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A survey of dioxin-like contaminants in fish from recreational fishing

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Abstract The dioxin and dioxin-like compounds are regarded as one of the most toxic group of environmental contaminants. Food for the commercial market is regularly monitored for their dioxin levels and the concentration allowed in food is strictly regulated. Less is known about locally caught fish from recreational fishing, which is often brought home for consumption. This can be fish caught from nearby lakes or streams or fish with marine origin close to industrial areas or harbours that are not regularly monitored for their dioxin levels. In this study, we established collaboration with schools in 13 countries. We received 203 samples of 29 different fish species of which Atlantic cod was the most abundant followed by brown trout and pollock. In general, the majority of samples from the participating countries had low concentrations (between 0.1 and 0.2 pg/g chemical-activated luciferase gene expression toxic equivalency wet weight (CALUX TEQ w.w.)) of dioxins and dioxin-like PCBs. Only 18 samples had concentrations above 1 pg/g CALUX TEQ w.w., and only 2 dab samples had concentration above maximum levels set by the European Commission. The Atlantic cod samples showed a significant reduction in the concentrations of dioxins with increasing latitude indicating less contamination of dioxin and dioxin-like compounds in the north of Norway. The results indicate that a moderate consumption of self-caught fish at presumed non-contaminated sites does not represent a major risk for exposure to dioxins or dioxin-like compounds at concentrations associated with adverse health effects. Recreational fishermen should, however, obtain knowledge about local fish consumption advice.

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Introduction

Persistent organic pollutants (POPs) have been shown
to enter the food chains and accumulate in fat deposits,
a process known as bioaccumulation, and thus become
concentrated in organisms that prey upon lower organisms (Borga et al. 2005). This phenomenon has particularly been evident in remote ecosystems in polar
regions, into which the pollutants have been
transported with winds and ocean currents. POPs are
also traced in common grocery goods, and consumers
can unintentionally be exposed for instance by consuming farmed fish (Hites et al. 2004). The dioxins
and dioxin-like compounds, such as chlorinated and
brominated furans, are groups of POPs that are byproducts of industrial processes such as combustion of
industrial waste. Other contaminants with dioxin-like
toxicity are the coplanar polychlorinated biphenyls
(PCBs) polychlorinated naphthalene (PCN) and
polyaromatic hydrocarbons (PAHs). The dioxins and
some of the dioxin-like compounds are considered to
be the most toxic man-made contaminants known
today, and they may have major impacts on human
health (Mukerjee 1998; Humblet et al. 2008). The
permitted levels of dioxins in food are strictly regulated in most countries, and a so-called toxic equivalent
factor system (TEF) is established, which enables authorities to set guidelines for intake that do not exceed
harmful levels (Van den Berg et al. 2006; Van den
Berg et al. 1998). Fish are especially prone to accumulate POPs and most fish on the commercial market,
both farmed and wild caught, are regularly monitored
for their dioxin levels. Usually, an investigation of
POPs in wild animals is restricted to certain areas
and/or to certain animal species. Less is known about
dioxin levels in locally caught fish from recreational
fishing, which is often brought home for consumption.
This can be fish caught from nearby lakes or streams
or fish of marine origin close to industrial areas or
harbours that are not regularly monitored for their
dioxin levels. There are several practical challenges
associated with making a national or worldwide
screening of the dioxin levels in fish caught from
recreational fishing, and the most significant is to

obtain fish from an adequate number of areas. The
international polar year (IPY) initiated by the Norwegian Research Council challenged the scientific environment in Norway to include different educational
and outreach aspects in their research. In this study,
we benefited from schools contributing with fish samples, data logging and their local knowledge about the
region and habits for recreational fishing for food
consumption. This enabled us to investigate trends
and patterns in dioxin levels within and across fish
species as well as across geographical regions. In
addition, the study added some information about the
risk of being exposed to dioxins and dioxin-like contaminants from consumption of self-caught fish. As far
as we know, this project was one of the very few IPY
projects where schools actively participated in a research project. Our experience with involving school
students in environmental research shows that students
are very attentive, meticulous and follow scientific
instructions very carefully (Heimstad et al. 2003;
Creilson et al. 2008; Nali and Lorenzini 2007).

Materials and methods

Chemicals

Hexane, dichloromethane, cyclohexane and toluene
(gas chromatography grade, respectively), concentrated
sulphuric acid, (analytical grade), sodium sulphate (anhydrous for analysis) and silica gel (0.063–0.200 mm,
for column chromatography) were purchased from
Merck (Germany). Diethyl ether (glass distilled grade)
was purchased from Rathburn (Scotland). Isopropanol
(chromosolv) and DMSO (for molecular biology) were
purchased from Sigma. FBS Gold, defined foetal bovine
serum, and Minimum Essential Medium (MEM) alpha
modification were purchased from PAA laboratories
(Germany). D-Luciferin was purchased from BioThema
AB (Sweden). ATP was purchased from Saveen Werner
AB (Sweden). The internal standards used for the chemical analysis of dioxins and PCBs in the study were from
Cambridge Isotope Laboratories (CIL) and purchased
from Promochem (Sweden). The dioxin standards used
in the chemical-activated luciferase gene expression
(CALUX) assay were purchased from Bio Detection
Systems BV (Amsterdam). All other reagents used were
analysis grade laboratory chemicals from standard commercial suppliers.

Sampling of fish

An existing website, www.sustain.no, which is maintained and developed by the Norwegian Centre for Science Education, was used as a platform where the participating schools could log their data. This website is a resource for promoting training in sustainable development at all levels, from elementary to secondary school. In total, 54 schools from 13 countries participated in the project during the years 2007–2010. The majority of the 54 participating schools came from Norway (36 schools), followed by Estonia (4 schools), France (3 schools) and 1 or 2 schools from Australia, Croatia, Czech Republic, Denmark, Finland, Iceland, Latvia, Liechtenstein, Poland and Sweden. We received 206 fish samples (Table 1S). Three samples of common whitefish from a Swedish school were discarded due to doubt of the accuracy of the analysis. Details about the fish samples received for analyses are shown in supplementary materials (Table 1S). Each school received a protocol for handling their fish samples,[1] which was carried out in accordance to the EMERGE sampling protocol (Rosseland et al. 2001). The species were identified, field data (GPS coordinates and description of field sampling site) were logged and weight and length of each individual fish were monitored. A muscle tissue sample was taken from the left side of the fish just above the pectoral fin and put into aluminium foil and frozen. The samples were then sent to NILU (Norwegian Institute for Air Research) and analysed for dioxins and dioxin-like compounds with the use of an in vitro bioassay (BDS DR CALUX® BioDetection System, The Netherlands) for Ah-receptor active compounds, such as dioxins and coplanar PCBs (Murk et al. 1996).

Analysis of dioxins and dioxin-like compounds
by CALUX bioassay

Sample preparation and analysis of dioxins and dioxin-like compounds were performed according to the standard procedure of the manufacturer (Bio Detection Systems BV, Amsterdam, The Netherlands; Stronkhorst et al. 2002; Husain et al. 2014). Briefly, an aliquot of homogenized fish muscle samples (≤0.5 g lipid) was mixed with 30 ml ultra-pure water and isopropanol (1:1 v. v.) followed by an extraction with 30 ml of hexane with 3 % diethyl ether. The hexane fraction was transferred to

a clean pre-weighed glass vial and the extraction repeated three times. The hexane fractions were pooled and evaporated to dryness under a gently stream of nitrogen. The glass vial was weighed with the residues and the lipid content of the sample was estimated gravimetrically. The sample was resuspended in a small amount of hexane (~1 ml), and cleanup was performed on a multilayer acidic silica column (5 g of 33 % sulphuric acid/silica followed by 5 g of 20 % sulphuric acid/silica and 1 cm of anhydrous Na_2SO_4). The dioxins and dioxin-like compounds were eluted with 40 ml n-hexane with 3 % diethyl ether. The cleaned extract was evaporated to near dryness and dissolved in DMSO to a final volume of 50 μL.

The DR CALUX analyses were performed by exposing a rat hepatoma H4IIE cell line stably transformed with an Ah-receptor controlled luciferase reporter gene. DR CALUX cells were obtained from Bio Detection Systems BV, Amsterdam, The Netherlands. The cells were cultured in α-MEM culture medium supplemented with 10 % (v/v) heat inactivated foetal calf serum at 37 °C, 5 % CO_2 and 100 % humidity. The cells were exposed to the fish extracts dissolved in DMSO in triplicates in 96-well plates for 24 h with a final DMSO concentration in the culture medium of 0.4 %. The 96-well plates also contained the standard 2,3,7,8-TCDD calibration solutions (0–20 pg TCDD per well), a DMSO blank and an additional reference sample of 2,3,7,8-TCDD. After exposure, the culture medium was removed, the cells were washed with PBS, and the cells were lysed with an aliquot of lysis buffer (10 % glycerol, 1 % triton x-100, 25 mM Tris, 2 mM DTT and 2 mM CDTA adjusted to pH 7.8). Luminescence was measured on a luminometer (BMG Lumistar Optima) by adding glowmix (33.3 mM DTT, 20 mM trycin, 2.67 mM $MgSO_4$, 1.07 mM $C_4H_2Mg_5O_{14}$, 530 μM ATP, 470 μM luciferin and 270 μM co-enzym A) to the lysed cell. The concentrations in the fish extract were calculated from the 2,3,7,8-TCDD calibration solution. Estimated limit of detection and limit (LOD) of quantification (LOQ) was 0.03 ± 0.009 (SD) and 0.09 ± 0.03 pg/g CALUX toxic equivalency wet weight (TEQ w.w.)), respectively.

Chemical analysis of dioxins and dioxin-like
compounds by gas chromatography high-resolution
mass spectrometry

Extraction and cleanup were performed at NILU with a semi-automated three-column system as described in

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[1] http://sustain.no/projects/globalpop/what_to_do.php

detail by Bengtson Nash et al. (2008). In brief, an aliquot of tissue was homogenized with anhydrous Na_2SO_4, spiked with internal standards (13C-labelled PCDD, PCDF and coplanar PCBs) and subjected to extraction and cleanup through three columns prepared with (a) activated silica and potassium silica, (b) silica and (c) activated carbon with dichloromethane (DCM) and cyclohexane (1:1) followed by DCM. Finally, the PCDD, PCDF and coplanar PCBs were eluted from the column with activated carbon with use of toluene. The toluene extracts were attributed to solvent exchange to hexane and further cleaned through consecutive sulphuric acid-coated silica column followed by potassium hydroxide-coated silica column with hexane followed by 1 % DCM in hexane. 13C-labelled 1,2,3,4-TCDD recovery standard was added prior to analysis by HRGC-HRMS-EI (an HP5890 GC coupled to a VG AutoSpec) by monitoring at m/z of the molecular ions. The separation of the congeners was carried out on a DB-5 ms (30 m, 0.25 mm, 11 μm film thickness) fused silica column.

Data analysis

Statistics (descriptive statistics, Mann-Whitney U, correlation analysis) were computed in GraphPad Prism 5 or Excel 2007. The non-parametric Mann-Mann–Whitney U was used to compare the difference in the concentration of dioxin-like compounds between fish from northern and southern Norway separated at 63° (cod and pollock) north and 60° north (trout), respectively. To evaluate the accumulated levels of dioxin-like compounds in fish (cod, pollock and trout) as a function of latitude, the Pearson correlation analysis was used. Normal distribution in the variables was assessed using the D'Augostino and Pearson omnibus normality test. If necessary, $\log_{(10)}$ transformation was applied to obtain normality.

Results and discussion

Of the 203 submitted fish samples, 49 were Atlantic cod, which was the most common species in this study. All of the cod samples came from Norway (Table 1S). Brown trout and pollock were the second and third most abundant fish species with 31 and 26 samples, respectively. Nine of the 203 samples were quantified below LOQ, but all samples were quantified higher than LOD. In the statistical analysis, the measured concentrations

of these samples were used even though they were below LOQ. Table 1 shows a summary of the measured CALUX TEQ in the collected fish on both wet weight (w.w.) and lipid weight (l.w.) concentration, in addition to size. Even though the dietary guidelines are based on wet weight concentrations of dioxins and dioxin-like PCBs, it can be convenient to report the concentrations of lipid-soluble compounds in fish in both wet weight and lipid weight, in order to do interspecies comparisons in accumulation. In general lipid-soluble compounds tend to accumulate in higher concentrations in fat fish compared to lean fish. Only one fish (7.4 CALUX TEQ/ g w.w.), a dab sample, had a concentration that was higher than the maximum limit set by the European Commission (EC 2011) for dioxins as a single group (3.5 pg TEQ/g w.w.) and for the sum of dioxins and dioxin-like PCBs (6.5 pg TEQ/g w.w.). To visualize the distribution of accumulated dioxin-like substances, the concentration data was separated in intervals of 0.1 units below 0.7 pg TEQ/g w.w. and with larger intervals above 0.7. The majority of samples had levels between 0.1 and 0.2 pg/g CALUX TEQ w.w. Only 12 samples had concentrations above 1 pg/g CALUX TEQ w. w. (Fig. 1). There were two samples of common dab from Norway with a high CALUX TEQ of 3.94 and 7.38 pg/g w.w., respectively. These two samples were caught at the harbour of Egersund, southwest of Norway. A survey by the National Institute of Nutrition and Seafood Research in Norway revealed that cod (liver) from this area had high levels of dioxin-like PCBs (Nilsen et al. 2011). The two dab samples were subjected to chemical analysis to get a detailed view of the content of dioxin-like compounds. The analyses revealed that the fish had even higher concentrations of dioxins and dioxin-like compounds than indicated by the bioassay, i.e., a sum WHO-TEQ 2005 of 6.72 and 13.76 pg/g w.w., respectively (Table 2). The CALUX bioassay is not a selective analytical method. All Ah-receptor inducers may in principle induce a response on the assay. These compounds include dioxins and furans, coplanar (dioxin-like) PCBs, PAHs and even PCN (Poland and Knutson 1982; Hanberg et al. 1991; Safe 1994; Okey 1990; Villeneuve et al. 2000). The sample preparation will, however, remove some of these substances, such as several of the PAHs. The possibility that there could have been compounds in the extracts that displayed antagonistic effects on the Ah-receptor should not be excluded. Comparative studies have shown that there are good correlations between traditional chemical

Table 1 List of fish species, sorted on number of samples

Fish species	Latin name	CALUX TEQ (pg/g w.w.)	CALUX TEQ (pg/g l.w.)	Weight (kg)	Length (cm)	Number[a]
Atlantic cod	*Gadus morhua*	0.25 (0.20)	64 (52)	1.5 (0.87)	49 (29)	49 (40)[a]
		0.07–0.65	18–180	0.10–6.5	6.6–78	
Brown trout	*Salmo trutta*	0.34 (0.28)	42 (33)	0.30 (0.24)	30 (29)	31 (28)[a]
		0.09–0.82	10–140	0.07–0.72	16–42	
Pollock	*Pollachius pollachius*	0.20 (0.15)	40 (36)	1.4 (1.3)	54 (39)	26 (23)
	Pollachius virens	0.07–0.54	9.5–130	0.18–3.4	12–124	
European perch	*Perca fluviatilis*	0.24 (0.16)	48 (40)	0.08 (0.08)	18 (19)	14 (6)
		0.10–0.54	21–89	0.05–0.14	13–23	
Arctic char	*Salvelinus alpinus*	0.49 (0.48)	45 (40)	0.45 (0.29)	40 (31)	10 (10)
		0.10–0.82	19–145	0.25–1.0	28–90	
Common carp	*Cyprinus carpio*	0.43 (0.32)	30 (18)	1.5 (1.4)	42 (43)	6 (6)
		0.17–1.1	6.6–104	1.3–1.9	37–49	
Pike	*Esox lucius*	0.35 (0.27)	130 (65)	0.81 (0.89)	47 (47)	6 (5)
		0.19–0.96	51–310	0.60–1.3	45–50	
Flounder	*Platichtys flesus*	0.52 (0.29)	66 (54)	n.d.	n.d	6
		0.27–1.1	32–110			
Atlantic mackerel	*Scomber scombrus*	0.87 (0.83)	58 (60)	0.23 (0.23)	31 (31)	5 (5)
		0.18–1.7	32–80	0.20–0.26	30–32	
Haddock	*Melanogrammus aeglefinus*	0.41 (0.37)	170 (190)	1.2 (1.4)	50 (52)	5 (5)
		0.06–1.1	12–420	0.4–2.1	41–59	
Fat chub	*Leuciscus cephalus*	0.61 (0.45)	47 (52)	0.52 (0.16)	29 (25)	4 (4)
		0.13–1.4	17–66	0.13–1.6	23–43	
Mullet	*Mugil cephalus*	0.43 (0.46)	170 (170)	1.2 (1.2)	47 (48)	4 (4)
		0.22–0.57	80–260	1.0–1.4	40–50	
Greater weever	*Trachinus draco*	0.34 (0.18)	83 (44)	0.17 (0.16)	34 (35)	4 (4)
		0.14–0.84	33–210	0.14–0.22	30–38	
Gilthead bream	*Sparus aurata*	2.9 (1.0)	2500 (340)	0.33	28	4 (2)
		0.42–9.1	190–9100			
Herring	*Clupea harengus*	1.9 (2.2)	86 (98)	0.16	29	4 (2)[a]
		0.36–2.9	17–130			
Burbot	*Lota lota*	0.23 (0.20)	28 (29)	0.43 (0.38)	36 (38)	3 (3)
		0.19–0.29	18–35	0.34–0.57	33–38	
Common dab	*Limanda limanda*	3.9 (3.9)	83 (89)	0.37 (0.37)	32 (32)	3 (3)
		0.5–7.4	65–97	0.35–0.38	31–33	
Common whitefish	*Coregonus lavaretus*	0.56 (63)	62 (57)	0.19 (0.21)	28 (29)	3 (3)
		0.36–0.68	51–78	0.17–0.21	27–30	
Roach	*Rutilus rutilus*	0.2	28	0.07	15	2 (1)
Yellowfin bream	*Acanthopagrus australis*	0.59	160	n.d	n.d	2
Narrowhead grey mullet	*Mugil capurrii*	0.92	150	n.d	n.d.	2
Red mullet	*Mullus surmuletus*	2.6	100	n.d.	n.d.	2
Red fish	*Sebastes marinus*	1.1	22	n.d.	n.d	2
European plaice	*Pleuronectes platessa*	0.14	18	0.56	37	1
Common ling	*Molva molva*	0.54	150	4.3	85	1
Southern black bream	*Acanthopagrus butcheri*	0.38	110	0.42	29	1

Table 1 (continued)

Fish species	Latin name	CALUX TEQ (pg/g w.w.)	CALUX TEQ (pg/g l.w.)	Weight (kg)	Length (cm)	Number[a]
Silver bream	*Blicca bjoerkna*	1.3	130	0.12	19	1
Eel	*Anguilla anguilla*	0.22	1.0	0.45	62	1

The mean concentration of dioxin and dioxins-like compounds in muscle tissue shown as CALUX TEQ in a lipid weight (l.w.) and wet weight (w.w.) concentrations. The median concentrations are shown in brackets in addition to the minimum and maximum concentrations

n.d. not determined

[a] The number of samples with reported weight and length data

analyses and the CALUX bioassay (van Leeuwen et al. 2007; Scippo et al. 2004; Schoeters et al. 2004). Scippo et al. (2004) reported, however, a relatively large variation in the estimated TEQ values of the same samples when comparing between the bioassay and chemical analysis. Some samples had estimated a higher CALUX TEQ than the estimated TEQ from the chemical analysis and vice versa. The CALUX TEQ method is, however, very suitable as a screening tool to identify samples with high concentrations of Ah-receptor inducers. Since this is a first tier screening method for dioxin-like compounds, it is recommended that all suspect samples need to be analysed by high-resolution GC-MS analysis (Hoogenboom et al. 2006). Samples with high TEQ response near the maximum limit set by the European Commission should be further investigated with more reliable and quantitative methods such as traditional chemical analysis before potential dietary advices are established (Vromman et al. 2012).

Two of the fish samples (two dab samples) from Egersund harbour which had a high concentration of dioxin-like compounds measured with CALUX were analysed by gas chromatography high-resolution mass spectrometry (GC HR-MS). The congener-specific analysis of the two dab samples showed highest concentration (pg/g w.w.) of PCB77, whereas PCB126 which has a TEF value of 0.1, contributed to over 90 % of the total measured TEQ concentration (Table 2). This is in accordance with previous studies showing that the coplanar dioxin-like PCBs are the dominating Ah-receptor inducers accumulated in marine organisms (e.g. Judd et al. 2004; van Leeuwen et al. 2007; Pandelova et al. 2008; Piskorska-Pliszczynska et al. 2012; Barone et al. 2014). This may be attributed to the ubiquitous spread of PCB due to their extensive industrial use, whereas the dioxins and furans are more regionally or locally distributed, as they are primarily by-products from combustion processes. Accordingly, fish from the Grenlands fjord in Norway, which have experienced extensive pollution from dioxins, have showed a higher proportion of the dioxins and furans contributing to the estimated TEQ-values (Knutzen et al. 2003). Also in juvenile dabs taken from various sites in Europe, it was found a higher proportion of the dioxins and furans contributing to the estimated TEQ values (Nunes et al. 2014).

Compared to similar studies that have used the CALUX bioassay method, the levels of dioxins in our samples were in general lower. We found 0.25 and 0.34 pg CALUX TEQ/g w.w. in Atlantic cod and brown trout respectively, compared to 0.85 and 1.04 pg CALUX TEQ/g w.w. in the same species from the Belgian market (Baeyens et al. 2007). Schoeters et al. (2004) reported a median concentration of 2.53 pg CALUX TEQ/g w.w. in herring, whereas we found a concentration of 1.9 pg CALUX TEQ/g w.w. van Leeuwen et al. (2007) analysed fish from the North Sea showing a median TEQ value of 0.6 pg/g w.w. in cod, between 0.8 and
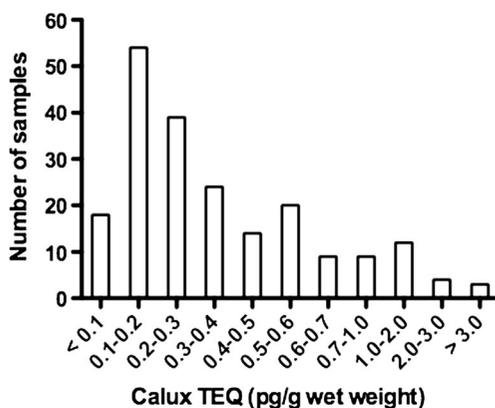
Fig. 1 Number of samples within increasing concentration intervals

Table 2 The concentration of dioxins and dioxin-like compounds in muscle tissue in two dab samples shown as wet weight concentrations and as WHO-TEQ$_{2005}$

Compound	Dab-1		Dab-2	
Dioxins	Concentration (pg/g w.w.)	WHO-TEQ$_{2005}$ (pg TEQ/g w.w.)	Concentration (pg/g w.w.)	WHO-TEQ$_{2005}$ (pg TEQ/g w.w.)
2378-TCDD	0.67	0.67	0.34	0.34
12378-PeCDD	0.90	0.90	0.40	0.40
123478-HxCDD	0.11	0.01	0.05	0.005
123678-HxCDD	0.58	0.06	0.22	0.02
123789-HxCDD	0.13	0.01	0.07	0.007
1234678-HpCDD	0.38	0.004	0.28	0.003
OCDD	0.58	0.0002	0.49	0.0001
SUM PCDD	3.35	1.65	1.84	0.78
Furanes				
2378-TCDF	10.6	1.06	5.79	0.58
12378/12348-PeCDF	1.16	0.03	0.61	0.02
23478-PeCDF	3.80	1.14	1.73	0.52
123478/123479-HxCDF	0.70	0.07	0.56	0.06
123678-HxCDF	0.49	0.05	0.35	0.04
123789-HxCDF	0.09	0.009	0.12	0.01
234678-HxCDF	0.37	0.04	0.19	0.02
1234678-HpCDF	0.72	0.007	0.87	0.009
1234789-HpCDF	0.22	0.002	0.34	0.003
OCDF	1.27	0.0004	2.17	0.0007
SUM PCDF	19.4	2.41	12.7	1.25
SUM PCDD/PCDF	22.8	4.06	14.5	2.03
nonortho-PCB				
3344-TeCB (PCB-77)	484	0.05	306.	0.03
3445-TeCB (PCB-81)	26.2	0.008	15.5	0.005
33445-PeCB (PCB-126)	92.4	9.23	44.7	4.48
334455-HxCB (PCB-169)	13.5	0.40	5.8	0.17
SUM no-PCB	616	9.70	373	4.69
Sum PCDD/PCDF/PCB	639	13.76	388	6.72

Samples were from the harbour of Egersund southwest of Norway

2.4 pg/g w.w. in mackerel and 1.3 and 5.7 pg/g w.w. in herring. The somewhat higher concentrations in fish from these studies may be attributed to the area of catch and size of the fish. A summary of concentrations of dioxins and dioxin-like compounds in different fish species caught in European waters relevant to our study are provided in Table 3. Here, it is evident that there are large variations both between and within species due to various fat content and geographical locations (Table 3). Our findings corroborate, however, the general findings that most

fish have dioxin concentrations below the levels recommended by the European Commission (EC 2011).

The northern parts of Europe (for instance above the 63° north parallel line) are less industrialized than the south, and even though a substantial long-range transport of contaminants to Arctic areas do occur, studies have found that fish from the northern areas in general are less contaminated by dioxins and PCBs than fish from the southern areas (Wania 1999; Wania and Su 2004). However, exceptions exist, for instance in the Gulf of Bothnia

Table 3 Data on concentrations of dioxins and dioxin-like compounds in fish from European waters

Species	Origin	Year	pg TEQ/g w.w.	Reference
Eel	Belgian market	2004–2006	1.16 ± 0.61[a]	Baeyens et al. (2007)
Eel	Ebro river Spain	2012	1.81[b]	Parera et al. (2013)
Eel	Southwest Italy	2008–2009	0.91–11.4[c]	Pacini et al. (2013)
Eel	Grenland fjords	2000–2001	26.2; 27.7[c]	Knutzen et al. (2003)
Eel	Latvian lakes	2009	6.70[b]	Zacs et al. (2013)
Cod	Belgian market	2004–2006	0.85 ± 0.64[a]	Baeyens et al. (2007)
Cod	Polish Baltic sea	2009–2010	0.85–0.86[b]	Piskorska-Pliszczynska et al. (2012)
Cod	Baltic sea	2009–2011	0.87–0.89[b]	Struciński et al. (2013)
Cod	North Sea	2000	0.3–0.8[c]	van Leeuwen et al. (2007)
Cod	Grenland fjords	2000–2001	1.9[c]	Knutzen et al. 2003
Cod	North Sea	1995–1997	0.024–0.074[e]	Karl et al. 2002
Brown trout	Belgian market	2004–2006	1.04 ± 0.67[a]	Baeyens et al. (2007)
Brown trout	Southwest Italy	2008–2009	0.22[c]	Pacini et al. (2013)
Herring	English channel	2000–2004	1.3–5.7[c]	van Leeuwen et al. (2007)
Herring	Belgian market	2004–2006	1.41 ± 0.4[a]	Baeyens et al. (2007)
Herring	Gulf of Finland	2005	2.19–2.47[b]	Pandelova et al. (2008)
Herring	Baltic sea	2002–2006	3.4–7.0[b]	Szlinder-Richert et al. (2009)
Herring	Baltic sea	2006–2011	1.19–7.28[b]	Struciński et al. (2013)
Herring	Polish Baltic sea	2006–2010	2.63–4.45[b]	Piskorska-Pliszczynska et al. (2012)
Herring	Norwegian Sea	2006–2007	0.24–3.5[b]	Frantzen et al. (2011)
Herring	Belgian market	2000–2001	2.53[ad]	Schoeters et al. (2004)
Herring	Grenland fjords	2000–2001	12.1[c]	Knutzen et al. (2003)
Flounder	Ebro river Spain	2012	1.11[b]	Parera et al. (2013)
Flounder	Baltic sea	2004	0.53–1.63[b]	Pandelova et al. (2008)
Flounder	Grenland fjords	2000–2001	3.5; 33.5[c]	Knutzen et al. (2003)
Common carp	Southwest Italy	2008–2009	1.18–1.74[c]	Pacini et al. (2013)
Common carp	Latvian lakes	2009	0.06; 0.16[b]	Zacs et al. (2013)
Fat chub	Southwest Italy	2008–2009	0.40–2.62[c]	Pacini et al. (2013)
Fat chub	Latvian lakes	2009	0.36[b]	Zacs et al. (2013)
Perch	Peipsi, Estonia	2004	0.185–0.303[b]	Pandelova et al. (2008)
Perch	Gulf of Finland	2004	0.32–1.37[b]	Pandelova et al. (2008)
Perch	Gulf of Riga	2004	0.868–1.605[b]	Pandelova et al. (2008)
Perch	Latvian lakes	2009	0.12; 2.06[b]	Zacs et al. (2013)
Roach	Latvian lake	2009	0.21[b]	Zacs et al. (2013)
Pike	Latvain lake	2009	0.15[b]	Zacs et al. (2013)
Mackerel	Spain market	2005	1.12[b]	Bocio et al. (2007)
Mackerel	North Sea	2000–2004	0.8–2.4[c]	van Leeuwen et al. (2007)
Mackerel	Belgian market	2000–2001	0.36[ad]	Schoeters et al. (2004)
Mackerel	Bay of Biscaya	1995–1998	0.15–0.66[e]	Karl et al. (2002)
Mackerel	Grenland fjords	2000–2001	7.4[c]	Knutzen et al. (2003)
Red mullet	Spain market	2005	4.65[b]	Bocio et al. (2007)

Table 3 (continued)

Species	Origin	Year	pg TEQ/g w.w.	Reference
Plaice	Belgian market	2000–2001	0.34[ad]	Schoeters et al. (2004)

[a] CALUX TEQ

[b] WHO-TEQ$_{2005total}$

[c] WHO-TEQ$_{1997total}$

[d] Median concentration

[e] WHO$_{1995}$-PCDD/F TEQ

where dietary advices exist for herring and other fish species due to high levels of dioxins and dioxin-like PCBs. We compared the CALUX TEQ in fish caught north and south of 63° north. The most abundant fish species in our sample was the marine species Atlantic cod. Cod caught north of 63° north showed a significantly lower average CALUX TEQ value (Mann-Whitney U, $p < 0.001$ w.w.; $p < 0.05$ l.w.) than fish caught further south (Fig. 2).
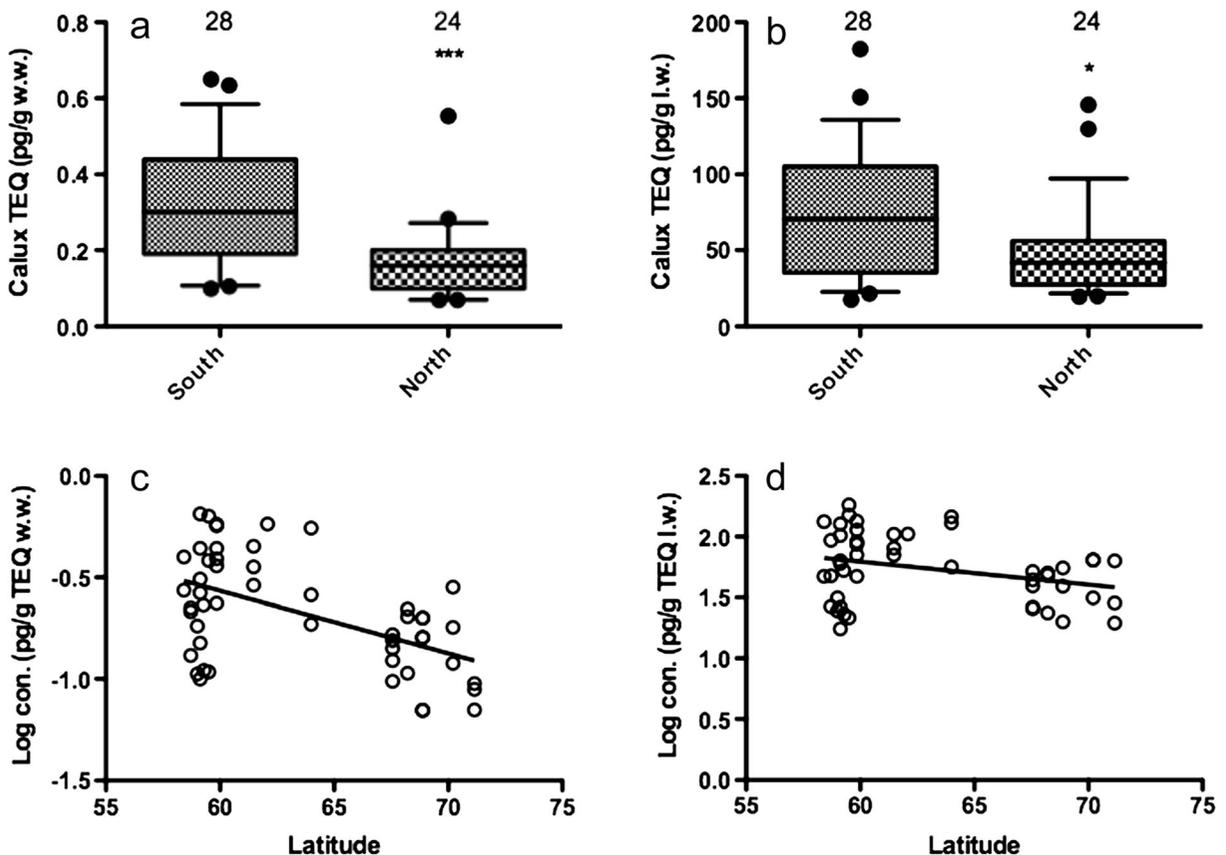
Fig. 2 Box plots of the concentration (max and min indicate the 10 and 90 % percentile and numbers of samples) on wet weight (**a**) and lipid weight (**b**) concentrations of dioxins and dioxin-like compounds between Atlantic cod from northern and southern Norway separated 63° north. *Asterisks* indicate statistical significant difference between groups (Mann-Whitney U *$p < 0.05$; ***$p < 0.001$). The concentrations are also shown by latitude of trapping site (**c**, **d**). Correlation analyses also showed that dioxin concentration decreased with increasing latitude (Pearson rank correlation (r_p) w.w. = -0.53 $p < 0.0001$, R^2 0.28; r_p l.w. = -0.31 $p < 0.03$, R^2 0.098)
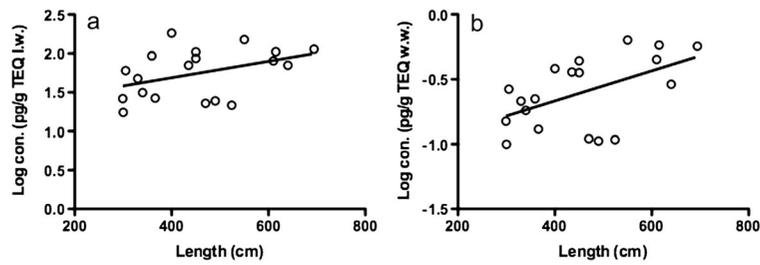
Fig. 3 The log transformed concentration of dioxins and dioxin-like compounds in Atlantic cod on wet weight (**a**) and lipid weight (**b**) concentrations from the southern parts of Norway (south of 63°

north) Correlation analysis showed a significant increase in the dioxin concentration with fish length for the wet weight concentration analyses (r_p w.w. = 0.53, $p < 0.02$, R^2 0.28)

Correlation analyses also showed that dioxin concentration decreased with increasing latitude (Pearson rank correlation (r_p) w.w. $= -0.53$ $p < 0.0001$; r_p l.w. $= -0.31$ $p < 0.03$).

Fish length is typically directly related to age, so within a species, larger fish in general represents older fish. Older fish also tend to have higher loads of
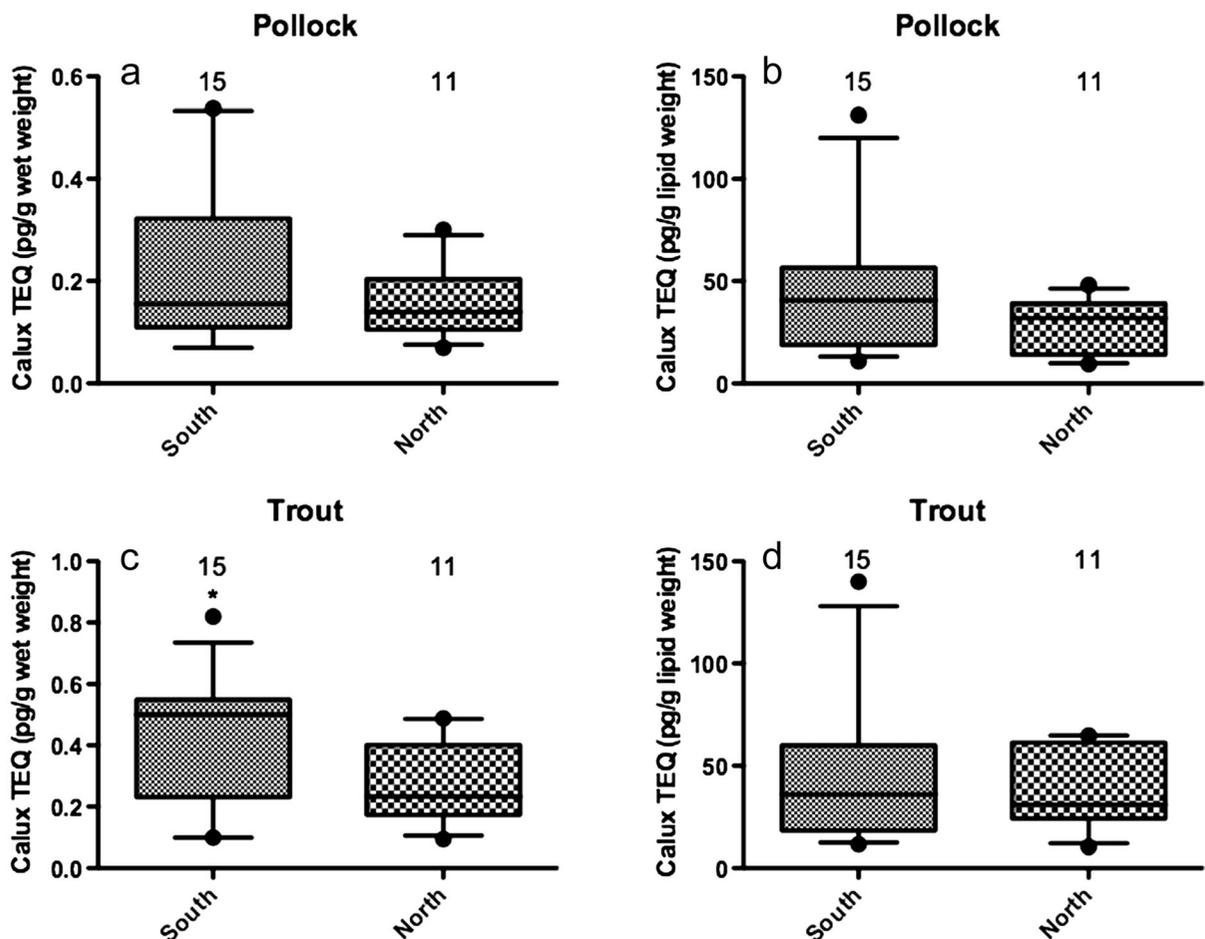
Fig. 4 A box plot of the concentration (max and min indicate the 10 and 90 % percentile and numbers of samples) of dioxins and dioxin-like compounds on wet weight and lipid weight concentrations of pollock (**a, b**) and brown trout (**c, d**) from northern and

southern Norway separated at 63° north and 60° north, respectively. *Asterisk* indicates statistical significant difference between groups (Mann-Whitney U *$p < 0.05$)

contaminants due to bioaccumulation over time. The north Norwegian fish was in general longer than fish from the south. In cod from southern Norway, fish length correlated significantly with the CALUX TEQ levels on the wet weight concentration (r_p w.w. = 0.53, $p < 0.02$) and close to significantly on the lipid weight concentration (r_p l.w. = 0.41, $p = 0.08$) (Fig. 3). This was not observed in fish from the northern part of Norway (r_p w.w. = −0.016, $p < 0.94$; r_p. l.w. = −0.14, $p = 0.50$). We also compared the pollock caught north and south of 63° north and the trout caught north and south of 60° north (Fig. 4). For the pollock samples, the difference in the CALUX TEQ concentration was near significant on the lipid weight concentration ($p = 0.078$, Mann-Whitney U) and for the trout samples only on wet weight concentration ($p < 0.05$, Mann-Whitney U). The CALUX TEQ concentrations showed a non-significant correlating trend f with the latitude for both of the species (data not shown). The sampling sites from south Norway were fjord areas closer to cities and more densely populated than the sampling sites in the north (Table 1S). This may explain the higher CALUX TEQ concentrations of the fish from south Norway compared to north Norway. In support of this, the two samples of common dab caught in the Egersund harbour in south-west Norway exhibited CALUX TEQ levels above maximum levels (EC 2011). Further, these two dab samples had relatively high fat content compared to other fish from the same area with lower TEQ concentrations (sea trout, pollock and a lean dab sample). In addition, common dabs are bottom-feeding fish and dioxins and PCBs are known to adsorb to sediments (Persson et al. 2002; Cornelissen et al. 2008).

Conclusions

The majority of fish samples from the participating countries had concentrations of dioxins and dioxin-like compounds lower than the maximum limits set by the European commission. The findings are in accordance with other surveys of dioxins in fish from European waters. The CALUX TEQ levels in the cod samples showed a significant reduction in the concentrations of dioxins with increasing latitude, indicating less contamination of dioxin and dioxin-like compounds in the north. Our results indicate that a moderate consumption of self-caught fish from presumed non-contaminated sites does not represent any major risk for exposure to

dioxins or dioxin-like compounds at concentrations associated with adverse health effects. Previous reports have however showed that high-level fish consumers, or consumption of fish from contaminated sites, may exceed recommended guidelines (e.g. Judd et al. 2004; Harris and Jones 2008.). In the Norwegian Mother and Child Cohort Study by Papadopoulou et al (2013), it was shown that dietary intake of dioxins and PCBs during pregnancy were negatively associated with foetal growth, even at intakes below the tolerable weekly intake of 14 pg TEQ/kg bw. One should also be aware of other contaminants in fish, such as mercury and non-coplanar PCBs. Elevated levels of mercury are reported in hair from recreational fishermen (Lincoln et al. 2011) and exposure to mercury from fish is associated with adverse health effects (e.g. Karagas et al. 2012). Even in fish caught at a remote site, the levels of mercury may exceed the guidelines for commercial sale (Evans et al. 2005). The non-coplanar PCBs are accumulated in higher concentrations in fish than the coplanar dioxin-like PCBs (Barone et al. 2014), and it has been claimed that the use of toxic equivalency factors that are based on dioxin-like activity is inadequate for estimating the total risk from exposures to PCBs (Fischer et al. 1998). In the study by Harris and Jones (2008), it was reported that 18 % had no knowledge about fish advisories in VA, USA. Recreational anglers should therefore obtain knowledge about local fish consumption advice.

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