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Effects of seafood consumption on mercury exposure in Norwegian pregnant women: A randomized controlled trial

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ABSTRACT

Background: Seafood provides nutrients that are important for optimal development of the unborn child. However, seafood is also a source of contaminants including mercury (Hg) and methylmercury (MeHg) that may have adverse effects on neurodevelopment of the fetus. Humans are predominantly exposed to MeHg through seafood consumption, however, levels of MeHg vary considerably between species.

Objectives: Investigate, in a randomized controlled trial (RCT) during pregnancy, if an increased intake of Atlantic cod (*Gadus morhua*), a fish species with relatively low levels of MeHg contamination, influences total hair Hg (THHg) concentrations in humans.

Methods: Pregnant women (n = 137) were enrolled in the RCT "Mommy's Food" (2016–2017), which was designed to increase iodine status. Participants were randomly assigned to intervention (400 g of cod fillets per week) or control (continued habitual diet) groups for 16 weeks (gestational week 20–36). THHg concentrations were measured at baseline and post-intervention using thermal decomposition, amalgamation, and atomic absorption spectrophotometry (US EPA method 7473). The trial is registered in ClinicalTrials.gov, NCT02610959.

Results: Post-intervention, the intervention group had median (inter-quartile range) THHg concentrations of 554 (392–805) μg/kg, and the control group 485 (341–740) μg/kg (p = 0.186). When adjusting for baseline THHg concentrations, there was a significant difference between the groups in those participants with baseline THHg concentrations below 534 μg/kg. Post-intervention, 8% of the study population exceeded the US EPA reference dose in hair (1,000 μg/kg) (intervention group: n = 6, control group: n = 4).

Conclusion: THHg concentrations were generally low in both study groups of pregnant women, despite the relatively high seafood intake. While the intervention with 400 g of cod per week slightly increased THHg concentrations, it did not lead to an increase in number of subjects exceeding the US EPA reference dose; a dose level at which no adverse effects are expected to occur over a period of lifetime exposure.

1. Introduction

Seafood is a dietary source of several important nutrients including high quality proteins, the marine long-chain omega-3 polyunsaturated fatty acids (n-3 LCPUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), vitamin D, vitamin B12, iodine, selenium and zinc (Aakre et al., 2019; Gil and Gil, 2015; Nerhus et al., 2018). These nutrients are important for optimal fetal development (Starling et al., 2015) and consumption of seafood during pregnancy has, in observational studies, been associated with beneficial fetal health and development (Brantsaeter et al., 2017; Daniels et al., 2004; Hibbeln et al., 2007; Leventakou et al., 2014; Mendez et al., 2009; Thorsdottir et al., 2004; Vejrup et al., 2014). However, seafood is also a source of mercury (Hg) primarily in the form of methylmercury (MeHg), a potent neurotoxin which can harm the human fetus (Karagas et al., 2012; Sheehan et al., 2014).

Abbreviations: BMI, body mass index; bw, body weight; DHA, docosahexaenoic acid; DMA-80, direct mercury analyzer; EFSA, European Food Safety Authority; EPA, eicosapentaenoic acid; FFQ, food frequency questionnaire; Hg, mercury; IMR, Institute of Marine Research, JNT, Johnson-Neyman technique; LOD, limit of detection; LOQ, limit of quantification; MeHg, Methylmercury, MoBa, The Norwegian Mother and Child Cohort; n-3 LC-PUFA, long chain n-3 polyunsaturated fatty acids; RfD, reference dose; RCT, randomized controlled trial; THHg, total hair mercury concentration; TWI, tolerable weekly intake; US EPA, United States Environmental Protection Agency; VKM, Norwegian Scientific Committee for Food Safety

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The European Food Safety Authority (EFSA) has established a tolerable weekly intake (TWI) level of MeHg of 1.3 μg/kg bodyweight (bw) based on neurodevelopmental outcomes after prenatal exposure in cohort studies from the Seychelles and the Faroe Islands (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012). The United States Environmental Protection Agency (US EPA) has developed an oral Reference Dose (RfD) of Hg of 0.1 μg/kg/day, corresponding to total hair Hg (THHg) concentrations of 1,000 μg/kg, which is estimated to be without any harmful health effects during a lifetime, including sensitive subgroups such as fetus (Hassett-Sipple et al., 1997; Keating et al., 1997; Mahaffey et al., 1997). This was based on data from the Faroes Island and New Zealand cohorts, using an uncertainty factor of 10 (WHO/UNEP, 2008). At levels substantially higher than the TWI from EFSA and the RfD from US EPA, severe toxicological effects on fetal development were observed in two major epidemic poisonings in Iraq and Japan (Bakir et al., 1973; Ekino et al., 2007). However, these studies were from environmental disasters, and the results cannot be extrapolated to general populations where MeHg exposure is much lower.

Humans are predominantly exposed to MeHg through fish and other seafood consumption (Bradley et al., 2017). Exposure of MeHg from seafood (in the vicinity of the TWI from EFSA) on child development are more inconclusive and studies have shown inconsistent results (Oken and Bellinger, 2008). These varying and inconsistent results may be explained by the beneficial effects of nutrients in seafood, such as selenium, EPA and DHA, on fetal neurodevelopment (Clarkson and Magos, 2006; Strain et al., 2008; Strain et al., 2012). Most European and American food based dietary guidelines recommend having 2–3 portions of seafood per week (EFSA, 2014; U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015). The rationale for the recommendations of fish consumption are predominantly based on the content of essential nutrients in fish and the evidence of preventing cardiovascular disease (EFSA, 2014). These guidelines also consider pregnant women and seem to be consistent with the premise that fish and seafood are beneficial with regard to fetal health outcomes (EFSA, 2014). However, pregnant women are advised to limit consumption of certain fish species and other seafood due to the potential for high levels of exposure to contaminants such as MeHg (EPA & FDA, 2019; The Norwegian Directorate of Health, 2011). Thus, pregnant women are faced with both risks and benefits of seafood consumption during pregnancy, which results in difficulties with risk communication and the interpretation of dietary recommendations (Leventakou et al., 2014; Taylor et al., 2018).

The Norwegian Mother and Child Cohort Study (MoBa) found a positive association between maternal seafood intake during pregnancy and increased birth weight, and language and communication skills, at three and five years of age (Vejrup et al., 2014; Vejrup et al., 2016; Vejrup et al., 2018). However, MeHg exposure from seafood reduced the beneficial effects and had a negative effect on birth weight and language and communication skills, even below the EFSA TWI (Vejrup et al., 2014; Vejrup et al., 2016). Thus, more knowledge is required to formally evaluate prenatal MeHg exposure below the TWI with regard to public health.

In Norway, according to a risk assessment performed by the Norwegian Committee for Food Safety and the Environment (VKM), fish and other seafood is the main dietary source of MeHg exposure (VKM, 2014; VKM et al., 2019). For pregnant women, the mean and 95th percentile exposures were estimated to be 0.17 and 0.39 μg/kg bw/week, respectively, with lean fish contributing to about 80% of total MeHg exposure (VKM, 2014). Norwegian Atlantic cod (*Gadus morhua*), the fish used in this intervention study, is a lean fish species with a relatively low content of Hg (n = 175 samples from 2018: mean: 0.094 mg/kg wet weight) (Institute of Marine Research, 2019) compared to many other fish species. However, dietary intake of Atlantic cod was found to be a significant source of MeHg exposure for pregnant women in Norway, since cod is among the most commonly consumed lean fish species (VKM, 2014).

To our knowledge, there are currently no other randomized controlled trials (RCTs) with fish during pregnancy investigating the impact on MeHg exposure. The aim of this sub-study was to investigate in an RCT, if an increased intake of Atlantic cod, a fish species with relatively low levels of MeHg contamination, influences total hair mercury (THHg) concentrations in pregnant women.

2. Methods

2.1. Study design and ethics

The Mommy's Food study is a two-armed RCT with dietary Atlantic cod in Norwegian pregnant women. The primary and secondary outcomes of the main study are to investigate if an increased intake of cod during pregnancy has an impact on maternal iodine status and infant development (Markhus et al., 2018). In this paper, THHg concentrations in the pregnant women were investigated. A total of 133 pregnant women from Bergen, Norway were randomized (1:1) to either receive cod twice a week (total of 400 g per week) or to continue with their habitual diet for 16 weeks (gestational week 20–36). The overall study design, including enrolment, randomization and study procedures are further described in detail elsewhere (Markhus et al., 2018).

The study was approved by the Regional Committees for Medical and Health Research Ethics West (REK 2015/879) and is registered in ClinicalTrials.gov (NCT02610959). The study was conducted and performed according to the Declaration of Helsinki. Participation in the study was voluntary and written informed consent was obtained from the pregnant women after giving both written and oral information about the study. The participants could withdraw from the study at any time without giving any reason, and this was highlighted in the declaration of informed consent.

2.2. Participants and recruitments

Participants were recruited through the Women's Clinic at Haukeland University Hospital in Health Region West in Norway from January 2016 to February 2017. In addition, information regarding the trial and invitation to participate were broadcasted online and through social media (Facebook, Instagram and online magazine for pregnant women in Norway (https://www.babyverden.no)). Inclusion criteria were prim parous, singleton pregnancy, gestational week ≤ 19 and Norwegian speaking and/or able to understand Norwegian writing (due to questionnaires and validated tests of the child in Norwegian). Exclusion criteria were fish allergies and diseases known to affect iodine status including hypothyroidism, hyperthyroidism, Grave's disease, thyroiditis and thyroid nodules.

2.3. Randomization, allocation and blinding

Baseline (pre-intervention) samples of biological material were collected in gestational week 18. Randomization occurred in gestational week 19. Participants and investigators were blinded to the allocation until baseline sampling was completed. The participants were randomized individually by lottery in blocks of ten to ensure approximately equal allocation to both groups. Owing to the nature of the study, further blinding of the pregnant women was not possible. Laboratory personnel and study investigators were blinded when analyzing data.

2.4. Intervention and procedures

After randomization, the allocated intervention group received frozen skin- and boneless cod fillets (Lerøy A/S, Bergen, Norway, bought after tender) of 200 g each and were instructed to consume two intervention meals weekly (a total of 400 g cod per week) for 16 weeks (a total of 32 meals). They also received cod for their partner, if any,

with the intention to increase compliance. The participants were free to choose preparations methods and recipes themselves, including choice of side dishes, but also received a recipe booklet for inspiration. To record dietary compliance the participants in the intervention group received a scale (Kitchen Scale, article no. 34–1207-16, ClasOhlson.com) and were instructed to weigh the cod fillet after thawing and before preparing the meal, and after the meal if there were any leftovers. The participants recorded the data in a weight registration form together with week number of the intervention and cooking method. There were no restrictions of foods or dietary supplements in either of the groups. The participants met for the post-intervention study visit in gestational week 36 when the intervention group had completed the intervention period, and biological material were again collected.

2.5. Outcomes

2.5.1. THHg concentrations

Collection of hair samples for Hg analyses of the mothers were performed by the study investigators in gestational week 18 (pre-intervention) and gestational week 36 (post-intervention). Hair samples were obtained by cutting a hair-bundle of 2–5 mm with a stainless-steel scissor, as close to the scalp as possible, from the occipital area of the head. A dental floss was tied around the hair-bundle closest to the hair root to make sure of using this end for analysis. Hair samples were stored in zip-lock bags at room temperature until laboratory analysis.

To investigate Hg-exposure during pregnancy, two cm of the hair closest to the scalp was used for analysis, which corresponds to Hg exposure 5.2 ± 0.8 to 12.4 ± 1.6 (95% confidence intervals (CI)) weeks prior to collection (LeBeau et al., 2011). Based on this calculation, a two cm hair sample from pre- and post-intervention corresponds to Hg exposure from gestational week 5.6 ± 1.6 to gestational week 12.8 ± 0.8 (95% CI) and from gestational week 23.6 ± 1.6 to gestational week 30.8 ± 0.8 (95% CI), respectively.

Each hair sample was measured precisely with a ruler and further cut off with a clean stainless-steel scissor. The hair sample was positioned in separate nickel boats and weighed on a calibrated four decimal scale from Sartorius (CP124S, USA) or Ohaus (Explorer Analytical, USA). The weight of the hair samples varied between 3.4 mg and 27.0 mg with a mean (standard deviation (SD)) weight of 12.8 (5.2) mg.

The hair samples were analyzed for total Hg by thermal decomposition, amalgamation, and atomic spectrophotometry (US EPA method 7473) using a Direct Mercury Analyzer (DMA-80, Milestone Srl, Italy). Prior to analysis in DMA-80 the metal boats were burned/cleaned in a muffle furnace, Carbolite ELF 11/14B at 650 °C in 30 min to avoid cross-contamination from previous samples. The hair samples from baseline and post-intervention were analyzed in similar analysis series. There were equal number of samples from the intervention and the control group in each analysis series.

Certified Reference Material for trace metals (Reference Material Human Hair, IAEA-086 (International Atomic Energy Agency, Austria)) were included in the analyses to assess the accuracy and quality of the analysis. The certified values of Reference Material Human Hair, IAEA-86 are 573 μg/kg. All registered values of Certified Reference Material Human Hair were within accepted area of the analysis (± 20%) with a mean accuracy of 90% (% relative SD: 2.5%). The DMA-80 instrument is calibrated in the linear range of Hg from 1.5 to 1000 ng. For samples in this range the accuracy is 80–120%. Of 242 hair samples analyzed from pre- and post-intervention, 2.5% (baseline: $n = 3$, post-intervention: $n = 3$) had a Hg content < 1.5 ng. All analyzed values were above the limit of quantification (LOQ) of 0.08 ng Hg and the limit of detection (LOD) of 0.02 ng.

2.5.2. Baseline characteristics

The participants filled out an electronic questionnaire in gestational week 18 and 36 (baseline and post-intervention, respectively). The questionnaire included questions of baseline characteristics such as age, gestational age, household income, education level, pre-pregnancy weight and height. If there was missing data in the questionnaire answered at baseline, data that were considered not to be significantly altered from pre- to post-intervention (such as education level, height and household income) were retrieved from the post-intervention questionnaire. Pre-pregnancy body mass index (BMI) was calculated as pre-pregnancy weight in kilograms (kg) divided by the square of the height in meters (kg/m^2).

2.5.3. Seafood intake

A food frequency questionnaire (FFQ) was included in the online questionnaire to acquire information about the participants' habitual diet. The FFQ was based on a validated semi-quantitative short seafood FFQ developed to analyze intake of seafood (fish and shellfish) and dietary supplements during pregnancy and postpartum (Markhus et al., 2013), and further modified specifically for this study. The FFQ included detailed questions about seafood intake and the participants were asked to give an estimate of their diet the past months (from pregnancy until gestational week 18 at baseline and last 16 weeks at post-intervention). The FFQ included 21 food items regarding frequency of seafood intake as dinner and warm lunch, and 14 food items regarding frequency of seafood intake as spread, focusing on type of seafood species and products. The seafood species included in the FFQ are specified in Appendices Table A1. Each question of the specific seafood species had follow-up questions concerning portion sizes per meal. More information regarding the processing of seafood intake from the FFQ are published elsewhere (Næss et al., 2019).

2.5.4. Estimated Hg-intake

To calculate the mean weekly intake of Hg from seafood reported from the FFQ, intake of the different seafood species in gram per week was multiplied by the average Hg concentrations of the specific species and further summarized. The Hg content of each specific seafood species was retrieved from the database 'Seafood data' from the IMR (Institute of Marine Research, 2019). Mean Hg concentrations of the specific species were retrieved from the study period (2015–2018) and analyzed further by averaging the means from each year. If data of the specific seafood species from these years were not available, data from analysis years closest to the study period were used. If no available data of a specific seafood species, Hg concentrations of a comparable seafood species were used. If concentrations of Hg were below the LOQ, an upper bound (UB) approach was used and the Hg concentration was set to the respective LOQ value. Information of Hg content of the specific seafood species are specified in Appendices Table A1. To calculate Hg intake per kg body weight per week, the total estimated Hg intake per week was divided by the self-reported pre-pregnancy body weight.

The intervention group received portion packed cod fillets of 200 g each. The cod fillets were caught in the Barents Sea in October 2015. Further, sub-samples (n = 30) of the received cod were analyzed for total Hg using DMA-80 as previously described. The samples were freeze-dried and homogenized prior to subsequent analysis. The cod samples (wet weight) had a mean (SD) total Hg concentration of 0.028 (0.014) mg/kg (min–max: 0.014–0.067 mg/kg). Thus, the cod received in the intervention group displayed lower levels of Hg compared to Norwegian Atlantic cod caught in previous years and compared to most other seafood species (Institute of Marine Research, 2019). In Europe, the maximum levels for Hg in muscle meat of fish is 0.5 mg/kg and is set by European Union Commission Regulation (EC) No. 1881/2006 (European Commission, 2006).

2.6. Sample size and power

The power calculation of sample size was based on daily iodine intake and urinary iodine concentration (UIC) from the "Little in Norway" cohort study (Dahl et al., 2018), as the primary outcome in the

Mommy's Food study was maternal iodine status. A sample size of 60 women per group were calculated to have a 95% power to detect a 30% higher UIC in the intervention group compared to the control group with a significance level of $\alpha = 0.05$. A dropout rate of 20% was expected and we aimed for a total of 144 participants to be enrolled in the study.

2.7. Statistical methods

Statistical analyses were performed using IBM SPSS Statistics version 25 (IBM Corporation) and R version 3.5.3 (R Core Team, 2019) run in RStudio Version 1.2.1335 (RStudio Team, 2018). *P*-values < 0.05 were considered statistically significant. Two-sided statistical tests were performed. Variables were tested for normality by using the Kolmogorov-Smirnov test and by visual inspection of Q-Q plots and histograms. Descriptive results are reported as frequency (%) for categorical variables. For continuous variables mean (SD), median (interquartile range (IQR)) or 5th and 95th percentile are reported as appropriate.

For categorical data, chi-square test or Fisher's exact test (if $> 20\%$ of expected values had frequencies < 5) were used to compare differences between the two groups. For continuous normally distributed data, parametric statistical tests were used. Independent *t*-test was used to compare differences between the two groups and paired sampled *t*-test was used for differences within the groups. Non-normal data were transformed using natural logarithm (lnTHHg) transformations, to correct for skewness. If data were not normally distributed after natural logarithm (ln) transformations, non-parametric statistical tests were used. Mann-Whitney *U* test was used to compare differences between the two groups and Wilcoxon signed-rank test was used for differences within groups.

The differences in THHg concentrations between the intervention and the control group at post-intervention was first assessed using an independent *t*-test. To adjust for possible interactions of the baseline THHg concentration, we also used pre-THHg concentrations as a covariate to adjust for differences in baseline THHg concentrations. This was also specified for the primary outcome in the study protocol article (Markhus et al., 2018) and is a recommended choice when studying group differences in RCT studies (Clifton and Clifton, 2019; Skovlund and Lydersen, 2018; Vickers and Altman, 2001). The one-way analysis of covariance (ANCOVA) did not meet the assumption of homogeneity of regression slopes. Thus, the Johnson-Neyman technique (JNT) (Johnson and Neyman, 1936) was performed in R adapting scripts available online (Ken Toyama, 2018; Marko Bachl, 2015) based on calculations described by White (2003). JNT does not require regression slopes to be homogenous across the entire dataset and allows for the identification of zones in which differences between groups are significant ($p < 0.05$).

Correlation between THHg concentrations and total seafood intake was performed using Spearman's rank order correlation coefficient, as the variables of THHg concentration and total seafood intake were not normally distributed and contained outliers. Compared to Pearson correlation coefficient, Spearman correlation coefficient is not as sensitive to extreme values and may be more reliable where outliers are present (Masson et al., 2003). The correlation coefficients strength (effect size) was considered small if < 0.30, moderate if 0.30–0.49 and strong/large if ≥ 0.50 in according to Cohen's (Cohen, 1992) and previously used dietary methods (Lombard et al., 2015).

3. Results

A flow chart of the study population is presented in Fig. 1. In total, 133 pregnant women were randomized to the intervention group ($n = 68$) or the control group ($n = 65$). Between randomization and post-testing, nine participants dropped out of the study (six in the intervention group and three in the control group). In addition, three participants were lost to follow up at post-intervention due to pre-term birth (one in intervention group and two in control group). Hence, 121 participants ($n = 61$ in intervention group and $n = 60$ in control group) were included in the main analysis. Baseline characteristics of the randomized pregnant women enrolled in the Mommy's Food study is presented in Table 1. Baseline characteristics were similar in both groups.

The mean (SD) and median (IQR) intake of the received cod in the intervention group were 306 (62), and 318 (275–356) g per week, respectively. The 5th percentile was 175 g of cod per week and less than 10% of the participants in the interventions group had a mean intake of less than 200 g cod per week.

The main outcome investigated in the present paper was the assessment of the difference in THHg concentrations between the control and intervention group at post-intervention. Post-intervention, the median (IQR) THHg concentration was 485 (341–740) μg/kg in the control group ($n = 60$) and 554 (392–805) μg/kg in the intervention group ($n = 61$) (Table 2). For the main analyses, there was no difference between the groups post-intervention ($p = 0.186$). When applying JNT and adjusting for baseline concentrations of THHg, a significant difference between the intervention and control group was observed in participants with baseline THHg concentrations below 534 μg/kg ($p < 0.05$) (Fig. 2). At baseline, $n = 34$ (52.3%) in the control group and $n = 35$ (51.5%) in the intervention group had THHg concentrations below 534 μg/kg. At baseline, in total 14 participants (10.5%) had THHg concentrations above 1,000 μg/kg ($n = 4$ in control (6.2%) group and $n = 10$ in intervention group (14.7%)). Post-intervention, in total 10 participants (8.3%) had THHg concentrations above 1000 μg/kg ($n = 4$ in control (6.7%) group and $n = 6$ in intervention group (9.8%)).

There was a moderate-to-large correlation between THHg concentrations and total seafood intake at both pre-intervention ($r = 0.39$, $p = < 0.001$, $n = 127$) and post-intervention ($r = 0.55$, $p = < 0.001$, $n = 106$). Additionally, there was a moderate-to-large correlation between THHg concentrations and estimated total Hg intake from seafood at both pre-intervention ($r = 0.36$, $p = < 0.001$, $n = 127$) and post-intervention ($r = 0.51$, $p = < 0.001$, $n = 106$).

Estimated Hg intake (μg) from total seafood intake, per week at baseline and post-intervention in the control and intervention group, is presented in Table 3. Post-intervention, the intervention group (median (IQR): 33 (27–40) μg/week) had a higher estimated intake of Hg from total seafood compared to the control group (median (IQR): 19 (11–29) μg/week) ($p < 0.001$). The median (IQR) estimated Hg intake from seafood per kg body weight post-intervention was 0.54 (0.42–0.63) μg/kg bw/week in the intervention group and 0.29 (0.17–0.45) μg/kg bw/week in the control group ($p < 0.001$). None of the participants exceeded the TWI, set by EFSA, post-intervention.

Visual box plots of total seafood intake and the categories of seafood at baseline and post-intervention are shown in Figs. 3 and 4. Seafood intake, presented for each seafood species and summarized in categories, at baseline and post-intervention in the control and intervention group are presented in Table A2. The mean (SD) total seafood intake in the control ($n = 60$) and the intervention group ($n = 65$) was 446 (221) and 456 (303) g/week, respectively. Post-intervention, the intervention group (mean (SD): 539 (214) g/week) had a higher intake of total seafood compared to the control group (mean (SD): 435 (282) g/week) ($p < 0.001$). During the intervention period, the intervention group reported a higher intake of lean fish compared to the control group ($p < 0.001$), while the control group reported a higher intake of fatty fish ($p < 0.001$), processed seafood ($p = 0.010$) and seafood as spread ($p = 0.014$). For the total seafood intake, at baseline, 68% of the total study group had seafood intake above 300 g/week (73% in the control group and 63% in the intervention group) and 44% had a total seafood intake above 450 g/week (45% in the control group and 40% in the intervention group). Post-intervention, 79% of the total study group had seafood intake above 300 g/week (65% in the control group and 93% in the intervention group) and 51% had a total seafood intake

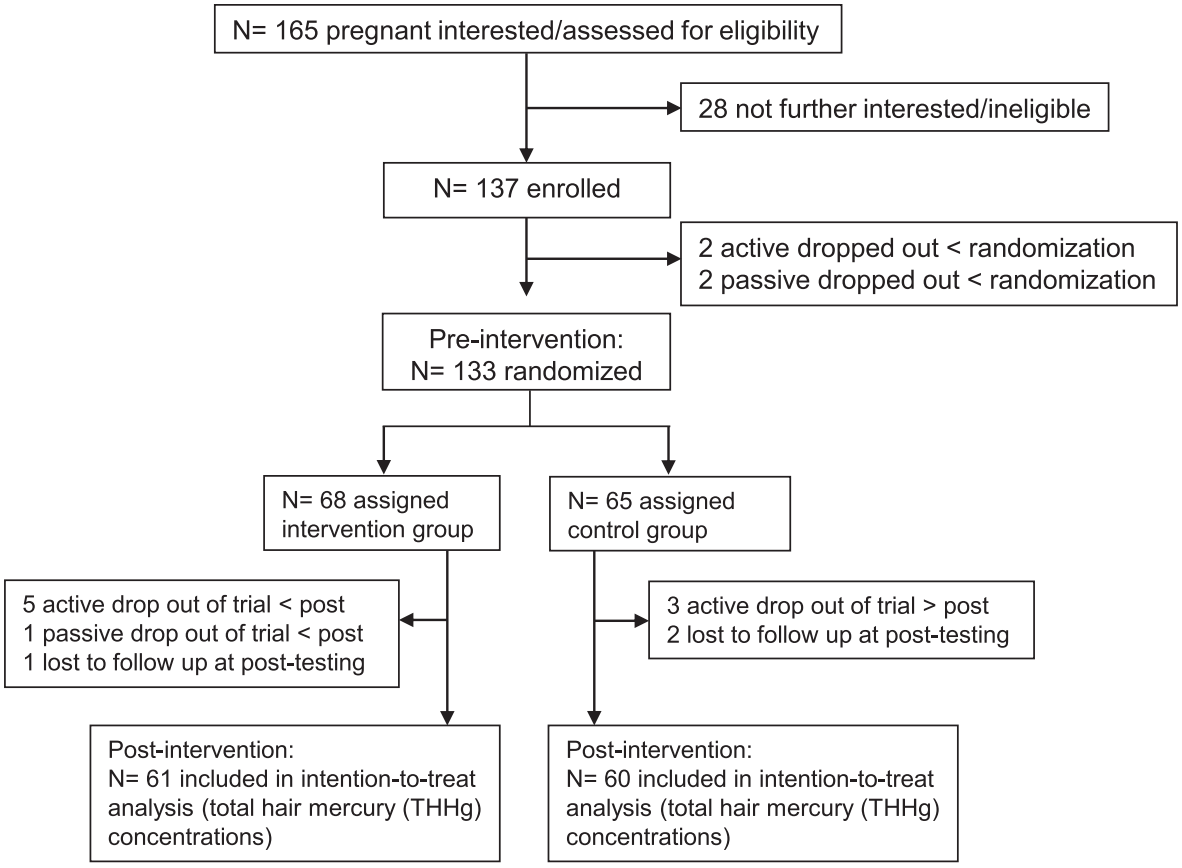

Fig. 1. Trial profile depicting the flow of participants through the intervention trial Mommy's Food (2016–2017).

Table 1
Baseline characteristics of pregnant women enrolled in Mommy's Food (2016–2017) by randomly allocated control and intervention group.

Characteristic	Control group (n = 65[a])	Intervention group (n = 68)
Age (years), mean (SD)	29.1 (3.5)	29.6 (4)
Pre-pregnancy BMI (kg/m^2), median (IQR)	22.5 (20.5–24.2)	21.9 (20.5–24.3)
Marital status, n (%)		
Married	20 (32)	23 (34)
Cohabiting	41 (65)	42 (62)
Other	2 (3)	3 (4)
Education level, n (%)		
Elementary school	0 (0)	1 (1)
High school	7 (11)	10 (15)
≤4 years university/college	15 (24)	18 (27)
> 4 years university/college	41 (65)	39 (57)
Total household income (NOK[b]), n (%)		
< 200,000–549,000	15 (24)	23 (34)
550,000–1,249,999	40 (63)	36 (53)
1,250,000– > 2,000,000	8 (13)	9 (13)

BMI, body mass index; IQR, interquartile range; NOK, Norwegian kroner; SD, standard deviation.

[a] Missing data: Pre-pregnancy BMI n = 3, marital status n = 2, education level n = 2, total household income n = 2.

[b] 100 NOK = approximately 11.6 USD/10.2 EUR.

Table 2
Total hair mercury (THHg) concentrations (μg/kg) pre- and post-intervention by control and intervention group in Mommy's Food (2016–2017).

	Pre-intervention	Post-intervention	
	THHg (μg/kg)	THHg (μg/kg)	Differences between groups post-intervention [a]
	Median (IQR) 5th, 95th percentile	Median (IQR) 5th, 95th percentile	*P*-value
Control group (n = 60)	517 (366–750) 131, 1413	485 (341–740) 124, 1166	0.186
Intervention group (n = 61)	542 (316–823) 154, 1178	554 (392–805) 248, 1134	

[a] Log transformed data of THHg are used for statistical analyses. Independent *t*-test for comparison of differences between control and intervention group post-intervention.

above 450 g/week (37% in the control group and 63% in the intervention group).

4. Discussion

To the best of our knowledge, this is the first RCT with fish investigating how an increased intake of fish (cod) during pregnancy has an impact on Hg exposure, using the biomarker THHg. This was explored by a two-armed RCT where pregnant women were randomized either to eat 400 g of cod per week or to continue with their habitual diet between gestational week 20–36. Post-intervention, the intervention group had a median THHg concentration of 554 μg/kg and the control group 485 μg/kg. While the intervention slightly increased THHg concentrations, it did not lead to an increase in number of subjects exceeding the US EPA reference dose; a dose level at which no adverse effects are expected to occur over a period of lifetime exposure. When adjusting for baseline concentrations of THHg, the difference between the groups increased. This may indicate that the participants

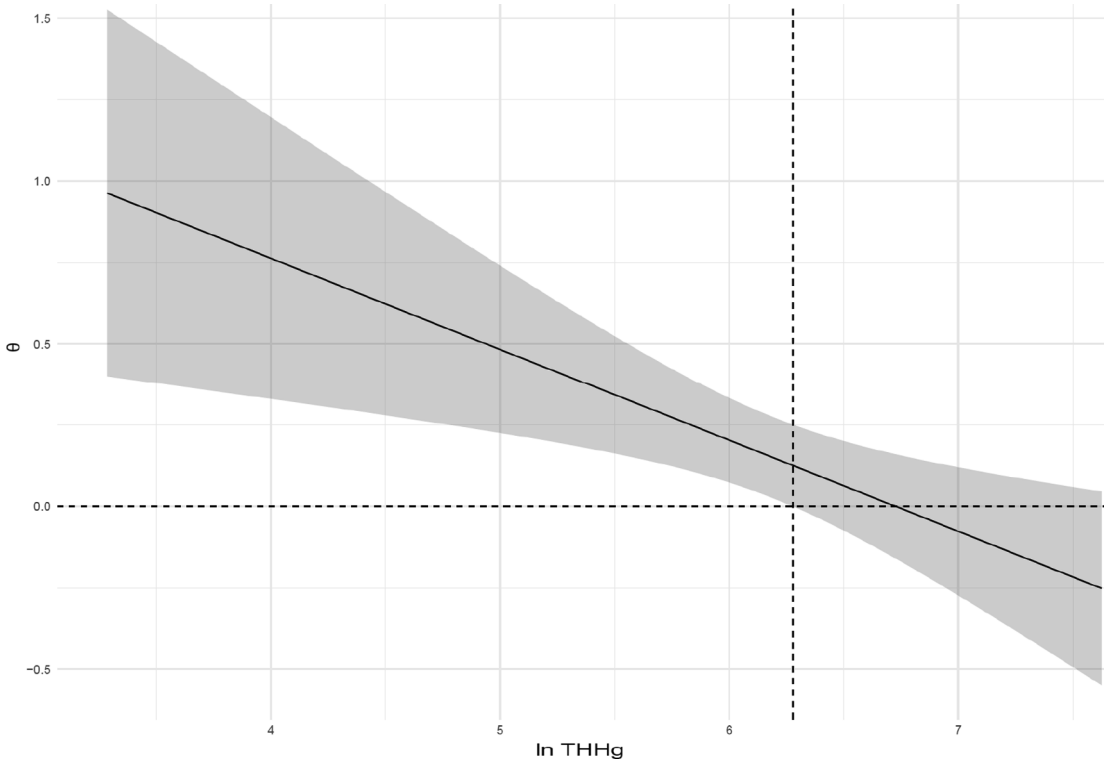

Fig. 2. Conditional effect plot of differences in total hair mercury (THHg) concentrations between control and intervention groups in Mommy's Food (2016–2017). The x-axis displays transformed (natural logarithm; ln) baseline THHg concentrations (ln THHg; μg/kg); the y-axis depicts the conditional effect (θ) of the intervention across values of ln THHg. Applying the Johnson-Neyman technique (JNT), a significant ($p < 0.05$) positive difference between intervention and control groups was observed in participants whose baseline THHg concentrations were below 534 μg/kg THHg (6.23 ln THHg).

with lower concentrations of THHg at baseline were more affected by the intervention with cod, compared to those with higher concentrations of THHg at baseline.

One might expect a larger difference between the control and the intervention group in THHg concentrations after the intervention, as the intervention group was given 400 g of cod per week; a relatively high intake of fish that corresponds with the dietary recommendations of fish intake in Norway. However, compared to the general seafood intake in Norway, and the seafood intake recorded in pregnant women (Brantsaeter et al., 2017; Markhus et al., 2013; The Norwegian Directorate of Health, 2012), the participants in this study had a relatively high seafood intake at baseline and almost twice as high than what was reported in the Norwegian cohort study MoBa (411 g/week vs. 234 g/week). The intervention group increased their seafood intake from baseline to post-intervention, and they had a higher seafood intake compared to the control group post-intervention (Figs. 3 and 4). However, this was mostly explained by a higher intake of lean fish, as the intervention group decreased their intake of fatty fish during the intervention period and the intake of fatty fish was higher in the control group post-intervention (Fig. 4). Hence, the participants in the intervention group with a relatively high seafood intake, might have replaced the intake of other fish species, (e.g. fatty fish) with the received intervention cod. This may be one reason as to why we did not observe any greater substantial difference in Hg concentrations between the groups. In addition, the measured Hg concentrations of the consumed cod (mean (SD): 0.028 (0.014) mg/kg) in the intervention group were lower compared to previously reported Hg concentrations in Atlantic cod (Institute of Marine Research, 2019). This may also have contributed to why we did not observe larger differences between the groups. It is possible that an intervention with a fish species with a higher Hg content would have resulted in a different finding, and one might likely have observed a larger increase in THHg concentrations in

Table 3
Estimated Hg intake (μg) from total seafood intake per week pre- and post-intervention by control and intervention group in Mommy's Food (2016–2017).

	Pre-intervention		Post-intervention		Difference between groups post-intervention
	Control ($n = 60$)	Intervention ($n = 65$)	Control ($n = 51$)	Intervention ($n = 56$)	
Hg intake (μg) per week	Median (IQR) 5th, 95th percentile		Median (IQR) 5th, 95th percentile		*P*-value[a]
From total seafood	20 (13–30) 5.7, 44	20 (12–30) 5.3, 57	19 (11–29) 6.7, 46	33 (27–40) 16, 51	< 0.001
From total seafood per kg bw[b,c]	0.30 (0.21–0.46) 0.12, 0.71	0.31 (0.20–0.45) 0.07, 0.94	0.29 (0.17–0.45) 0.12, 0.45	0.54 (0.42–0.63) 0.28, 0.89	< 0.001

bw, body weight; IQR, interquartile range.
[a] Mann-Whitney *U* test for differences between groups post-intervention.
[b] Self-reported pre-pregnancy body weight.
[c] n = 59 in control group due to missing pre-pregnancy body weight, n = 1.

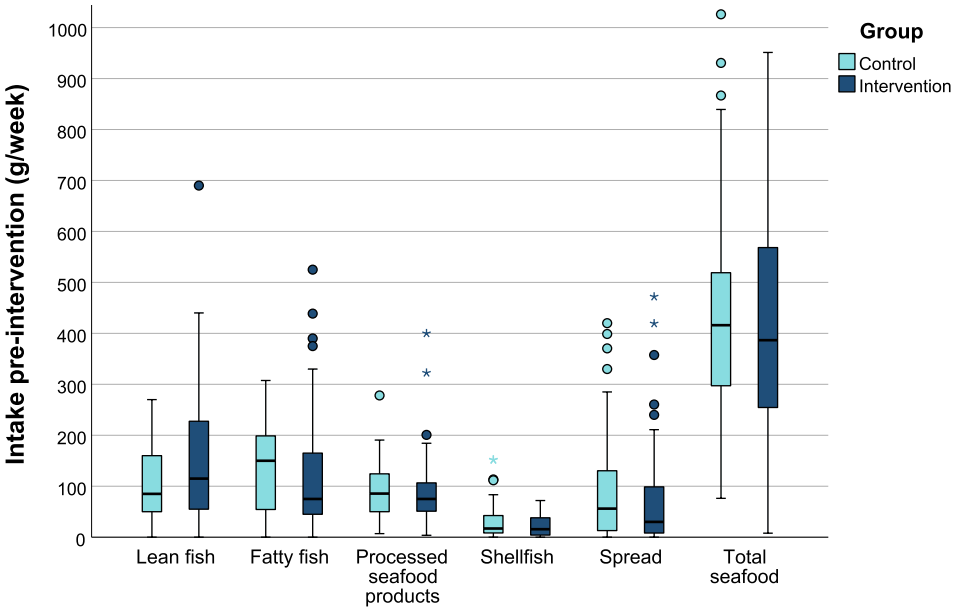

Fig. 3. Box plot of seafood intake by categories at baseline in control (n = 60) and intervention group (n = 65) in Mommy's Food (2016–2017). Boxes indicates the upper (75 th percentile) and lower (25 th percentile) quartile with the thick black line giving the median (50 th percentile). The T-bars indicate 1.5 × length of the box (inter quartile range). The filled circles are outliers defined as a value > 1.5 length of the box. The asterisks are extreme outliers defined as a value > 3.0 length of the box. Values above 1,000 g/week (n = 3 in intervention group) are not included in figure (but in statistics).

the intervention group.

The increased intake of cod during pregnancy did not lead to an increase in subjects exceeding the reference value set by US EPA of 1,000 μg/kg, a level that is considered to be without risk of adverse effects. Of the participants in the intervention group with THHg levels above 1,000 μg/kg at baseline (n = 10), all participants decreased their THHg concentrations from baseline to post-intervention. Nevertheless, there are some discussions that the reference levels of 1,000 μg/kg in hair should be set at lower level for optimal prevention of MeHg toxicity (Bellanger et al., 2013; Grandjean et al., 2012; Pichery et al., 2012). The TWI set by EFSA of 1.3 μg/kg per week is based on maternal THHg concentrations of 11,500 μg/kg, derived from the No Observed Effect Level (NOEL) in the Seychelles cohort (maternal THHg 11,000 μg/kg) and the Benchmark Dose (BMD_{05}) from the Faroe Islands cohort (maternal THHg 12,000 μg/kg). An uncertainty factor of 6.4 was used to derive the TWI value from EFSA, corresponding to THHg concentrations of ~1,800 μg/kg. None of the participants in this study, either of the intervention or the control group, exceeded this value post-intervention. In addition, neither of the participants post-intervention exceeded the TWI of 1.3 μg/kg from EFSA. A recent report from VKM in Norway (VKM et al., 2019), concluded that if eating fish with a low Hg concentration (defined as below 0.051 mg/kg), this will not lead to an exposure exceeding the TWI, even in high consumers of seafood (> 1,000 g/week). However, if consuming fish with a high Hg concentration (defined as above 0.33 mg/kg), this will lead to an exposure exceeding the TWI when consuming more than one portion of fish per week (150 g). The mean Hg concentrations in the analyzed cod given in this intervention would here be defined as fish with a low Hg concentration, if using these definitions.

As there are no other similar RCTs in pregnant women, the results from this study are not directly comparable to other studies. There are few studies measuring Hg concentrations of pregnant women in Norway, and this study gives new knowledge of Hg exposure in this vulnerable population group. The analyzed THHg concentrations, showed a moderate-to-large correlation with seafood intake, confirming that seafood is a major exposure of MeHg exposure. This is also found in

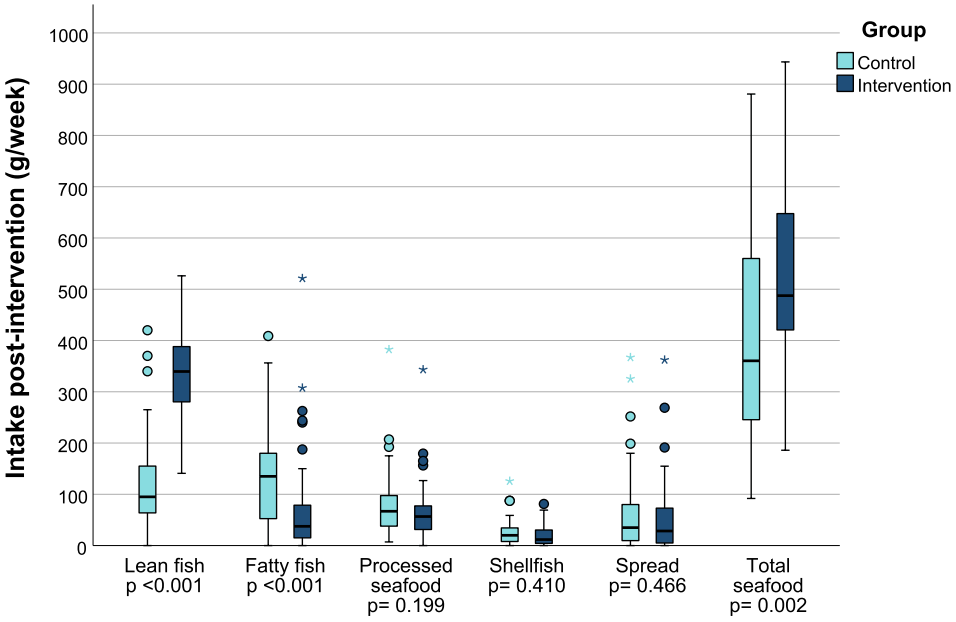

Fig. 4. Box plot of seafood intake by categories post-intervention in control (n = 51) and intervention group (n = 56) in Mommy's Food (2016–2017). Boxes indicates the upper (75th percentile) and lower (25th percentile) quartile with the thick black line giving the median (50th percentile). The T-bars indicate 1.5 × length of the box (inter quartile range). The filled circles are outliers defined as a value > 1.5 × length of the box. The asterisks are extreme outliers defined as a value > 3.0 length of the box. Values above 1,000 g/week (n = 2 in control group, n = 1 in intervention group) are not included in figure (but in statistics).

other studies, and also confirms that THHg concentration can be considered a valid biomarker to measure MeHg exposure from seafood (Berglund et al., 2005; Den Hond et al., 2015; Okati and Esmaili-Sari, 2018; Sanzo et al., 2001). Hg concentrations in blood have been investigated in a sub-population in the Norwegian cohort study MoBa (gestational week 17, $n = 2,239$) (Vejrup et al., 2018). Using a conversion factors of 250 between blood and hair (Clarkson and Magos, 2006), the concentrations of THHg in MoBa were median (range) 258 (0–3,450) μg/kg and thus lower compared to this study. In addition, the seafood intake and estimated Hg intake from seafood were similarly lower in MoBa, compared to this study, which most likely explain the difference. Compared to reported concentrations of THHg in Swedish (median: 350 μg/kg, $n = 127$ and 220 μg/kg, $n = 100$) (Bjornberg et al., 2003; Gerhardsson and Lundh, 2010) and Danish pregnant women (median: 340 μg/kg, $n = 146$) (Kirk et al., 2017), THHg concentrations (both pre- and post-intervention) were higher in this study. However, the reported intake of seafood was also higher in our study compared to the studies from Sweden and Denmark. A study investigating Hg concentrations in hair of women of reproductive age in Europe revealed data from 17 countries ($n = 1,875$) involved in the DEMOCOPHES (DEMOnstration of a study to COordinate and Perform Human biomonitoring on a European Scale) project and literature 8 countries ($n = 6,820$) (Bellanger et al., 2013). Including all countries, median THHg concentrations were 465 μg/kg; however, there were large variations between the countries with the highest levels being observed in Southern Europe. Comparing the results from our study, the concentrations of THHg were generally higher than most other countries in Europe, yet lower when compared to Spain, Portugal, Italy, Faroe Islands, Croatia, Greece and Malta. The variations of Hg concentrations are most likely related to type and quantity of seafood consumed. The pregnant women in this study generally consumed seafood species with a relatively low content of Hg (Tables A1 and A2). In contrast, some of the countries in DEMOCOPHES showing higher concentrations of THHg compared to this study are known to consume seafood species with a higher content of Hg such as long-lived, predatory fish species (Carvalho et al., 2008; Jacobs et al., 2017; Llull et al., 2017; Nunes et al., 2014; Storelli and Barone, 2013).

As seafood is the predominantly source of MeHg exposure in humans (Bradley et al., 2017), some countries (e.g. USA and Norway) have specific advise for pregnant women to avoid species high in Hg. Reducing consumption of seafood during pregnancy would decrease Hg exposure further, but at the same time would also reduce the intake of beneficial nutrients important for optimal fetal neurodevelopment (Starling et al., 2015). A recent systematic review including 44 studies, concluded that no adverse effects of seafood consumption on neurocognition were found, and consumption of seafood during pregnancy have beneficial associations with neurocognitive outcomes (Hibbeln et al., 2019). This conclusion is also in consistent with risk-benefit assessments performed by other risk assessment bodies such as EFSA (EFSA, 2014, 2015), FAO/WHO (FAO/WHO, 2011), the United States Food and Drug Administration (FDA, 2014) and in Norway by VKM (VKM, 2014) and other research groups (Cohen et al., 2005; Hellberg et al., 2012; Hoekstra et al., 2013; Thomsen et al., 2018). However, the beneficial effects of seafood are reduced by exposure to MeHg and other fish contaminants thus creating a complex challenge for risk communication to pregnant women and their dietary choices to support healthy neurodevelopment of the fetus (FAO/WHO, 2011; VKM, 2014). Furthermore, additional knowledge of low level MeHg exposure to pregnant women will be required to further refine and improve risk communication to this vulnerable population sub-group.

The strengths of this study are its innovative study design using an RCT with cod during pregnancy, detailed measurement of compliance (weighing of cod fillet) and the detailed registration of seafood intake using an FFQ and estimation of Hg intake. There are also some study limitations that should be considered. As the primary outcome of the study was iodine status, the power calculation was not based on THHg concentrations as an outcome. We used hair as a biomarker to measure Hg exposure, which has been considered a valid measurement of long-term exposure of MeHg (Berglund et al., 2005; Nuttall, 2006). Analyzing Hg in blood reflects more recent exposures, compared to hair and may have been a more precise method for assessing and estimating Hg exposure in individuals (Nuttall, 2004). However, Hg blood concentrations reflect Hg intake at the steady state and may not be accurate in populations with unstable dietary patterns (Sirot et al., 2008). In addition, THHg concentrations correlated well with estimated Hg intake from seafood, confirming that THHg concentrations can be used as an appropriate biomarker of Hg exposure from seafood.

5. Conclusion

The intervention with 400 g cod per week slightly increased THHg concentrations, however, it did not lead to an increase in number of subjects exceeding the US EPA reference dose; a dose level at which no adverse effects are expected to occur over a period of lifetime exposure. When adjusting for baseline concentrations of THHg, the difference between the groups increased, indicating that the participants with lower concentrations of THHg at baseline were more affected by the intervention with cod, compared to those with higher concentrations of THHg at baseline. The study population generally had a high seafood intake, mostly consisting of seafood species with relatively low Hg content. THHg concentrations and estimated MeHg intake were generally low in both study groups of pregnant women.

6. Availability of data sharing

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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Synnøve Næss: Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Marian Kjellevold:** Methodology, Project administration, Writing - review & editing. **Lisbeth Dahl:** Methodology, Writing - review & editing. **Ive Nerhus:** Investigation, Writing - review & editing. **Lisa Kolden Midtbø:** Investigation, Writing - review & editing. **Michael S. Bank:** Writing - review & editing. **Josef D. Rasinger:** Formal analysis, Visualization, Writing - review & editing. **Maria Wik Markhus:** Methodology, Visualization, Project administration, Supervision, Writing - review & editing.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105759.

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