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Effects of cooking and freezing practices on the distribution of cadmium in different tissues of the brown crab (*Cancer pagurus*)

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ABSTRACT

Increased cadmium concentrations in claw meat were demonstrated after cooking and freezing practices of whole brown crabs. This was investigated in crabs from two different locations along the Norwegian coast, one with normal and one with high cadmium concentration. For both locations, in whole crabs, samples of fresh raw claw meat showed lowest values followed by raw-boiled and frozen-thawed-boiled. Cadmium levels in separately cooked claws were comparable to the low values in claws from raw whole crab. Claws taken from frozen crabs before thawing had low values compared to claws taken off the carapace after thawing. This clearly indicates a transfer from hepatopancreas to claw meat, which potentially induces biases when measuring and monitoring Cd levels in crabs. Further, different cooking and storing practices might have profound effects on cadmium intake from eating crabs since concentrations above regulatory limits were found following common household and commercial practices.

1. Introduction

The edible crab *Cancer pagurus* is an appreciated food item in different countries and its fishery is of significant economic value in different European countries. In the English channel, the catch of crustaceans steadily increases, partly replacing the catch of large finfish (Molfese, Beare, & Hall-Spencer, 2014) which shows the future importance of the crab fishery. In Norway, the annual catch of brown crab tripled within a decade (1993–2003) and has been stable around 5–6000 tons ever since (Norwegian Directorate of Fisheries, 2016). White crab meat from claws and legs is a well-balanced nutritious food, rich in proteins, amino acids and essential elements with a low cholesterol and fat content (Barrento et al., 2009b; Barrento et al., 2009c). However, findings of high values of cadmium above the legal limit of 0.5 mg/kg ww set by the European Commission (EU, 2006) in claw meat (Julshamn, Nilsen, Valdersnes, & Frantzen, 2012), and even higher values in the commonly consumed hepatopancreas (HP) (Maulvault, Cardoso, Nunes, & Marques, 2013), have raised concerns about food safety (Maulvault et al., 2012a; Noël et al., 2011). High values of cadmium have been found in claws and HP in crabs harvested in Norway (Julshamn et al., 2012), Scotland (Davies et al., 1981; Maulvault et al., 2012a) and the English channel (Barrento et al., 2009a). Measurements along the Norwegian coast have shown a clear pattern with higher values of cadmium in brown crab meat in the north of Norway (Julshamn et al., 2012) which eventually led to a breakdown of the crab fishery in the Salten region. However, crabs are also caught north of this area and commercially processed to different products. In food processing, crabs are either steamed as whole, or claws are taken off and the different body parts processed separately. The traditional and most frequently applied method for preparing crabs in private homes in Portugal is boiling crabs as a whole (Maulvault et al., 2013), which also applies to other European countries.

The toxicity of cadmium towards humans is well known with

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renal and bone diseases as most common symptoms (Järup & Åkesson, 2009). Further evidence also implicates cadmium as a risk factor for diseases in other tissues and organ systems, and also at low concentrations (Satarug, Garrett, Sens, & Sens, 2010). The European food safety authority EFSA has determined a tolerable weekly intake (TWI) for Cd of 2.5 μg/kg body weight as a long term intake limit to avoid harmful effects (EFSA, 2009). At present, the intake of Cd by the average European is already close to the TWI without considering consumption of crabs (EFSA, 2012).

Most studies focusing on toxic elements in crabs as well as the current legal limits consider raw tissues, even though they are almost exclusively consumed cooked. The cadmium content varies substantially between different organs in many consumed crab species, with much higher concentrations in HP than in muscle (Davies et al., 1981; Noël et al., 2011; Nørum, Bondgaard, Pedersen, & Bjerregaard, 2005; Rouleau, Gobeil, & Tjälve, 2001). Mobility of Cd under different cooking processes has been shown in fish (Atta, El-Sebaie, Noaman, & Kassab, 1997; Ersoy, Yanar, Küçükgülmez, & Çelik, 2006), mussels (Houlbrèque et al., 2011; Metian et al., 2009) and crustaceans (Abd-Allah & Abdallah, 2006; Jorhem, Engman, Sundström, & Thim, 1994). Although located in different body parts of the crab, there is limited physical barrier between the HP, located in the carapace and the claw meat. Thus, there is a risk in the edible crab that cadmium from HP may contaminate claw meat and other tissues while cooking. Contamination of claw meat by Cd during cooking may impose an increased risk for reaching the legal limit and thereby a critical intake level of Cd. So far, only one study looked at the effect of cooking on the Cd concentration in crab claw meat (Maulvault et al., 2012a) and no difference between raw and cooked crabs was found. However, the brown meat cadmium level was relatively low (mean 5.6–8.4 mg/kg wet weight (ww)) compared to crabs from Northern Norway (mean 16–18 mg/kg ww) (Julshamn et al., 2012). Further, the process of freezing also needs to be considered as it has been shown to influence the cadmium level in saucer scallop (Francesconi, Moore, & Joll, 1993) and sunfish samples (Ney & Martin, 1985). Studies focusing on the natural level of Cd in crabs and processes underlying the accumulation of cadmium, have not always contemplated the effect of sample treatments like freezing. If a transfer occurs during sample treatment, it could have a significant effect on analytical results and thereby study outcomes.

This study was conducted to assess the influence of different cooking and sample preparation methods on the level of cadmium measured in the claw meat of crabs. The aim was to assess 1) the difference in Cd concentrations in HP and claw meat between raw, whole boiled and thawed and whole boiled crabs from geographical areas with high and normal Cd levels, 2) the effect on Cd concentrations in separately boiled or steamed claws and 3) the effect of freezing and thawing on Cd concentrations.

2. Material and methods

2.1. Biological material

Three experiments were performed to elucidate the effects of different cooking and sampling methods. In experiment 1, 60 female crabs were sampled in Vesterålen, Northern Norway (68.7 N, 15.1 E) 05 Nov 2013, where earlier investigations have shown that crabs contain elevated levels of cadmium, and around Hitra, Southern Norway (63.5 N, 9.2 E) 01 Dec 2013. For experiments 2 and 3, 18 crabs were sampled 18 Nov 2014 and 12 crabs were sampled 19–22 Sep 2015, respectively, in Vesterålen, Northern Norway (68.7 N, 15.1 E). Crabs were captured using baited crab pots.

2.2. Experiment 1: effect of freezing and cooking crabs

In each treatment of experiment 1, we used fifteen crabs. Samples of claw meat and brown meat were taken from each crab. Brown meat consisted solely of HP and gonad at different maturation states. In treatment 1 ('raw'), samples were taken from raw, fresh crabs. Before samples were taken, crabs were euthanized by sticking according to best practice regulations Codex Alimentarius (WHO/FAO, 2012). Gill samples were also taken. In treatment 2 ('boiled fresh'), samples were taken after boiling the whole crabs for 15–25 min in 8 L of salted water (50 g NaCl/L). In treatment 3 ('thawed, boiled sw') and treatment 4 ('thawed, boiled fw'), whole crabs were frozen and thawed before being boiled in salted water (50 g NaCl/L) or fresh water respectively. In treatments 2 to 4, several crabs were cooked together in one pot and the water was renewed after each cooking.

2.3. Experiment 2: effect of cooking claws separately

In experiment 2, we investigated, whether the exoskeleton could serve as a source of cadmium, and used frozen crab carapaces and claws cooled on ice. The experiment consisted of two trials. In trial A ($n = 10$), claw meat of one of the two claws from each crab was analyzed raw ('raw A') and the second claw was boiled ('boiled sw A') separately in salted water (50 g NaCl/L) before sampling. The boiling water was renewed after each cooking to avoid contamination. We took HP samples from the thawed carapace ('defrosted, raw A'). In trial B ($n = 8$), one claw was boiled ('boiled sw B') for 20 min in salted water (50 g NaCl/L) and the remaining claw was steamed ('steamed sw B') for 15 min over boiling salted water (50 g NaCl/L). The complete inner meat, consisting of HP, gonad and connective tissue (excluding stomach), of the thawed carapace was taken as raw sample ('defrosted, raw B'), as this was done in earlier studies on Cd levels in crabs in Norway (Julshamn et al., 2012). In addition, we took samples of the exoskeleton from all claws after the respective treatments.

2.4. Experiment 3: effect of freezing and thawing

In experiment 3, the aim was to determine if leakage of cadmium occurs during freezing and thawing. Whole crabs ($n = 12$) were received frozen. One claw was removed from the carapace while still frozen and thawed separately ('claw taken frozen') ($n = 12$), while the second claw was removed after the crab had thawed ('claw taken thawed') ($n = 8$). HP was taken after thawing ('defrosted raw'). Samples from claw meat and HP were analyzed.

A schematic illustration of the different treatments in the experiments is given in Fig. 1. In all three experiments, all liquid was collected when dissecting the raw claw meat, and the carapace was emptied of liquid before samples of HP or brown meat were taken. To prevent contamination, all used stainless steel dissection instruments were cleaned between each sample and samples were directly transferred to sampling tubes, avoiding contact with other external surfaces.

2.5. Chemical analyses

After freeze-drying and homogenization of the samples, the analysis of cadmium was performed using ICP-MS as described by Julshamn et al. (2007). In brief, we used ICP-MS (Agilent 7500c) after digestion of samples in a microwave oven (Milstone-MLS-1200). 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

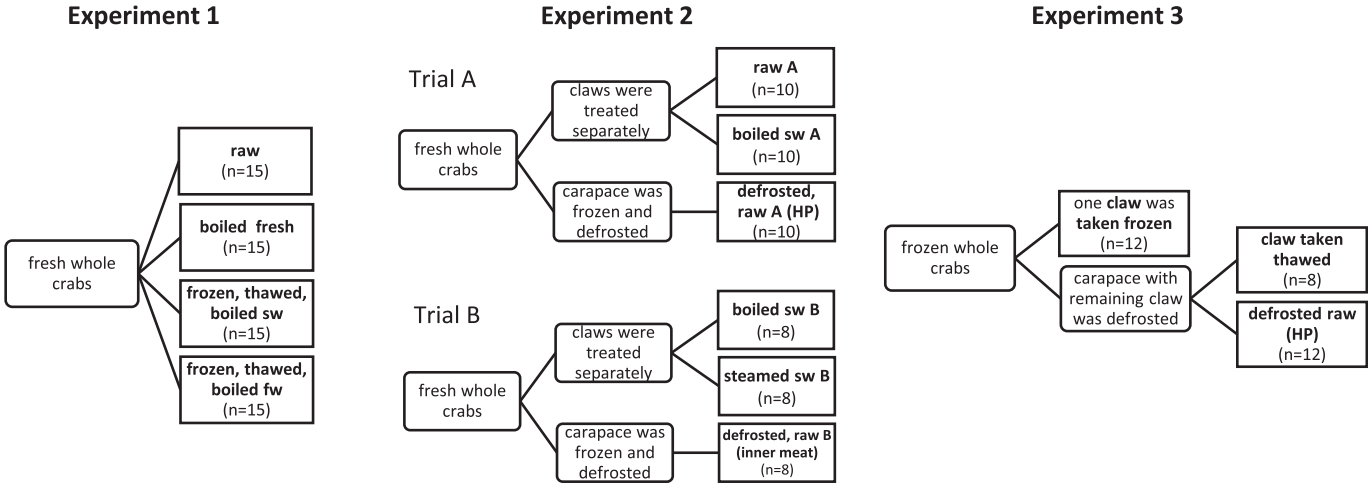

Fig. 1. Schematic illustration of the cooking and freezing practices applied in the different experiments.

Council, Canada). The LOQ_{dw} was set to 0.005 mg/kg dw with standard sample size (0.2 g). The wet weight based LOQ for each individual samples (LOQ_{ww}) was determined as: $LOQ_{ww} = LOQ_{dw}$ x % dry $matter_{sample}/100$.

2.6. Data treatment

In a pilot study it was found that the Cd concentration in gonads represented less than 1% of the Cd concentration in brown meat of raw crabs (0.031 ± 0.032 mg/kg ww (mean ± SD)). Thus, for experiment 1, Cd concentration in HP was calculated using the ratio of dry weight of brown meat neglecting the Cd concentration in gonad:

$$Cd_{HP} = Cd_{brown\ meat} \times \text{dry weight}_{brown\ meat}/\text{dry weight}_{HP}$$

With Cd_{HP} being the calculated concentration of Cd in HP and $Cd_{brown\ meat}$ being the actual measured concentration of Cd in brown meat consisting of HP and gonad. The same was assumed for boiled and thawed and boiled crabs.

Differences between the Cd concentration in the different treatments were tested with ANOVA and whenever necessary followed by multiple comparison testing (Tukey HSD). The significance level was 0.05. If homoscedasticity requirements were not fulfilled, data was log-transformed before use. For experiment 1, Pearson's linear correlation coefficient was calculated for Cd in HP and Cd in claws. All statistical analyses were performed with STATISTICA 12 (©Statsoft, Tulsa, USA).

3. Results

The results from all three experiments are presented in Table 1.

A clear difference in Cd concentrations between crabs from Northern and Southern Norway was found for both, claw and brown meat, with higher concentrations in crabs from Northern Norway. This is in line with the results from the national monitoring program (Julshamn et al., 2012).

3.1. Experiment 1: effect of freezing and cooking crabs

The different treatments in experiment 1 led to significantly different Cd values in claw meat. In crabs from Northern Norway, claw meat from raw crabs was lowest with 0.024 mg/kg ww followed by boiled crabs with 0.30 mg/kg ww and highest in crabs thawed and boiled in either freshwater (0.84 mg/kg ww) or salted water (1.0 mg/kg ww). The difference between boiling crabs in fresh or salted water was not significant. The same pattern was found for crabs from Southern Norway with 0.007 mg/kg ww for raw, 0.065 mg/kg ww for boiled and 0.16 mg/kg ww and 0.10 mg/kg ww for crabs thawed and boiled in freshwater and salted water respectively.

In crabs from Northern Norway, the different treatments caused no statistically significant difference in the Cd concentration in the HP. However, there was a trend with lower concentrations in HP of crabs that were thawed and boiled than in HP of raw crabs. In crabs from Southern Norway, the concentration in HP in freshly boiled crabs was significantly higher than in thawed and boiled crabs in freshwater. Otherwise, no clear trend was seen.

For both locations, the total Cd content in brown meat (consisting of both gonads and HP) showed higher values in raw crabs compared to thawed and boiled crabs. In crabs from Northern Norway, boiling of fresh crabs also resulted in lower values than in raw crabs. Gills showed a significantly higher Cd concentration in crabs form Northern Norway with 0.54 ± 0.14 mg/kg ww (mean ± SD) compared to Southern Norway with 0.36 ± 0.09 mg/kg ww (mean ± SD).

In the crabs from Northern Norway, with generally higher Cd concentrations in HP, strong correlations between the concentration of Cd in HP and claw meat were found in all treatments (coefficients of correlation between 0.65 and 0.95, Table 1). In crabs from Southern Norway, only the correlation between the Cd concentrations in HP and claw meat from raw crabs was statistically significant ($r = 0.70$).

3.2. Experiment 2: effect of cooking claws separately

Meat from claws, which were cooked separately from the carapace in trial A and B, showed no elevated Cd concentrations compared to raw claw meat with values between 0.020 and 0.027 mg/kg ww.

The Cd concentration in HP in thawed crabs was significantly higher than the concentration in the whole inner meat consisting of HP, gonads and other edible tissues, excluding the stomach.

We found very low Cd concentrations in the exoskeleton of the separately cooked claws with only three values above LOQ (0.04 mg/kg ww) and a maximum of 0.013 mg/kg ww.

3.3. Experiment 3: effect of freezing and thawing

In frozen claws thawed separately from the carapace, the

Table 1
Weight, carapace width (CW) and measured cadmium concentrations in the different tissues of the brown crabs and the correlation between HP and claw meat concentrations. Different letters indicate statistically significant differences within each experiment and column.

Treatment	Weight [g]	CW [cm]	Claw Cd [mg/kg ww]	HP Cd [mg/kg ww]	Total brown meat Cd [mg]	Correlation between HP Cd and Claw Cd	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	r	p
Experiment 1							
Northern Norway							
Raw	589 ± 76 ac	15.6 ± 0.7 ac	0.024 ± 0.012 a	38 ± 28 a	1.15 ± 0.76 a	0.72	<0.01
Boiled fresh	473 ± 51 b	14.4 ± 0.5 b	0.30 ± 0.29 b	44 ± 42 a	0.45 ± 0.26 b	0.95	<0.01
Thawed, boiled sw	553 ± 68 c	15.1 ± 0.7 ac	0.84 ± 0.66 c	22 ± 16 a	0.41 ± 0.28 b	0.95	<0.01
Thawed, boiled fw	592 ± 104 c	15.8 ± 1.0 c	1.0 ± 1.1 c	26 ± 16 a	0.43 ± 0.25 b	0.65	<0.01
Southern Norway							
Raw	492 ± 63 abc	15.1 ± 0.7 abc	0.007 ± 0.005 a	8.4 ± 4.9 ac	0.21 ± 0.14 a	0.70	<0.01
Boiled fresh	555 ± 86 b	15.6 ± 0.8 b	0.065 ± 0.075 b	12 ± 11 ab	0.16 ± 0.12 a	0.33	0.23
Thawed, boiled sw	462 ± 39 c	14.7 ± 0.4 c	0.16 ± 0.09 c	6.8 ± 5.1 ac	0.08 ± 0.07 b	0.39	0.15
Thawed, boiled fw	450 ± 51 c	14.5 ± 0.7 c	0.10 ± 0.08 c	5.7 ± 2.3 c	0.08 ± 0.10 b	0.16	0.58
Experiment 2							
Raw A			0.027 ± 0.047 a				
Boiled sw A			0.020 ± 0.019 a				
Boiled sw B			0.022 ± 0.012 a				
Steamed sw B			0.027 ± 0.019 a				
Defrosted, raw A	459 ± 99 a	14.9 ± 1.1 a		15 ± 9.5 a			
Defrosted, raw B	390 ± 114 a	14.3 ± 1.2 a		5.7 ± 2.3 b			
Experiment 3							
Claw taken frozen			0.011 ± 0.007 a				
Claw taken thawed			0.24 ± 0.23 b				
Defrosted raw	379 ± 93	14.0 ± 0.9		11 ± 11			

concentration of cadmium in the meat was low with a mean of 0.011 mg/kg ww and thereby significantly lower than the concentration in the meat from the claw that was thawing while attached to the carapace with 0.24 mg/kg ww.

4. Discussion

4.1. Effect of cooking method and sample preparation

4.1.1. Claw meat

Our Cd levels found in raw claw meat (Table 1) are in line with other findings of 0.01 (Maulvault et al., 2012a) and levels of about 0.03 mg/kg ww (Barrento et al., 2009c).

Both, cooking and thawing significantly increased Cd values in the claw meat. In Experiment 1, for both locations, boiling whole fresh crabs resulted in about ten–fold higher claw meat Cd concentrations compared to raw claw meat. Freezing and thawing before boiling enhanced this effect, causing at least a further doubling of the claw meat Cd concentrations. In experiment 3, the freezing and thawing process also increased the Cd concentration in claws that were attached to the carapace during thawing. This clearly indicates a transfer and redistribution of Cd from HP, with its high concentrations, into the claw meat during these processes. Cd in raw crabs is mainly found in the soluble cytosolic fraction bound to metallothionein (MT) (Pedersen et al., 1994). During freezing, water expands and ice crystals form, leading to cell bursting and leakage of the cadmium during thawing. In cooking processes, additionally free Cd ions are partly released, as, although MTs are described as heat stable, the metal link (metal-SH) is vulnerable to heat (Bragigand & Berthet, 2003). As no rise in Cd levels was found once claws were thawed and boiled separately, it can be concluded that the Cd is not transferred from the exoskeleton. In accordance with our findings, Jorhem et al. (1994) noticed a reduction of cadmium concentrations in crayfish HP and a rise of Cd in abdominal muscle comparing raw and boiled crayfish. As high brown meat to white meat Cd ratios are known for other widely consumed crustaceans like Lobster *H. gammarus,* with 101, spider crab *M. squinado* with 28.5 (Noël et al., 2011), and snow crab *Chionoecetes opilio* with 10 (Rouleau et al., 2001), a transfer of Cd into the white meat during cooking is probable for all these important crustacean species.

In similar experiments with bivalves, the Cd concentration in the cooking water increased and cooking processes concentrated Cd in the soft tissue by loss of water (Houlbrèque et al., 2011; Metian et al., 2009). When boiling crayfish, Jorhem et al. (1994) found no changes in heavy metal concentration in the water. The differences in anatomy of bivalves and decapod crustaceans might explain this difference. Bivalves, once the shell opens during boiling, expose a big surface of the inner organs to the cooking water. The almost closed exoskeleton of crabs instead, only allows a limited movement of boiling water in and out of the exoskeleton. Cadmium from HP may therefore to a higher degree redistribute inside the crabs.

4.1.2. Brown meat

Experiment 1 showed that for both locations, the total cadmium content in brown meat (taking into account the wet weight of the total brown meat consisting of both gonad and HP), is highest in the raw crabs with lower values in boiled and thawed and boiled crabs, although not significantly for freshly boiled crabs from Southern Norway. In HP, the levels of Cd were lower in the thawed, boiled treatments, however, not significantly and the values in freshly boiled crabs were even slightly higher than in raw crabs. In an earlier study by Maulvault et al. (2011), an increase in brown meat Cd levels was found after cooking, while in another study conducted by the same group no difference in the brown meat Cd content after cooking was found (Maulvault et al., 2012a). The degree of change in Cd levels in HP due to cooking seems to be less clear. Probably, this is partly related to the large inter-individual variation in Cd levels (see 4.2). The assumption made in calculating the concentration of Cd in HP, that the gonad of the crabs does not contain any Cd might bias the results in the freshly boiled and thawed and boiled crabs. As seen with the claw meat, it is possible that there was some transfer of Cd from HP to the gonad while cooking and thawing. Thereby, the concentrations in HP

might be overestimated and the effect of the treatments underestimated. The degree of leakage from HP to gonad should be investigated further in future studies, as the female gonad is commonly consumed as a part of the brown meat or could be used separately if taken from raw crabs.

In crabs from Northern Norway, concentrations of Cd in HP and claw meat were strongly correlated in all treatments. In crabs from Southern Norway, correlations between Cd in claw meat and HP were not that pronounced, probably because of lower concentrations in the HP. High variations between the individual crabs might also mask the statistical significance of the effects. Furthermore, several crabs were cooked together in one pot, and although the leakage out of the body is not expected to be pronounced while boiling crabs, it might be enough for some interaction of the concentrations between different crabs through the boiling water. Other factors during crab handling could also influence the final cadmium levels in the different organs. The position and orientation of the crabs while freezing, thawing and cooling off after cooking, as well as lost legs and claws while and before thawing and cooking, could influence the distribution of the liquids and hereby Cd concentrations. Hence, the need to have a detailed standardized protocol for how to process crabs for analysis is evident. There was no difference between the salted water and freshwater treatment, showing that the variation in salt used for preparation in households is of no importance for the Cd content.

4.2. Natural variation of Cd in the edible crab

Cd measurements exhibit a high variability with a mean SD of 80% in raw HP and 87% in raw claw meat. This is confirmed by findings in other studies measuring Cd in the edible crab with a mean SD of 61% in raw HP and 63% in raw claw meat (Barrento et al., 2009b) and 137% in raw HP and 93% in raw claw meat (Maulvault et al., 2012a). Noël et al. (2011) measured Cd levels in crabs originating from France, United Kingdom, Netherlands and Ireland. The values for white meat ranged from <0.020 mg/kg ww and up to 0.587 mg/kg ww underlining the high variability. However, the sample preparation is not explicitly described in this study and the leakage processes demonstrated in our study might have contributed to the high variation. In an investigation by the Food Standard Agency UK (2013) of crabs caught and retailed in UK, cadmium concentrations in HP from whole crabs ranged from 0.61 to 16 mg/kg ww. Here, cooked and fresh samples of different crab species were evaluated together. The high variation might be explained by different biological and natural factors. In the green crab *Carcinus maenas* the accumulation of cadmium depends on different physiological parameters like ion concentrations, hydration level and volume of tissues which can be interpreted as condition of the crab (Bjerregaard, 1991; Bjerregaard & Depledge, 2002) and is thereby linked to feeding conditions. Also the stage in the moulting cycle and ovarian maturation influences the Cd accumulation in green crabs. In post-moult stages, they accumulated Cd at much higher rates and accumulation decreased during ovarian maturation when exposed to Cd in water (Bondgaard, Nørum, & Bjerregaard, 2000; Nørum et al., 2005). Furthermore age and growth rate could influence the values like seen in fish (Giguère, Campbell, Hare, McDonald, & Rasmussen, 2004).

Our mean value in HP in raw crabs from Southern Norway of 8.4 mg/kg ww is in line with earlier findings of 5.6 mg/kg ww (Maulvault et al., 2011), about 8 mg/kg ww (Maulvault et al., 2012a) and 6–28 mg/kg ww (Barrento et al., 2009b) in crabs from Scotland, and the findings of Noël et al. (2011) in crabs from different countries (UK, FR, IE) with a mean value of 12.8 mg/kg ww. However, our values for Northern Norway with an average of 38 mg/kg ww and a maximum of 87.8 mg/kg ww were exceptionally high. As similar differences were found earlier along the Norwegian coast (Julshamn et al., 2012), it seems to be a consistent pattern. Further investigations are needed to clarify why this is the case. Dry weight contents in the different tissues were quite stable and showed no significant differences within the different trials and experiments. Accordingly, dry weight and wet weight based results show the same pattern. In contrast, Maulvault et al. (2012a) found higher dry matter contents after steaming compared to boiling (summer 19% and 15%, spring 9 and 5%) and argues for a leaching of water during the cooking process. Measurement in gills of raw crabs showed significantly higher Cd values in Northern Norway compared to Southern Norway. This might be caused by higher concentrations of Cd in the North, as it has been shown in fish that higher Cd concentrations in water results in higher concentration in gills (Giguère et al., 2004). The higher values in the gastro-intestinal tract, here HP, than in gills however, suggest a more important uptake of Cd from food than water. This has been demonstrated in lab experiments in brown crabs (Davies et al., 1981) and green crabs (Bjerregaard, Bjørn, Nørum, & Pedersen, 2005).

4.3. Implications for human consumption and study design

The Cd concentrations in all our analyzed HP and brown meat samples were higher than the EU limit of 0.5 mg/kg set for unprocessed white meat from crab appendages (EU, 2006, 2011), with a total mean value exceeding the EU maximum level by a factor of 35. Considering the high values of Cd found in brown meat in crabs from Norway, a person of 70 kg only needs to consume as little as 4 g of HP from a freshly boiled crab from Northern Norway or 15 g from a freshly boiled crab from Southern Norway, to reach the TWI of 2.5 μg/kg body weight set by EFSA (EFSA, 2009). Also the mean levels in claw meat from the thawed and boiled crabs from Northern Norway and some single values in the cooked crabs of experiment 1 and thawed crabs of experiment 3 exceeded the legal limit of 0.5 mg/kg ww. In the Portuguese and Norwegian population, crabs are mostly consumed in the coastal area with portions of up to 200 g of muscle and inner meat consumed per meal (Bergsten, 2004; Maulvault et al., 2013). Taking into account these consumer habits together with the potential of high catch rates in some regions, people fishing crabs for recreation and consuming their own catch, are in high danger of heavy Cd exposure. In industrial processing, meat of crabs from different origin is often mixed. This, however, is not the case in small-scale recreational fishing, and a repeated exposure to values as high as our maximum individual value of 174 mg/kg ww in HP of a freshly cooked crab can take place. A questionnaire amongst recreational fishermen in the inner Oslofjord showed that over 45% did not know about any contamination in different kinds of fish (Holt, 2015). Thus, low risk perception or lack of knowledge might fortify the risk of high Cd exposure.

The risk of exceeding the TWI is highest when brown meat is consumed (99.6% of the consumers in Portugal (Maulvault et al., 2013)), but should not be neglected either, if white meat in crabs from Northern Norway is consumed, given that crabs are prepared in the traditional way of boiling the whole crabs. An assessment based on the existing consumer data in the Norwegian population, considering the Cd intake from other sources then crab meat, concluded that consumers of high amounts of crab brown meat and especially adolescences are at high risk of exceeding the TWI (VKM, 2015). In contrast, treatments based on cooking the claws separately, result in a safe product in regards to Cd. We agree with the conclusion of Maulvault et al. (2013) that white crab meat is a healthy food item if adequate processing methods are used. Considering our findings, even crabs with high values in the HP can be processed if claws are treated separately from HP. Furthermore,

gonads of the females could be safely consumed if taken from raw crabs. Our findings emphasize the importance of choosing the right sample preparation method coinciding with the aim of the study. If conclusions about food safety regarding to Cd are drawn, samples must be prepared according to common household or commercially used practices. Otherwise, values are prone to overestimation (HP) or underestimation (white meat) and cannot serve as the basis of risk assessment. Similar findings are present for other heavy metals in seafood (Atta et al., 1997; Ersoy et al., 2006; Jorhem et al., 1994) and also other characteristics like chemical composition, fatty acids profile, macro and trace elements (Maulvault et al., 2012b).

5. Conclusions

This study evidenced the influence of cooking and freezing on the Cd content in claw and brown meat of the edible crab. The results strongly suggest that the process of cooking crabs whole leads to a leakage of Cd from HP to claw meat. In crabs with high levels of Cd in HP this resulted in values in claw meat above the maximum legal limit. Freezing and thawing enforced the effect with even higher values after cooking the crabs whole. Claw meat from separately cooked claws had Cd values comparable to raw claw meat, which were low and unproblematic for human consumption. Furthermore, the freezing and thawing process of whole crabs from Northern Norway with high levels of Cd in HP led to values of Cd in raw claw meat above the maximum legal limit. Thus, if natural levels of Cd are investigated, care should be taken if freezing of the samples is necessary. Our results strongly suggest the consideration of the cooking process when assessing food safety of the edible crab regarding Cd.

Conflict of interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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