	100
Chile	mean	n. d.	0.21	0.04	n. d.	n. d.	0.13	0.12	n. d.	0.51
n = 2	range	-	0.14-0.28	0.02-0.06	-	-	0.06-0.19	n. d.-0.24	-	0.23-0.79
	freq. of det. (%)	0	100	100	0	0	100	50	0	100
wild	mean	n. d.	n. q.	n. d.	n. d.	n. d.	n. q.	n. d.	n. d.	0.04
n = 12	range	n. d.-0.02	n. d.-0.02	n. d.-0.01	n. d.-0.04	n. d.-0.002	n. d.-0.04	-	-	n. d.-0.10
	freq. of det. (%)	25	42	8.3	50	17	33	0	0	75
	MDL	0.004	0.002	0.005	0.017	0.0001	0.008	0.002	0.012	
	MQL	0.02	0.008	0.01	0.04	0.002	0.02	0.006	0.04	

[a]Resmethrin (MDL = 0.025 ng/g ww) and fluvalinate (MDL = 0.035 ng/g ww) were not detected.

[b]To calculate mean and total concentrations, n. q. were given the MDL value and n. d. were considered 10 % of the MDL.

[c]n. d. means not detected (< MDL) and n. q. means not quantifiable (< MQL).

most occurring pyrethroids in farmed salmon were cypermethrin (100 % of the samples), bifenthrin (93 %) and deltamethrin (83 %). Cypermethrin and deltamethrin dominated the pyrethroid profiles of farmed salmon representing 77 ± 27 % of the total contamination. These two compounds are the active ingredients of anti-sea lice pesticide formulations [Haya *et al.*, 2005; Burridge *et al.*, 2010]. No pyrethroid showed significantly more frequently or in higher quantities in wild salmon samples.

Pyrethroids that are not typical active ingredients of anti-sea lice formulations are present at lower concentrations similar to those usually found in environmental samples. As mentioned before, pyrethroids account for 25 % of the insecticides used worldwide [Casida and Quistad, 1998; Shafer *et al.*, 2005]. Being used for household, commercial, medical and farming purposes, these pesticides reach the environment through wastewater and runoffs or are even applied directly on the fields and water bodies (e.g. farming).

Concentrations of pyrethroids are expressed in wet weight (ww) as this unit relates to the state in which salmon reaches the consumer. This allows for a more realistic risk assessment that could be easily translated into regulation if it were needed.

Mean concentration in farmed salmon was $1.31 \pm 1.39\,ng\,g^{-1}$ ww (0.03 to $5.75\,ng\,g^{-1}$ ww) and the mean for wild salmon was $0.02 \pm 0.03\,ng\,g^{-1}$ ww (n. d. to $0.09\,ng\,g^{-1}$ ww) (Figure 1a). Concentrations in farmed salmon were statistically higher than in wild salmon (t-test $t = 5.78$, df = 49, $p < 0.01$). This proved that the treatment against sea lice performed in fish farms has an effect on the pyrethroids concentration of the fish. Moreover, there seemed to be differences between farmed salmon's concentrations according to their place of origin (Figure 1a). Farmed salmon from Norway and Scotland showed similar concentrations ($t = 0.84$, df = 26, $p > 0.1$): $1.76 \pm 1.61\,ng\,g^{-1}$ ww and $1.35 \pm 0.79\,ng\,g^{-1}$ ww, respectively. These concentrations were higher than pyrethroids concentrations of the farmed salmon from the other locations ($t = 4.51$, df = 37, $p < 0.01$): $0.40 \pm 0.24\,ng\,g^{-1}$ ww. However, these samples with less contamination still contained more pyrethroids than the wild salmon samples ($t = 5.03$, df = 21, $p < 0.01$). No difference was observed between wild salmon from Alaska and the Pacific Ocean ($t = 0.52$, df = 10, $p > 0.1$) (Figure 1b).

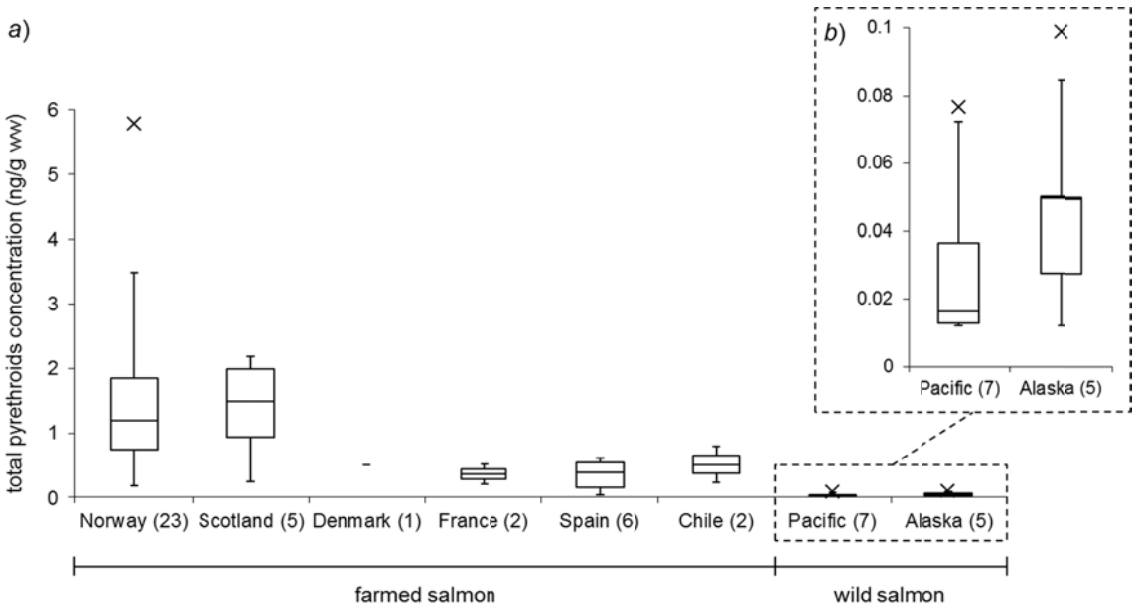

Figure 1. (*a*) Mean pyrethroids concentration (ng/g ww) of farmed and wild salmon according to place of origin. (*b*) Enlarged wild salmon's box plots. Outliers (×) are shown

All Norwegian farmed salmon available in stores were the same species, *Salmo salar*. On the other hand, the farmed salmon samples from Scotland ($n = 5$) and Spain ($n = 6$) included both *Oncorhynchus mykiss* and *Salmo salar* species. Although concentrations in *Salmo salar* were slightly higher than in the other species for the Spanish samples, no difference was observed for the Scottish ones. A greater number of samples would be required to assess whether total pyrethroids accumulation differs in different species.

This study also evaluated if the processing of salmon altered the pyrethroids concentration. The farmed salmon samples were divided into three groups: marinated, smoked and non-processed (Figure 2). The last group included fresh, refrigerated and frozen salmon. No significant differences were found between the non-processed samples and the marinated ($t = 1.30$, df = 14, $p > 0.1$) or the smoked samples ($t = 1.04$, df = 33, $p > 0.1$). However, comparing the box plots of the concentrations in ww with the box plots of the concentrations in lw, a greater coincidence of the values can be seen in the latter. This can be explained as marinating or smoking the salmon reduces its water content. Processed samples' water content was 60 % ± 5.8, while non-processed samples contained a 72 % ± 4.5 of water. Thus, the conversion of the values

to ww produces a slight increase of dispersion between the concentrations of the three groups, although —as stated above— not a significant one.

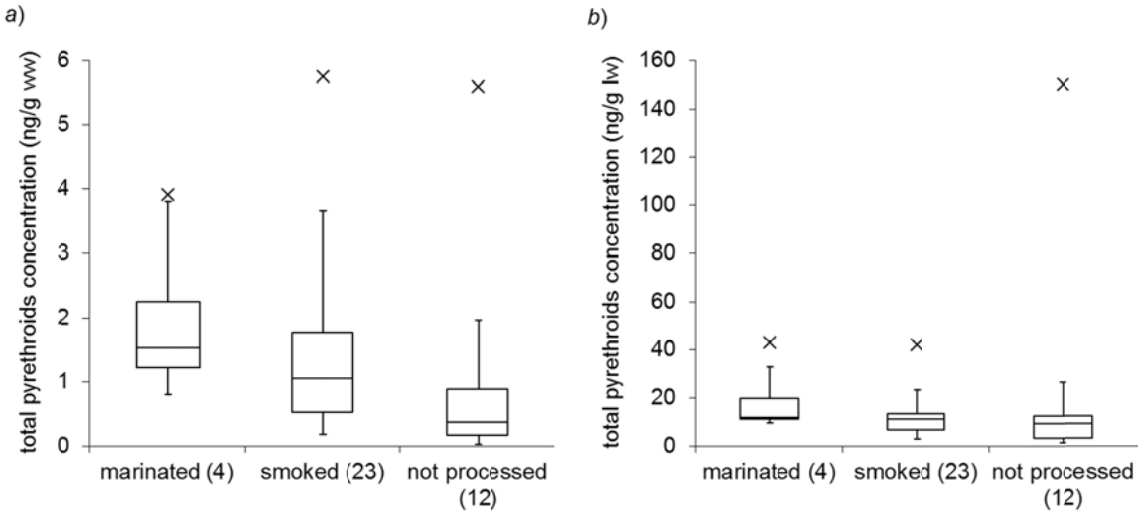

Figure 2. Mean pyrethroids concentration (ng/g) in (a) ww and (b) lw of farmed salmon according to processing. Outliers (×) calculated as values above Q3 + 1.5 IQR

3.2. Risk assessment

Since it has been proved that pyrethroids have toxic effects and accumulate in mammals, including humans [Zehringer and Herrmann, 2001; Corcellas *et al.*, 2012], it is important to evaluate whether the use of these pesticides on a seafood item poses a threat to the consumers' health. Daily intakes for bifenthrin, cypermethrin, deltamethrin and permethrin through farmed salmon were calculated according to the formula:

$$\text{(eq. 1) average daily intake} = \frac{\text{concentration level} \times \text{fish consumption}}{\text{body weight}}$$

In the formula, concentration level is the concentration of a pyrethroid in the sample. Fish consumption is the average fish consumption per adult person per day in Spain (94.4 g) and body weight is set to a consumer of 68.5 kg [AESA, 2006]. For this study, it was assumed that all fish consumption corresponded to salmon.

Mean estimated daily intakes (EDIs) expressed in $\text{pg}\,(\text{kg bw})^{-1}\,\text{day}^{-1}$ for bifenthrin, cypermethrin, deltamethrin and permethrin were, in the same order, 0.1 ± 0.1, 0.7 ± 1.4, 0.9 ± 0.8 and 0.1 ± 0.4. The highest individual EDI was $6.2\,\text{pg}\,(\text{kg bw})^{-1}\,\text{day}^{-1}$ for

cypermethrin in one sample. For consumers aged 7-12, the EDIs increased by 33 % (fish consumption = 63.3 g, body weight = 34.5 kg [AESA, 2006]). These values were several orders of magnitude below their respective ADI: 0.02, 0.02, 0.01 and 0.05 mg (kg bw)$^{-1}$ day^{-1}. Therefore, pyrethroids consumption through salmon roughly equals 0.002 % of the ADI, meaning that the pyrethroids treatment on salmon should not have a negative impact on the health of the consumer.

3.3. Enantiomeric study

Because of their relevance in the pyrethroid profiles, cypermethrin and bifenthrin underwent an enantiomeric analysis. For reference in this article, cypermethrin enantiomers are going to be referred to with the following Roman numerals according to their order of elution: I_{cis}, 1R-3R-αR; II_{cis}, 1S-3S-αS; III_{trans}, 1S-3R-αS and 1R-3S-αR; IV_{cis}, 1R-3R-αS; V_{cis}, 1S-3S-αR; VI_{trans}, 1R-3S-αS and 1S-3R-αR [35]. Additionally, I_{cis} and II_{cis} are called the first *cis* pair, *cis*1, and IV_{cis} and V_{cis} are the second *cis* pair, *cis*2.

No differences were observed comparing the enantiomeric factors (EF) and diastereoisomeric ratios (*R*) obtained for farmed salmon of the same species in different countries. This suggests that the pesticide formulations used might be the same commercial product or have the same enantiomeric composition. On the other hand, some differences were found comparing different species (Figure 3). Farmed salmon's values were grouped according to species (i. e. *Oncorhynchus mykiss* or *Salmo salar*) regardless of their country of origin.

While $EF_{bifenthrin}$ values were similar for both species, $EF_{cypemethrin}$ values for both the first and the second *cis* enantiomeric pairs were lower for *Salmo salar*. Regarding the *cis*1 pair, *Salmo salar* showed a preference to accumulate II_{cis} over I_{cis} ($EF_{cis1} = 0.33 \pm 0.09$), while *Oncorhynchus mykiss* accumulated I_{cis} better ($EF_{cis1} = 0.60 \pm 0.06$). As for the *cis*2 pair, both species had a preference for V_{cis} over IV_{cis}. However, this tendency was stronger for *Salmo salar* ($EF_{cis2} = 0.18 \pm 0.06$ versus $EF_{cis2} = 0.29 \pm 0.06$).

$R_{trans1/trans2}$ values were similar for both species, showing a preference for III_{trans} over VI_{trans}. Nevertheless, $R_{cis/trans}$ (including all *cis* and *trans* enantiomers) revealed that

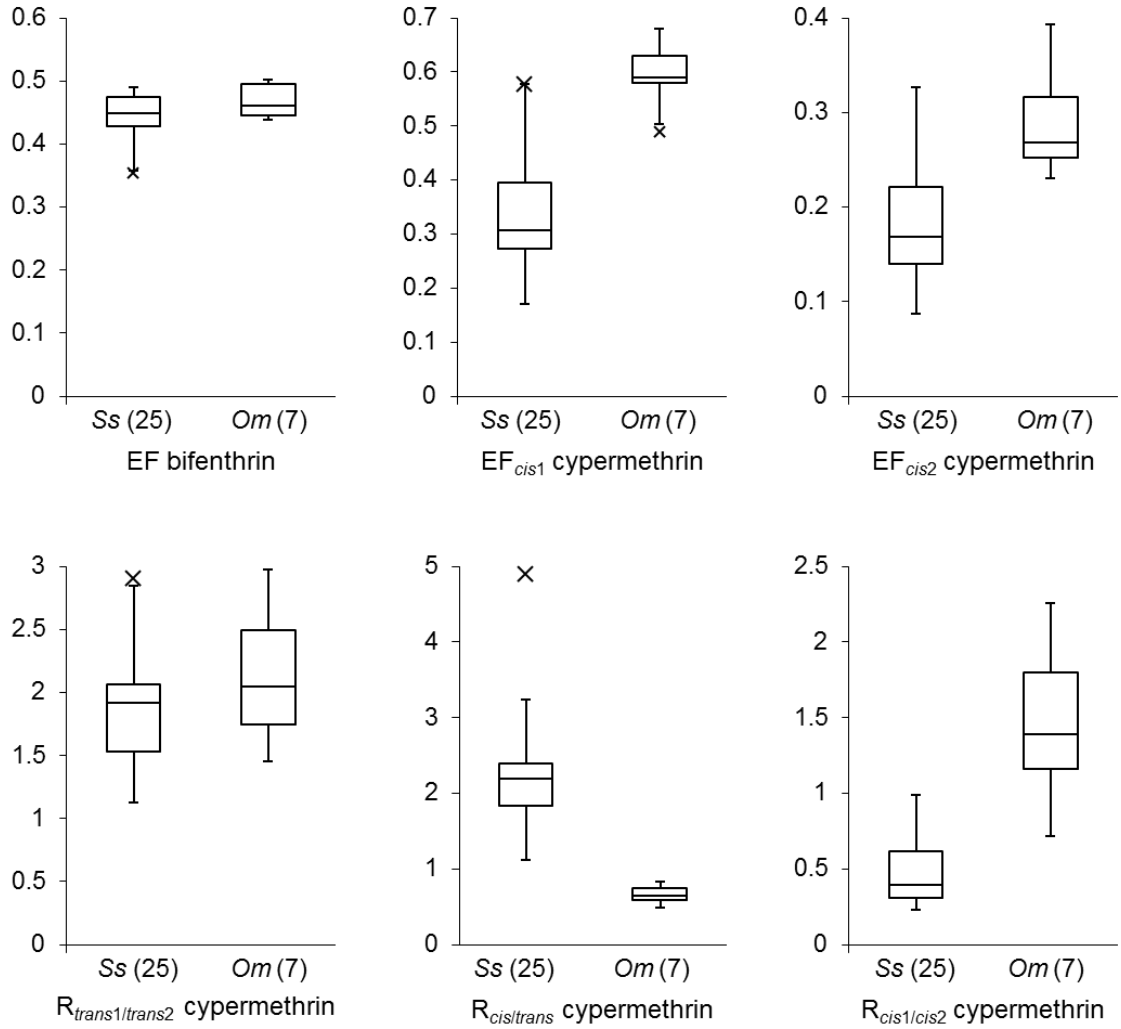

Figure 3. Enantiomeric factors (EF) and diastereoisomeric ratios (*R*) obtained for farmed salmon according to species (*Ss* = *Salmo salar*; *Om* = *Oncorhynchus mykiss*). Outliers (×) calculated as values above Q3 + 1.5 IQR and below Q1 − 1.5 IQR (Q1 = first quartile)

Salmo salar had more affinity for *cis* enantiomers ($R_{cis/trans} = 2.33 \pm 0.90$), while *Oncorhynchus mykiss* was the opposite ($R_{cis/trans} = 0.66 \pm 0.13$). $R_{cis1/cis2}$ values also showed opposed tendencies as *Salmo salar* had more affinity for the *cis*1 pair ($R_{cis1/cis2} = 0.49 \pm 0.24$), while *Oncorhynchus mykiss* accumulated *cis*2 better ($R_{cis1/cis2} = 1.47 \pm 0.54$).

These different selective accumulations of isomers between species might be caused by differences in their metabolisms. If the toxicity of the isomers was characterised and some showed greater negative effects than others, this information might become relevant to the seafood industry, policy makers and the consumers.

4. Conclusions

Pyrethroids were detected in 100 % of the farmed salmon with a mean concentration of $1.31 \pm 1.39\,ng\,g^{-1}$ ww and in 50 % of the wild salmon with a mean of $0.02 \pm 0.03\,ng\,g^{-1}$ ww. Concentrations in farmed salmon were higher proving that the treatment against sea lice performed in fish farms has an effect on the pyrethroids concentration of the fish. Additionally, cypermethrin and deltamethrin were in over 80 % of the farmed salmon representing 77 ± 27 % of the total contamination. These two compounds are the active ingredients of the anti-sea lice pesticide formulations AlphaMax® and Excis®. Salmon farmed in Norway and Scotland showed higher concentrations than salmon farmed in any other locations. On the other hand, no difference was observed between concentrations of samples from different species or between processed (marinated or smoked) and non-processed salmon.

Although farmed salmon had higher concentrations of pyrethroids than wild salmon, the EDI of pyrethroids through salmon consumption was several orders of magnitude below the ADI. Thus, the pyrethroids treatment on salmon should not have a negative impact on the health of the consumers.

Finally, no differences were observed in the enantiomeric analysis for farmed salmon of the same species in different countries, as the pesticide formulations used might be the same commercial products. Conversely, the species *Oncorhynchus mykiss* and *Salmo salar* showed enantiomeric selectivity for the accumulation of cypermethrin that was characteristic for each species.

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