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Cadmium in brown crab *Cancer pagurus*. Effects of location, season, cooking and multiple physiological factors and consequences for food safety

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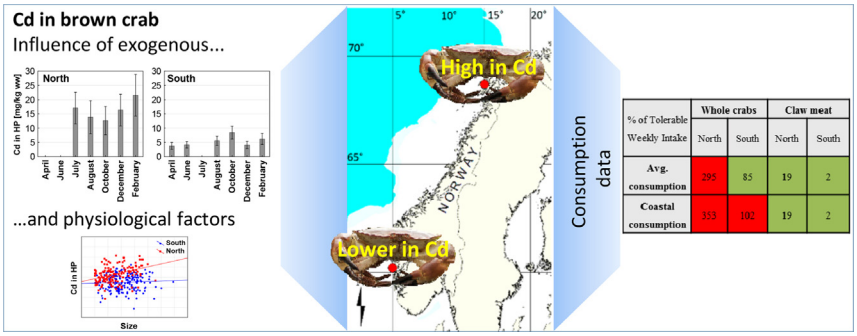
HIGHLIGHTS

- Brown crab from Northern Norway had significantly higher Cd levels.
- Crab size was correlated with the total amount of Cd.
- Accumulation over time combined with slower growth may cause higher Cd in the North.
- The consumption of claw meat does not display a consumer health risk regarding Cd.
- Consumption of inner meat may pose a health risk due to levels of Cd.

GRAPHICAL ABSTRACT



% of Tolerable Weekly Intake	Whole crabs		Claw meat	
	North	South	North	South
Avg. consumption	295	85	19	2
Coastal consumption	353	102	19	2

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ABSTRACT

Brown crab *Cancer pagurus* is appreciated as seafood in several European countries. However, cadmium levels in crabs can be elevated and their consumption may pose a hazard for human health. To assess if cadmium poses a threat to food safety in Norway, crabs were sampled at two different locations along the Norwegian coast: one in the South of Norway and one in the North of Norway. Cadmium levels were determined in different tissues (claw meat, hepatopancreas and inner meat). To highlight specific risk factors for cadmium, the concentration of cadmium was related to different exogenous (location, cooking and season) and physiological (size, sex, moulting stage, gonad maturation stage, condition) factors. The results confirmed previous findings of much higher cadmium levels in brown crab sampled in the North of Norway compared to the South. Cooking of crabs further led to higher concentrations in claw meat. The effect of season on cadmium levels was different in the North and South and no clear patterns could be identified, probably due to a high inter-individual variation in cadmium levels. Size showed a correlation with the total amount of cadmium for crabs in the North indicating an accumulation of cadmium over time; together with a slower growth, this may lead to the higher cadmium levels, observed in the crabs from Northern Norway.

The risk connected to cadmium exposure when consuming brown crab mainly depends on the consumption pattern, the parts of the crab consumed and the origin of the crab. Regardless of origin, the consumption of claw meat does not display a consumer health risk. However, the consumption of meals consisting of inner meat only and inner meat of brown crab from Northern Norway may pose a health risk.

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1. Introduction

The brown crab *Cancer pagurus* is a highly appreciated seafood in several European countries. In Europe, the total annual commercial catch is about 50,000 metric tons (FAO, 2017). The Norwegian annual catch volume has been stable at around 5000 metric tons since 2008 (Norwegian Directorate of Fisheries, 2017; Søvik et al., 2017). When assessing the risks the consumption of this seafood poses for human health, the trace element content of crabs is an important factor to consider as environmental contaminants like arsenic, cadmium (Cd), mercury and lead gave rise to concern due to their potential to cause severe human health disorders (Jaishankar et al., 2014).

The elemental composition of brown crab has previously mainly been investigated in brown crabs from the Scottish coast and the English Channel. Cadmium was identified as main risk factor when consuming brown crab, especially inner meat containing hepatopancreas (HP) (Barrento et al., 2009a,b; Maulvault et al., 2012; Noël et al., 2011). Renal and bone diseases are the most commonly observed symptoms of chronic cadmium toxicity (Järup and Åkesson, 2009). However, cadmium has also been identified as risk factor for diseases in other tissues and organ systems (Satarug et al., 2010). The European Food Safety Authority has established a tolerable weekly intake for Cd of 2.5 μg/kg bw (EFSA, 2009).

Measurements of Cd in brown crab caught along the Norwegian coast in 2011 revealed a geospatial pattern with significantly higher concentrations of Cd detected in crabs from the Salten region (ca. 67 N) and northwards (Julshamn et al., 2012). In cooked claw meat, concentrations of Cd were found to exceed the maximum level of 0.5 mg/kg wet weight set by EU (EU, 2006). The Norwegian food safety authority therefore advised not to consume crabs from the affected region. As a consequence, the commercial crab fishery and relevant processing industry suffered substantial economic losses (Jensen and Wasmuth, 2010). Additional surveys performed in subsequent years revealed that Cd levels similar to those observed in the Salten region also were detected further north, in the Vesterålen region (Julshamn et al., 2013) and the Troms region (Bakke et al., 2016). It is not yet clear yet what has caused this sudden spatial increase. However, several factors have been hypothesized to play a role in the uptake of Cd in crabs.

Earlier studies, sampling brown crabs in the field, investigated the influence of different exogenous factors like area, season and cooking on the Cd levels in different crab tissues (Barrento et al., 2009a,b; Maulvault et al., 2012; Wiech et al., 2017). While clear differences were found between areas and tissues in all these studies, the influence of season was not clear and the results for cooking were not consistent between the studies.

In shore crab *Carcinus maenas* different physiological factors including moulting stage (Bondgaard and Bjerregaard, 2005; Bondgaard et al., 2000; Nissen et al., 2005; Nørum et al., 2005), ovarian maturation (Bondgaard et al., 2000) and condition (water content) of the crab (Bjerregaard, 1991) were found to influence the accumulation of Cd and so did tissue hydration, crab size (Bjerregaard and Depledge, 2002) and sex (Knutsen et al., 2018).

In the present study we investigated the influence of exogeneous and physiological factors affecting Cd levels in brown crab along the Norwegian coast and provide data, which will allow for more differentiated food safety advice related to the consumption of brown crab in Norway. To investigate spatial and seasonal variation, we sampled brown crab throughout one year at one station in the North of Norway, Guvåg, known for high Cd levels, and one station in the South of Norway, Sotra, where levels are known to be lower. Hepatopancreas (HP) and claw meat of raw crabs and claw meat and inner meat of cooked crabs were analysed for Cd. Furthermore, the effects of the physiological factors size, sex, moulting stage, ovarian maturation stage and condition of the crab on Cd levels were examined. Lastly, the risk of consuming brown crab in the Norwegian population was assessed in different scenarios according to different consumption patterns and stratified Cd concentrations.

2. Material and methods

2.1. Biological material

A total of 545 brown crabs above the minimum size limit of 13 cm carapace width were sampled from two sites along the Norwegian coast. One in the North, Vesterålen (68.7 N, 15.0 E), known for high Cd values in the crabs, and one in the South, Sotra (60.2 N, 5.0 E) (Fig. 1). The intention was to catch crabs every second month from both locations covering one year. This was viable in the South with samplings in April, June, August, October, December 2015 and February 2016. In the North, however, it was despite considerable effort not feasible to get hold of a sufficient number of crabs in the months of April, May and June 2015. Instead, crabs in the North were caught in July, August, October, December 2015 and February 2016. Crabs were caught with baited pots at depths between 6 and 85 m, depending on season. At each sampling occasion, 30 crabs were dissected and processed freshly. In addition, between 18 and 20 crabs were cooked; boiled for 15 min in salted water (50 g NaCl/L). The latter method was included due to food safety considerations, since boiling is the most frequently used cooking method and has previously been shown to affect Cd concentrations in brown crabs (Wiech et al., 2017).

2.2. Physiological factors and tissue sampling

Freshly handled crabs were sacrificed according to best practice regulations (WHO/FAO, 2012) by piercing the two main nerve ganglia as described by Baker (1955).

For each crab, carapace width, whole wet weight, sex, number of missing legs, moulting stage, gonad maturation stage, visual meat filling, and weight of HP were recorded. In females, in addition, the presence of sperm plugs, spermathecae and external roe were recorded.

The moulting stage of the crabs was assigned to one of four stages: '1': early postmoult, '2': recent moult, '3': intermoult or '4': degraded, by examination of carapace hardness, levels of biofouling and visual indices according to Haig (2016).

For dissection, crabs were opened around the outside margin of the ventral carapace using a stainless steel knife. Liquid present in the carapace was allowed to run off for 5 s before the stomach was removed and remaining liquid allowed to run off for 15 s afterwards.

In fresh crabs, HP was sampled and weighed and gonad maturation stage was estimated using visual observations according to Ungfors (2007) and Haig et al. (2016). In female crabs, ovaries were classified as '1': immature (thin translucent gonad, white and pale) '2': undeveloped (lobes present, greyish pink) '3': developing (slight pink appearance, covering <50% of cavity), '4': ripe/mature (orange, red obvious ovaries. Covers >50% of cavity) or '5′: resting (whitish ovary with loose appearance, remnant eggs). The male testes were classified as '1': immature (testes small and transparent or undetectable), '2': developing (testes obvious and white) and '3': mature (Testes and vas deferens swollen and white).

In cooked crabs, the whole inner meat, except stomach, mainly consisting of HP, gonad and connective tissue was taken as sample.

Visually examined measurements were recorded by the same observer to minimize bias.

Samples of claw meat were dissected from both fresh and cooked crabs. Only claw meat from chela and merus were taken as sample, and all liquid present in chela and merus was included in the sample.

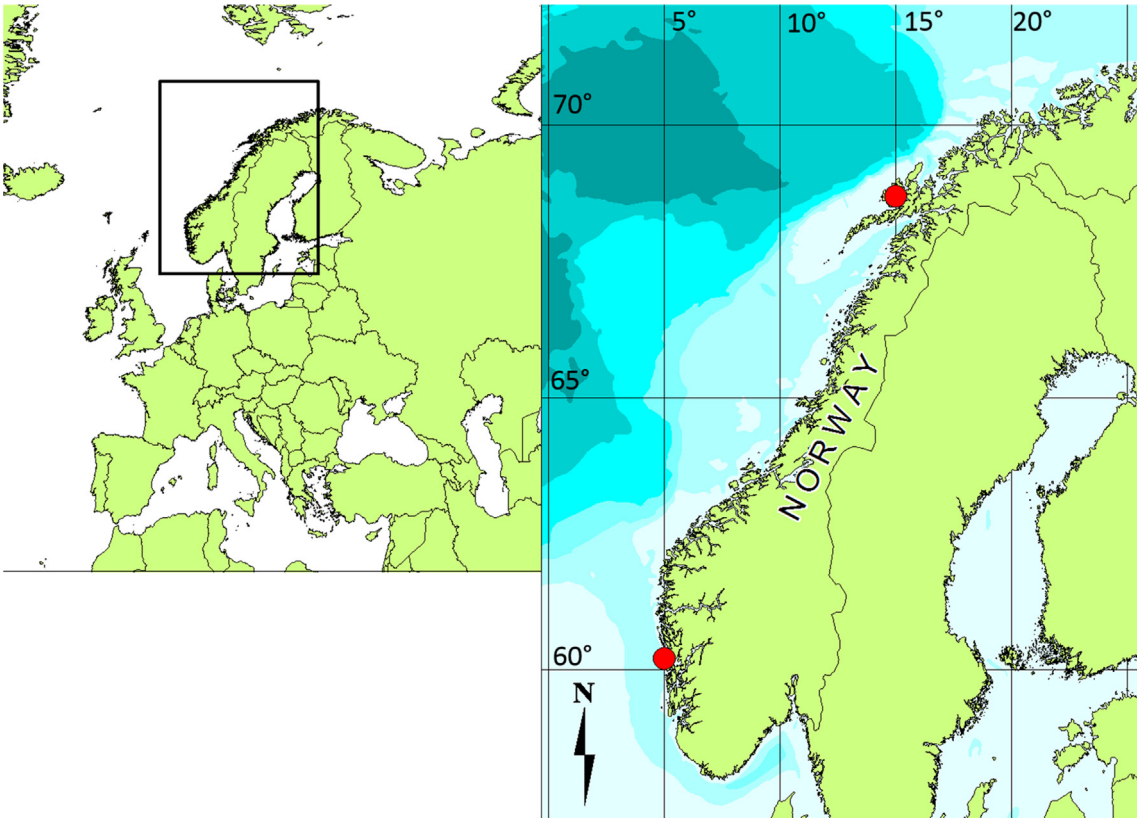

Fig. 1. Map of the sampling area. The two sampling locations "North": Vesterålen (68.7 N, 15.0 E) and "South": Sotra (60.2 N, 5.0 E) are marked with a red dot.

Claw meat samples from cooked crabs were excised just after cooling. Before excising the claw meat of the raw crabs, the claws were removed from the carapace and left for at least 24 h at room temperature or frozen and thawed. This was necessary, as the claw meat in fresh raw claw is firmly attached to the carapace, making it laborious and difficult to remove all present muscle.

The hepatosomatic index (HSI), was calculated as:

$$HSI = \frac{m_{HP}}{CW^2} \times 100 \quad (1)$$

where m_{HP} is the individual hepatopancreas dry weight and CW the carapace width.

All factors representing the condition of the crab, including dry matter content in HP and claw meat (Bjerregaard, 1991) and HSI were strongly correlated ($r > 0.6$, $p < 0.001$). Consequently, only HSI was used as condition factor for the statistical analysis.

2.3. Analytical determination

Hepatopancreas from all freshly dissected crabs and claw meat from 20 of these crabs were analyzed for trace elements. From boiled crabs, all inner meat and claw meat samples were analyzed. Before analysis, all samples were freeze-dried (Freezone 18 L by Labconco, Kansas, USA), the dry matter content determined and homogenized. The determination of Cd was performed using ICP-MS as described by Julshamn et al. (2007). In brief, acidified samples were digested in a microwave oven (Milstone-MLS-1200) and analyzed using ICP-MS (Agilent 7500c). The method is accredited by the Norwegian Accreditation Authority according to NS-EN 17025 and was controlled by use of standard reference material (CRM, Tort 2, National Research Council, Canada) and participation in proficiency tests. The limit of quantification (LOQ) for Cd was set to 0.005 mg/kg dry weight with standard sample size (0.2 g).

2.4. Data treatment

To detect significant differences in Cd levels or physiological stages between groups, one-way ANOVA was performed followed by Tukey's honest significant difference test. Pearson's linear correlation coefficient was calculated to analyze correlations between continuous variables. Variance component analysis (ANOVA with consecutive overall levels) was performed on the total amount of Cd and Cd concentrations in hepatopancreas of raw crab based on wet weight and dry weight. First, location was selected as random effect factor only to determine the variance explained by location. To analyze the variance explained by the factors shown by ANOVA or correlation to have an influence on Cd levels, month and the continuous factors HSI and width, divided into quintiles, were used as random factors and analyzed for each location separately. If homoscedasticity requirements were not fulfilled, data was log-transformed before analysis. The statistical significance threshold was set to $p < 0.05$. All statistical analyses were performed using STATISTICA 13 (©Statsoft, Tulsa, USA). Factor levels with $n < 5$ were not considered in the analysis. For the analysis of the influence of gonad maturation stage on Cd levels, eight female individuals had to be excluded because of difficulties to determine the gonad maturation stage.

As HP is the organ holding about 90% of the total Cd body burden in crab (Bjerregaard and Depledge, 2002), we focused on raw HP to investigate the seasonal variation and influence of the physiological factors on Cd levels in brown crab.

2.5. Risk assessment

To assess the risk of exceeding critical intake levels for Cd when consuming crab from northern or southern Norway the here measured Cd tissue concentrations were combined with consumption

data for crab in the Norwegian population. Different scenarios of Cd intake were compared with the tolerable weekly intake (TWI) for Cd of 2.5 μg/kg body weight set by EFSA (2009). In line with EFSA reports, a body weight of 70 kg was assumed (EFSA, 2012b) and brown crab was regarded the only source of Cd exposure. In addition, the risk also was assessed assuming that the exposure to Cd from brown crab comes in addition to the intake from other Cd contaminated foodstuff. Due to lack of Norwegian exposure data, for this scenario, the mean Cd exposure in adult Europeans of 1.77 μg/kg body weight (EFSA, 2012a) was subtracted from the total TWI and the exposure from Cd in brown crab was compared to the resulting TWI of 0.73 μg/kg body weight.

Cd concentrations measured in inner meat and claw meat of cooked crabs were considered separately and in combination, considering the consumption of whole crabs. In the latter case, it was assumed that the same amount of claw and inner meat was consumed (portion size of 260 g) with the concentration ($C_{whole\ crab}$) being calculated as:

$$C_{whole\ \ crab} = \frac{C_{claw\ \ meat}}{C_{inner\ \ meat}}/2 \quad (2)$$

To calculate exposure, consumption data for the Norwegian population was taken from two surveys on food frequency habits, which include data on brown crab (Bergsten, 2004; Meltzer et al., 2002). One survey targeted the overall population (Meltzer et al., 2002); the second focused on high consumers of fish and game, which already were identified in the first survey as people living at the coast (fish) and inland (game) (Bergsten, 2004). Both studies, assessed the consumption for whole crab and claw meat only separately. Here we also assumed the same consumption for inner meat only and whole crabs, respectively.

For consumption data, three different representative patterns were chosen: (i) Average consumption in the Norwegian population, (ii) average consumption for people living along the coast of Norway and (iii) the high consumption group (95th percentile) of people living along the coast. This corresponded to a weekly consumption of 35, 42 and 147 g meat of whole crab and 35, 35 and 147 g claw meat, respectively.

3. Results and discussion

3.1. Physiological characteristics

The main physiological characteristics of the crabs are shown in Table 1; crabs are grouped according to treatment (raw or cooked), location and sex.

Considering raw crabs, the mean carapace width of crabs in the different groups varied between 145 and 152 mm, with a mean weight between 466 and 596 g. The carapace widths were relatively similar within the different groups. However, raw crabs from the South were significantly wider (mean 152 mm) than those from the North (mean 145 mm). Furthermore, the crabs were significantly heavier in the South (mean of 545 g) than in the North (mean of 494 g).

Regarding sexes, male crabs were significantly heavier than the female crabs, while the average width was the same in both males and females. Males had larger claws than females, which was reflected in the measured claw meat weight of 34.4 g compared to females with 18.4 g ($p < 0.001$). In addition, HSI was significantly higher in males than in females. This could be due to the fact that in females a larger portion of the available energy is allocated to the gonads as they mature, and hence less energy will be available to be stored in HP.

Concerning all sampled crabs, 78% were intermoult crabs, while 19% were recent moult crabs and 4% early-postmoult. No clear seasonal trend was seen for the moulting stages at either location (Supplementary Fig. 1).

Condition measured as HSI increased with moulting stage ($p < 0.05$) in crabs from the North and South (Supplementary Fig. 2). In parallel, the dry matter content was lowest in early postmoult crabs and increased during intermoult until premoult. This has previously been found for shore crab (Nissen et al., 2005; Nørum et al., 2003) and most probably is the result of constant feeding and building up of tissue after ecdysis.

No statistically significant ($p > 0.05$) seasonal variation in HSI was seen at any location (Fig. 2). Mean HSI in crabs from the North, however, was lowest in July, the earliest point of time it was possible to catch crab after a period of several months. This indicates a seasonality in feed intake in the North, close to brown crabs' northern distribution limit. This seasonality is probably driven by low temperatures, too low for active foraging, during certain parts of the year and may explain why brown crab seem to grow more slowly in the North of Norway (Bakke et al., 2018).

The HSI of female crabs increased significantly with increasing gonad maturation stage from stage 2 to stage 4 (Supplementary Fig. 3). For males, there was only two maturation stages, and although mean HSI was higher in stage 3 than stage 2, the difference was not significant. The increase in HSI with increasing gonad maturation in females indicates pronounced foraging in the period of ovarian maturation. The gonad maturation stage determined for raw crabs, was 3 for 63% of the male crabs and 37% were in stage 2. For female crabs, <1% were in stage 1, 46% were in stage 2, 31% in

Table 1
Weight, carapace width, hepato-somatic index (HSI), dry matter of hepatopancreas (HP) and claw meat of male (m) and female (f) brown crab sampled at two different locations; "North": Vesterålen (68.7 N, 15.0 E) and "South": Sotra (60.2 N, 5.0 E). Mean ± SD is given. Different letters indicate significant differences ($p < 0.05$) within lines. A difference between sexes is indicated with an asterisks (*).

		Raw		Cooked	
	Sex	North	South	North	South
n	m	69	79	46	29
	f	85	100	56	84
Weight	m	530 ± 128^{a*}	596 ± 156^{b*}	537 ± 113	501 ± 145
[g]	f	466 ± 115^{a*}	503 ± 98^{b*}	516 ± 127	498 ± 107
Width	m	145 ± 10	152 ± 12	145 ± 8	145 ± 11
[mm]	f	146 ± 11	152 ± 10	149 ± 12	151 ± 12
HSI	m	0.58 ± 0.30*	0.63 ± 0.30*	0.62 ± 0.39^{a}	0.93 ± 0.54^{b}
[g/mm^2]	f	0.50 ± 0.20*	0.55 ± 0.25*	0.67 ± 0.53^{a}	0.80 ± 0.44^{b}
Dry matter HP	m	27.6 ± 9.7	29.0 ± 9.7*	24.2 ± 5.6^{a}	26.9 ± 6.8^{b}
[%]	f	25.2 ± 7.6	26.4 ± 8.8*	24.7 ± 4.6^{a}	28.7 ± 8.6^{b}
Dry matter claw	m	18.6 ± 6.0	18.8 ± 5.3^{a}	18.6 ± 5.3	23.1 ± 5.6^{b}
[%]	f	18.2 ± 4.1	19.0 ± 3.4^{a}	18.1 ± 4.6	23.4 ± 2.9^{b}

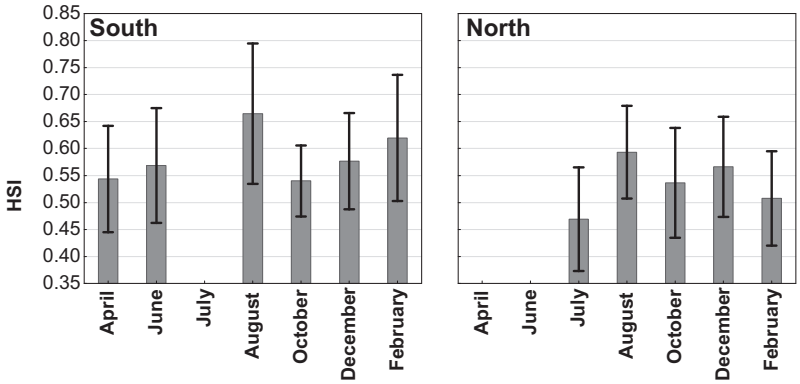

Fig. 2. Seasonal variation of the condition of the sampled brown crab shown as hepato-somatic-index (HSI) at both sampling locations; "North": Vesterålen (68.7 N, 15.0 E) and "South": Sotra (60.2 N, 5.0 E). Bars denote means and whiskers 95% confidence intervals.

stage 3, 18% in stage 4 and for 5% no exact gonad maturation stage could be determined.

Only 3% of the female crabs had an external sperm plug, whereas all had spermathecae and none were ovigerous.

3.2. Influence of the different factors on cadmium levels

3.2.1. Exogeneous parameters

Different concentrations of Cd based on wet weight and dry weight were found in the different tissues, both in cooked and raw crabs ($p < 0.05$) (Table 2). HP and cooked inner meat displayed much higher concentrations than claw meat (Table 2), which is in accordance with earlier findings (Barrento et al., 2009a; Barrento et al., 2009b; Maulvault et al., 2012; Maulvault et al., 2013). HP, as main digestive organ in crabs, is the main site of dietary nutrient and contaminant uptake. This leads to a high abundance of different trace metals including non-essential and potentially toxic trace metals. HP plays an important role in the detoxification of trace metals since it contains high levels of the protein metallothionein (MT), efficiently binding and detoxifying different trace element ions.

Cooking the crabs significantly increased the wet weight and dry weight based concentrations of Cd in claw meat ($p < 0.05$) and the concentrations of Cd (dry weight and wet weight based) were higher in raw HP than in cooked inner meat ($p < 0.05$) (Table 2). This effect of cooking in brown crab was demonstrated earlier (Wiech et al., 2017). The difference between Cd levels in HP and inner meat can partly be explained by the fact that the inner meat taken from cooked crabs also consisted of organs other than HP, which most likely contain lower Cd concentrations. As HP is the organ with the highest Cd concentration in crab (Bjerregaard and Depledge, 2002), an increased proportion of organs other than HP in inner meat will lead to a dilution and decrease of the Cd concentration. Furthermore, it has been shown that boiling crabs does result in a loss of Cd from HP, as it is mainly present in the soluble protein fraction and therefore labile (Wiech et al., 2017). A redistribution of Cd during cooking explains why cooked claw meat contained elevated Cd concentrations when compared to raw claw meat.

Crabs from the North of Norway showed much higher levels of Cd than crabs from the South both in claw meat and HP/inner meat in raw and cooked tissues ($p < 0.05$) (Table 2) and the factor location explained 40% of the total variance in the Cd concentrations (Table 3). Samples were taken at slightly different times of the year at the two locations. However, this was not affecting the overall pattern.

The elevated Cd concentrations in the North are in accordance with earlier findings of higher concentrations of Cd in crabs sampled north of 67°N on the Norwegian coast than in crabs sampled further south (Julshamn et al., 2012; Wiech et al., 2017). When comparing the measured concentrations of Cd in raw HP (Table 2) to findings from the Scottish Coast (SC) and English Channel (EC) with mean values of 26 and 15 (SC) and 15 mg/kg wet weight (EC) (Barrento et al., 2009a; Barrento et al., 2009b), our mean Cd concentrations in the North of 16 mg/kg ww, were in the same range, while the mean concentration in the South of 5.4 mg/kg wet weight, was a bit lower. This indicates that our crabs sampled in the South of Norway might actually have lower Cd concentrations than crabs caught elsewhere. However, previously reported values for raw crabs sampled in the same areas in Norway, also showed higher values than the ones measured in the present study, with mean concentrations of 38 ± 28 mg/kg wet weight and

Table 2
Cadmium concentrations on wet weight (ww) and dry weight (dw) basis in the different tissues hepatopancres (HP), muscle meat of claws (Claw) and inner meat of brown crab, analyzed raw or cooked, originating from two different locations; "North": Vesterålen (68.7 N, 15.0 E) and "South": Sotra (60.2 N, 5.0 E). Mean ± SD, median and the total range (Min-Max) is given. All the ww and dw based concentrations were statistically significantly different ($p < 0.05$) regarding tissue (HP/inner meat vs claw meat), treatment (raw vs cooked) and origin (North vs South).

	Raw				Cooked			
	HP		Claw		Inner meat		Claw	
	North	South	North	South	North	South	North	South
n	154	179	103	106	99	113	95	103
	Mean ± SD/Median/(Min-Max)							
Cd [mg/kg ww]	16 ± 16	5.4 ± 4.6	0.006 ± 0.002	0.003 ± 0.002	8.1 ± 8.1	2.5 ± 2.2	0.28 ± 0.49	0.028 ± 0.062
	12	4.0	0.004	0.003	5.8	1.8	0.15	0.008
	(0.75–90)	(0.15–30)	(0.001–0.036)	(0.0008–0.0094)	(0.67–59)	(0.23–13)	(0.008–3.7)	(0.0015–0.41)
Cd [mg/kg dw]	68 ± 67	23 ± 25	0.035 ± 0.040	0.016 ± 0.010	36 ± 40	9.9 ± 9.4	1.7 ± 3.3	0.13 ± 0.30
	49	16	0.024	0.014	24	6.5	0.78	0.032
	(2.8–480)	(0.65–240)	(0.008–0.23)	(0.005–0.082)	(3.1–260)	(0.63–51)	(0.029–23)	(0.0057–1.8)

Table 3
Results of variance components analyses with consecutive ANOVA. The effects of location, month, width and condition (measured as hepatosomatic index, HSI), on the variance of log-transformed total amount of Cd and Cd concentrations based on dry weight (dw) and wet weight (ww) in hepatopancreas. The components of variance are given as absolute values with percentage of the total variance in brackets. Significance is denoted with an asterisks (*).

	Components of variance mean squared type: 1		
Source	Log Cd dw	Log Cd ww	Log Tot Cd
Location	19.0* (39%)	17.4* (40%)	13.1* (33%)
	North		
Month	0.47* (5%)	0.35 (4%)	0.31 (2%)
Width	0.61* (8%)	0.43* (6%)	1.11* (18%)
HSI	0.78* (12%)	0.14 (0%)	0.26 (2%)
	South		
Month	0.39 (4%)	0.49* (8%)	0.33* (4%)
Width	0.21 (1%)	0.22 (2%)	0.16 (0%)
HSI	1.45* (21%)	0.16 (1%)	0.06 (0%)

8.4 ± 4.9 mg/kg wet weight in North and South, respectively (Wiech et al., 2017). This difference between studies might be due to high inter-individual variation in Cd concentrations in brown crab and also the lower number of samples in previous studies (about 20–25) compared to a sample size of 333 samples in the present dataset. Furthermore, physiological differences between the crabs in the different studies could also have led to the differences observed, as except from this study, crabs were purchased from local fish mongers, who are unlikely to sell crabs in very varying condition.

The mean concentrations of Cd in raw claw meat of 0.003 and 0.006 mg/kg wet weight in the South and the North, respectively (Table 2), were lower than the earlier reported mean values of 0.04 mg/kg wet weight (Maulvault et al., 2012), 0.02 mg/kg wet weight (Barrento et al., 2009b) and 0.015 mg/kg wet weight (Barrento et al., 2009a). In case of the highest value (Maulvault et al., 2012), it was not clearly stated if only claw meat was measured, as the term "muscle meat" was used. Muscle meat inside the cephalothorax, at the base of the appendages, is located close to HP, and could contain higher amounts of Cd than muscle meat from claws does. As the term "brown meat" is used without further specification of what it actually consisted of, it is difficult to compare the mean value of 8.3 mg/kg wet weight to the values for HP in the present study. Therefore, future work on element concentrations in crabs would profit from clear definition and consequent use of the terms HP, brown meat, inner meat, claw meat and muscle meat.

The seasonal pattern in Cd levels was not very distinct (Table 3, Fig. 3). Components of variance analysis showed that in the North, the factor "month" only had a significant effect on the dry weight based Cd concentration, and explained only 5% of the variance. Crabs from the North showed a trend to higher Cd concentrations in HP on dry weight basis in July and February (Fig. 3), where crabs collected in February had significantly higher concentrations ($p < 0.05$) than those collected in August. The concentrations on wet weight basis showed no significant differences ($p = 0.06$). Relatively high Cd concentrations were observed in the month with lowest condition (July, Fig. 2). This indicates that higher Cd levels early in the season were related to a poorer physiological condition, which in turn possibly is linked to lower water temperatures in the North.

For crabs from the South, components of variance analysis showed that "month" had a significant effect on Cd concentrations on a wet weight basis and total Cd content, explaining 8 and 4% of the variance, respectively (Table 3). Significantly higher wet weight based Cd concentrations in October compared to April, June and December ($p < 0.05$) were found (Fig. 3). The concentrations on dry weight basis showed no significant seasonal changes ($p = 0.054$). Although the mean dry weight concentration in October was highest, this difference was not significant. The seasonal variations in Cd concentration in the South did not correspond to any variation in condition.

Barrento et al. (2009b) found in brown crab harvested at the Scottish coast, purchased at a local fish monger, a similar pattern to our crabs from the South with low Cd concentrations in HP based on wet weight in April. However, Barrento and co-workers found the highest Cd concentration in summer. Their measurements also showed a large variation making it difficult to detect a consistent pattern. Maulvault et al. (2012) found no difference between crabs sampled in spring and summer along the Scottish coast. The Cd concentration at one location during the year might be influenced by many different environmental factors like salinity, temperature, chelating agents in water as well as physiological factors of the crab such as condition, moulting and gonad maturation. Furthermore, migration takes place, which in turn might influence the diet and exposure to environmental conditions. All these factors may differ with season and sampling location. This complexity

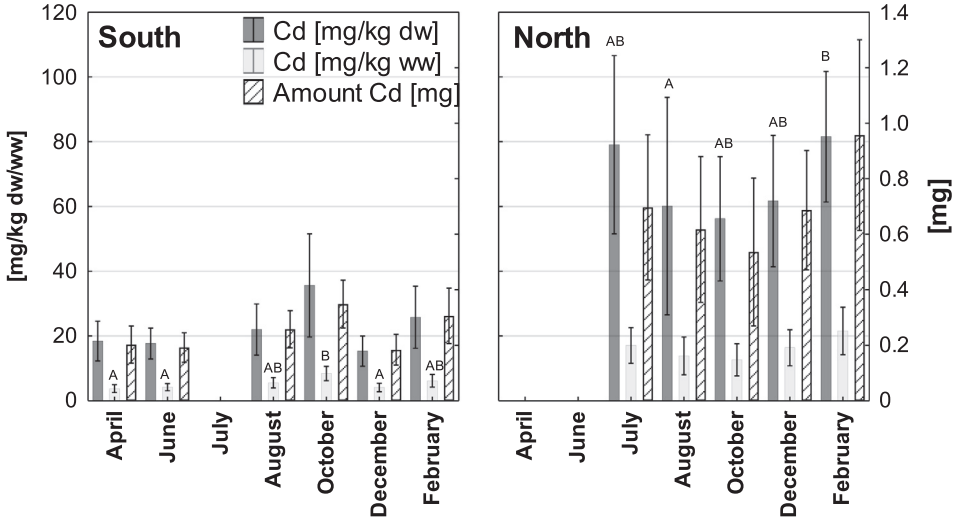

Fig. 3. Dry and wet weight based cadmium concentrations and total amount of Cd in the hepatopancreas of brown crabs sampled at the different months at two different location: "North": Vesterålen (68.7 N, 15.0 E) and "South": Sotra (60.2 N, 5.0 E). Bars denote means and whiskers 95% confidence intervals.

makes it difficult to draw clear conclusions from the observed patterns. In addition was it not possible to sample crabs at both locations at the same time which makes it more difficult to compare the patterns.

3.2.2. Physiological factors

Carapace width of brown crab sampled in the North of Norway was significantly correlated with Cd concentrations based on wet weight ($r = 0.22$, $p < 0.05$), dry weight ($r = 0.26$, $p < 0.05$) and the total amount of Cd ($r = 0.38$, $p < 0.05$) in HP, while there was no correlation for crabs caught in the South (ww: $p = 0.14$; dw: $p = 0.69$; total Cd: $p = 0.42$) (Supplementary Fig. 4). In another investigation on brown crab from the North and South of Norway, where brown crabs with a much larger size range were included, a similar pattern was found (Lindborg, 2017). Based on wet weight concentrations, no significant correlation with carapace width was found in crabs from the South and a rather weak correlation was detected in crabs from the North ($r = 0.24$) (Supplementary Fig. 4). However, based on the total amount of Cd, the correlation in the North was much stronger ($r = 0.61$) and also for the South the correlation was clear ($r = 0.55$). These findings suggest that the changes in dry matter and mass of HP can mask the correlation between size and Cd concentrations, even though the total amount of Cd increased with size. The lack of a correlation for crabs from the South in the present study, might be due to less variation in size and lower Cd values.

Furthermore, age can be assumed to be more relevant for trace metal accumulation over time than size and recent findings indicate that brown crab in the North of Norway has a lower moulting frequency than brown crab in the South (Bakke et al., 2018), suggesting a lower growth rate. This indicates that the size range of crabs sampled in the North represents a larger age range, making the detection of a correlation between size and Cd levels more likely.

A correlation between size and Cd levels has also been observed in shore crab *Carcinus maenas*. Bjerregaard et al (2002) compared small (body wet weight: 34 ± 7 g) and large (body wet weight: 108 ± 11 g) crabs, and found differences in HP for dry weight based Cd concentrations but not for wet weight based concentrations. In shore crab from Norwegian waters, the correlation with size was, as in brown crab, strongest for the total amount of cadmium (Knutsen et al., 2018).

Condition of the crab, measured as HSI, was negatively correlated with Cd dry weight and wet weight concentration both in crabs sampled in the North (dw: $r = -0.36$, $p < 0.05$; ww: $r = -0.25$, $p < 0.05$) and South (dw: $r = -0.43$, $p < 0.05$; ww: $r = -0.35$, $p < 0.05$) (Fig. 4), and Cd concentration in the North was highest in July and February when HSI was the lowest (Fig. 2, Fig. 3). In general, a better condition results from a higher food intake and therefore an increase in total amount of Cd can be expected with better condition, as crabs are very efficient in accumulating Cd from their food (Bjerregaard et al., 2005). However, dry matter content of HP, and in general the crab's condition, is low after ecdysis and early in the feeding season. Earlier accumulated Cd will be highly concentrated in the remaining dry matter of HP. The condition and dry mass of HP increases due to foraging, causing growth dilution, which is likely to be more important than recent accumulation of Cd in determining the Cd concentration.

As the total amount of Cd and Cd concentration on wet weight basis were not correlated to condition, the recent accumulation of Cd might be proportional to HP tissue growth.

There were no statistically significant differences in Cd levels in HP between males and females in either of the two locations (Supplementary Fig. 5). Differences in migratory habits (Hunter et al., 2013; Ungfors et al., 2007) and sexual dimorphism with bigger claws in males, connected to allometric growth (Öndes et al., 2017), could be expected to cause differences in feeding habits and thereby Cd levels. However, also earlier studies found no difference between males and females in HP Cd levels in brown crab (Barrento et al., 2009a) and burrowing crab *Neohelice granulate* (Beltrame et al., 2010). Only in the shore crab *Carcinus maenas*, sex differences have been observed earlier (Knutsen et al., 2018, Bjerregaard et al., 2005). In brown crab, for muscle meat and gills, higher concentrations were found in females (Barrento et al., 2009a). We also found higher Cd concentration in claw meat of female than in male crabs while the total Cd content was the same (Supplementary Fig. 5). Male brown crab generally has larger claws than the females. Faster growth with the same Cd intake would lead to enhanced growth dilution of Cd in male claws compared to female claws.

The influence of gonad maturation stage was not pronounced and only male crabs from the North had a lower total amount of Cd in HP in gonad maturation stage 2 (developing) compared to stage 3 (mature) ($p < 0.05$) (Supplementary Fig. 6). In females, sampled in the North, there was a tendency towards lower Cd in the

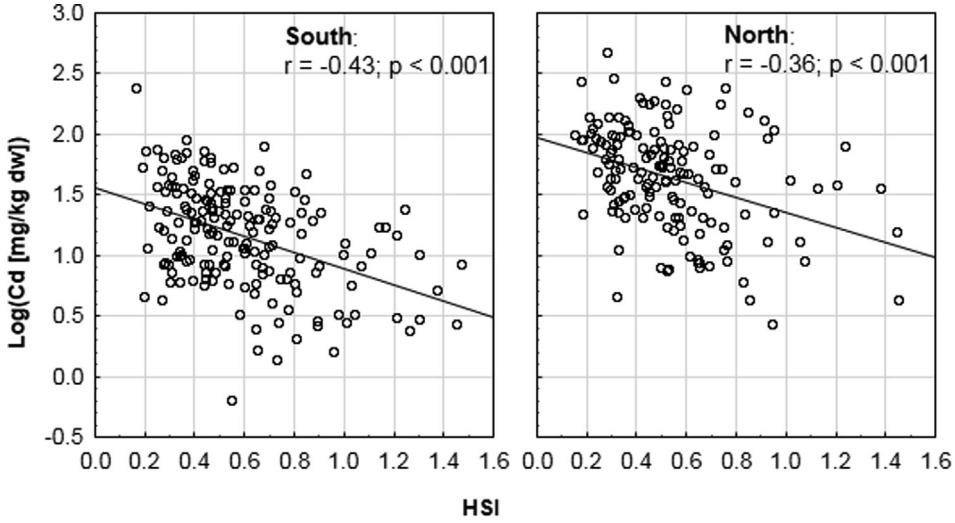

Fig. 4. Cadmium concentrations in the hepatopancreas versus hepatosomatic index (HSI) of brown crabs at two different locations: "North": Vesterålen (68.7 N, 15.0 E) and "South": Sotra (60.2 N, 5.0 E). Log transformed cadmium values are shown.

most mature stages. High variation and low number of samples for some stages may have affected these findings. Literature on the influence of gonad maturation on the uptake of Cd is scarce. Bondgaard et al. (2000) investigated the uptake of Cd in female shore crabs at different gonad maturation stages by exposing them to 1 mg Cd/L seawater. They found a decrease of the uptake of Cd in HP, gills, hemolymph and gonads measured as concentration in crabs with increasing gonad maturation stage. The Cd uptake measured as total content decreased in hemolymph and HP.

No statistically significant difference was found in Cd concentrations or total Cd content in HP between different moulting stages of brown crab sampled in the North (Supplementary Fig. 7). In the South, in males, only dry weight based Cd concentrations in recent moult crabs were higher than in intermoult crabs ($p < 0.05$). In females the moulting stage did not influence the Cd concentrations or amount. This is in contrast to earlier findings in shore crab, where the influence of moulting was more pronounced. Nissen et al. (2005) found higher dry weight based Cd concentrations in HP of intermoult compared to late premoult crabs. Furthermore, the Cd accumulation in hemolymph was increased in late premoult crabs compared to early postmoult and intermoult (Bondgaard and Bjerregaard, 2005). The hemocyanin concentration in hemolymph behaved correspondingly (Nørum et al., 2003). It was discussed that Cd in hemolymph is bound to hemocyanin and that a high abundance of hemocyanin in hemolymph will decrease the amount of free Cd. This in turn might regulate the uptake in the organs of the crab and especially in HP, meaning that early postmoult crabs with a low protein concentration in hemolymph could have a high uptake of Cd in HP (Bondgaard et al., 2005). The uptake in muscle actually followed the predicted pattern, while it was not observed in HP, where it should be pronounced because of the high Cd affinity. One reason why the Cd concentration in HP did not follow the predicted pattern might be that the volume of hemolymph is increasing in parallel to the increase of concentration of hemocyanin (Nørum et al., 2003) and this dilution not necessarily leads to a reduction of the total amount of hemocyanin and thereby capacity to bind Cd.

An explanation as to why the reported findings of effects of gonad maturation and moulting on the uptake and accumulation of Cd in crab, measured in laboratory studies (Bondgaard et al., 2005; Nørum et al., 2005), were not reflected in the Cd levels measured in crabs sampled in the present study, might be the unknown history of Cd accumulation in crabs from the sea. A crab sampled at a specific moulting stage, also accumulated Cd in previous moulting stages and cycles. Therefore the measured amount of Cd is actually the sum of the uptake in all previous moulting stages and consequently all the undergone moulting cycles. In a laboratory study with high exposure concentrations, only the Cd uptake at a given stage is measured, neglecting earlier accumulation.

The influence of the physiological factors on the Cd levels measured in the present study was rather weak and a maximum of 25% of the total variance within one location could be explained. Also in comparison to findings reported in earlier studies, the explanatory power of the factors assessed in the present work was rather low. However, in most of the earlier studies conducted, aqueous uptake was investigated and it might be questioned how relevant this approach is for the overall accumulation measured in a field study. A laboratory study on brown crab comparing the uptake of Cd from food and water in brown crabs from Norway concluded that dietary uptake will contribute most to the total uptake (Wiech et al., 2018).

Furthermore, Cd levels in brown crab show a high inter-individual variation (Fig. 3), which can potentially mask effects and the marked inter-individual variation may have many causes. Considering the efficient Cd uptake from food, factors linked to foraging may play an important role, such as large variation of Cd in prey items (Ness, 2014) and opportunistic feeding (Woll, 1995).

In a field study, it is difficult to ensure that the same population is sampled all the time due to migration. Ovigerous crabs are known to avoid pots (Howard, 1982) and it is assumed that also early postmoult crabs are avoiding traps.

Our findings underline the importance of investigating trace metal concentrations both, based on dry weight and wet weight as well as the total amount of trace metals when physiological effects on levels of a trace metal is studied, as already suggested by other authors (Bjerregaard and Depledge, 2002; Nissen et al., 2005; Nørum et al., 2003). In crab, especially HP undergoes tremendous changes in dry matter content during the moulting cycle and also dry weight and wet weight based mass will change, potentially masking changes based on wet weight concentrations, as was seen for condition in the present study.

3.3. Cadmium levels in the North and South of the Norwegian coast

The results from this study confirm previous findings of much higher levels of Cd in brown crab from the North compared to the South of Norway (Julshamn et al., 2012; Wiech et al., 2017). Some of our findings in the present paper support explanatory features for these findings:

The correlation between Cd levels and size suggest an accumulation of Cd over time. Combined with a lower moulting frequency in the North (Bakke et al., 2018), indicating a slower growth, this might contribute to higher Cd levels observed in crabs sampled in the North. A slower growth in the North would lead to crabs at the same size being older, which would give the crab more time to accumulate Cd resulting in higher values at the same size compared to the South.

As indicated earlier, the uptake from food and foraging factors may be more important than physiological factors investigated in the present study. Cadmium levels found in crab might rather reflect Cd levels accumulated from different prey items, than the influence of a certain physiological stage of the crab. Which prey items are consumed might further be connected to migratory patterns. In the present study, it was not possible to catch a sufficiently high number of crab in the North of Norway during spring at fishing spots used during autumn, the main fishing season. This means, crabs might be migrating to other areas, where they were not available for the here used gear. Crabs may be dwelling below 140 m where water temperatures would be highest at that time of the year or, they may not actively be moving and foraging due to low temperatures and therefore not enter the traps, as demonstrated for ovigerous crabs (Howard, 1982). In the South, similar spots in shallow water down to 50 m delivered good catches all year round. This indicates differences in the migratory pattern between crabs from North and South. As brown crab is known as an opportunistic feeder, an inhabitation in different habitats will lead to an ingestion of different prey items (Woll, 1995) and hence, different amounts of Cd. Moreover, deep-sea water is known to be rich in Cd (Janssen et al., 2014), which might be reflected in higher values in prey consumed in the deep areas.

Site specific differences in Cd concentrations in brown crab have also been reported earlier around the UK (Barrento et al., 2009a; Davies et al., 1981; Falconer et al., 1986). However, in an investigation of the shore crab Carcinus along the Norwegian coast, no clear geo-spatial pattern was found (Knutsen et al., 2018). Cadmium has been shown to be a rather mobile element and accessibility from sediment and bioaccumulation increases with contamination (Amiard et al., 2007) and physicochemical properties of the sediment (Signa et al., 2017a), especially in benthic food webs (Signa et al., 2017b). In accordance, earlier studies have been investigating the run-off of cadmium from bedrock in ground

water and surface water (Finne, 2013) as well as anthropogenic contamination in sediment (Falk, 2012) in the region with high concentrations of cadmium in the crab. However, they could not find any correlation between run-off and sediment contamination and high values of Cd in crab.

Spatial variation was also found in other decapod species from the North-East Atlantic with higher values in the North compared to further south (Zauke and Schmalenbach, 2006; Zauke et al., 1996). Zauke and Schmalenbach (2006) found increasing Cd levels from south to north in the Barents Sea in two different shrimp species. They hypothesized that the higher Cd levels in crustaceans from polar regions could be connected to Cu deficiency and the insufficient selectivity of the uptake process, possibly resulting in a higher uptake of Cd.

3.4. Risk assessment

To assess the potential of Cd exposure for adult Norwegian consumers of brown crab, measured Cd concentrations in the inner and claw meat of cooked brown crab were combined with publicly available consumer data in Norway.

The consumption of crab claw meat from both areas were estimated to not lead to an exposure of Cd above the recommended TWI, regardless of consumption pattern and consideration of Cd exposure from other foodstuff, respectively (Table 4). Claw meat from brown crab can therefore be considered a safe food in terms Cd contamination, which is in accordance to earlier findings (Maulvault et al., 2013; Wiech et al., 2017).

Considering brown crab as the only source of Cd, an average adult Norwegian consumer would exceed the TWI if exclusively the inner meat of crabs from Northern Norway was consumed at the same frequency as whole crabs. People living along the coast, having a higher than average consumption of both whole crabs and only inner meat from crabs from North, would be exceeding the TWI. For high coastal consumers, eating whole brown crabs or the inner meat, would lead to a high Cd exposure exceeding the TWI, regardless of the origin of the crabs. Considering the contribution from other foodstuff, also average adult Norwegian consumers would exceed the TWI consuming whole crabs from the North and coastal adult consumers when consuming whole crabs from North and South of Norway. Furthermore, consumption of inner meat solely presents a risk for all adult consumers regardless of where the crab is caught. The consumption of the inner meat of brown crab should therefore be limited. Furthermore, whole crabs from the North should be consumed occasionally only. This finding is in accordance with earlier studies assessing the risk from Cd in brown crab in the Portuguese (Maulvault et al., 2013) and Norwegian population (VKM, 2015).

When investigating the other factors influencing the concentration of Cd in the crab, we identified only the geographical origin of the crab as crucial, which is reflected in the risk assessment. Considering the consumption in the average Norwegian population, the TWI would only be exceeded if whole crabs or only inner meat of crabs from the North would be eaten; a scenario which is not very likely. While only a minor fraction of the crabs sold on the Norwegian market is caught in the North (Søvik et al., 2017), the risk of high Cd exposure can be evaluated as moderate on the average coastal consumer level. However, it was shown that many of the coastal consumers actually obtain their seafood by fishing themselves (VKM, 2015). This puts especially consumers at high risk living close to the coast in the region known for high concentrations of Cd in crab.

Due to lack of exposure data, other susceptible sub-groups including children, adolescent and young adults were not assessed, although they might be at elevated risk due to their low body weight.

On average, a person with a body weight of 70 kg could consume weekly a portion of 40 g of crab meat of a whole cooked brown crab from the South and 12 g from a crab from the North before exceeding the TWI taking into account the average contribution from other food.

4. Conclusion

In accordance with earlier findings, the Cd levels were much higher in brown crabs sampled in the North of Norway compared to in the South, and cooking resulted in increased Cd concentrations in claw meat and reduced Cd concentrations in cooked inner meat compared to raw hepatopancreas. The data showed further that season and the investigated physiological factors only had a limited influence on Cd levels, and the variation within and between locations was difficult to explain only by considering physiological factors. This may be due to the large inter-individual variation in field sampled crab, where physiological factors and past and present Cd exposure could not be controlled. A correlation between crab size and amount of Cd in hepatopancreas suggested an accumulation of Cd over time, potentially explaining the difference between areas to some extent, as crabs have been shown to have a reduced growth rate in the North compared to further South. At both sampling sites condition was negatively correlated with dry weight based Cd concentrations probably due to growth dilution. Moulting and gonad maturation stage were not correlated with Cd levels. Other factors, such as variation in foraging due to different migration patterns or possibly other geographically varying factors not considered in this study may be more important in forming Cd levels in brown crab and explain the dif-

Table 4
Calculated exposure of Cd in the Norwegian population considering different consumption patterns and concentration in brown crab along the Norwegian coast, according to spatial origin (North/South) and consumed tissue. Red indicates an exceedance of the tolerable weekly intake (TWI) of 2.5 μg Cd/kg body weight from the consumption of brown crab only. Yellow indicates an exceedance of the TWI of 0.73 μg Cd/kg body weight, considering the mean contribution of Cd from other foodstuff in the European population. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Cd [μg/kg bw/week]	**Whole crab**		**Claw meat**		**Inner meat**	
	North	South	North	South	North	South
Avgerage consumption	2.15	0.62	0.14	0.01	4.06	1.25
Coastal consumption	2.58	0.75	0.14	0.01	4.88	1.50
High coastal consumption	9.03	2.61	0.60	0.06	17.07	5.26

ferences between different areas better, which should be taken into account in future studies.

The consumption of claw meat does not display a food safety issue, while meals consisting of inner meat only and whole crabs from Northern Norway may put consumers at risk of exceeding the TWI for Cd.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.134922.

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