Aquatic Toxicology 201 (2018) 198–206

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aqtox

Tracing simultaneous cadmium accumulation from different uptake routes in brown crab *Cancer pagurus* by the use of stable isotopes

Martin Wiech[a,d,*], Heidi Amlund[a], Karl Andreas Jensen[b], Tom Aldenberg[c], Arne Duinker[a], Amund Maage[a,d]

[a] Institute of Marine Research, P.O. Box 1870, Nordnes, NO-5817 Bergen, Norway
[b] Institute of Environmental Sciences, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway
[c] National Institute for Public Health and The Environment, P.O. Box 1, NL-3720 BA Bilthoven, The Netherlands
[d] University of Bergen, P.O. Box 7800, NO-5020 Bergen, Norway

ARTICLE INFO

Keywords:
Cancer pagurus
Cadmium
Accumulation
Stable isotope
Gavage feeding
Kinetic modelling

ABSTRACT

High concentrations of cadmium in brown crab are an issue of food safety, and large variations between different areas have been found. To investigate the relative importance of dietary and aqueous uptake regarding the overall accumulation in brown crab, we used stable isotopes to trace the uptake from both routes simultaneously in the same animals. We demonstrated that the analytical challenges regarding background concentrations of natural isotope distribution and polyatomic interferences in the different matrices can be overcome with an appropriate analytical setup and modern mathematical corrections using a computer software. Cadmium was accumulated via both routes and was found in all measured organs at the end of the exposure phase. The obtained data were used to establish accumulation curves for both uptake routes and estimate accumulation parameters for hepatopancreas, as the most important organ in crab regarding total cadmium body burden. Using the estimated parameters in combination with naturally relevant cadmium concentrations in seawater and diet in a model, allowed us to predict the relative importance of the aqueous and dietary uptake route to the total hepatopancreas burden. According to the prediction, the dietary route is the main route of uptake in brown crab with a minimum of 98% of the accumulated cadmium in hepatopancreas originating from diet. Future studies addressing the source and accumulation of cadmium in crab should therefore focus on the uptake from feed and factors connected to foraging.

1. Introduction

The brown crab (*Cancer pagurus*) is an appreciated seafood species with an increasing value and a global catch of about 50 000 t (FAO, 2018) with about 5 000 tons harvested in Norway in 2016 (Søvik et al., 2017). However, elevated concentrations of cadmium (Cd) in the hepatopancreas (HP) and claw meat of cooked brown crab in several European countries (Barrento et al., 2009; Julshamn et al., 2012; Maulvault et al., 2012) have become a food safety concern (Maulvault et al., 2012; Noël et al., 2011). In the North-East Atlantic Ocean, an interesting pattern was seen in crabs caught at the Norwegian Coast. The highest Cd values were found in crab in the North and claw meat concentrations have regularly been found to exceed the current legal limit of 0.5 mg/kg ww set by European Union (Julshamn et al., 2012; Wiech et al., 2017). The coast of Northern Norway is regarded a rather pristine area and the occurrence of high concentrations of Cd in crab therefore arouse public concern and scientific interest in finding the reason for the high levels. In general terms, trace elements, except methyl-mercury are not expected to biomagnify along the food chain (Fisher & Reinfelder, 1995). To elucidate the cause of the high Cd levels, it is important to understand how Cd is taken up and retained in brown crab. The uptake of metals in crab can occur via two different routes: from water over the gills, or via the dietary route from ingested diet. The importance of these routes regarding the overall metal concentration at steady-state can be determined using a kinetic model when assimilation efficiency, ingestion rate, and unidirectional uptake and elimination rate constants are known for the species in question (Luoma & Rainbow, 2005; Wang et al., 1996). To produce data sufficient for a reliable parameter estimation, radioisotopes have often been used to trace the accumulation of metals. However, the use of radioisotopes has some drawbacks (see Croteau et al (2004)) and as a result of the recent developments in inductively coupled plasma mass

* Corresponding author at: Institute of Marine Research, P.O. Box 1870, Nordnes, NO-5817 Bergen, Norway.
E-mail addresses: Martin.Wiech@hi.no (M. Wiech), Heidi.Amlund@hi.no (H. Amlund), Karl.Jensen@nmbu.no (K.A. Jensen), Tom.Aldenberg@rivm.nl (T. Aldenberg), Arne.Duinker@hi.no (A. Duinker), Amund.Maage@uib.no (A. Maage).

https://doi.org/10.1016/j.aquatox.2018.05.015
Received 14 February 2018; Received in revised form 17 May 2018; Accepted 23 May 2018
Available online 24 May 2018

spectrometry instrumentation (ICP-MS), the use of stable isotopes has become a good alternative. The use of stable isotopes has proven to be adequate to investigate the uptake of metals from water and feed in bivalves (Croteau et al., 2004; Strady et al., 2011). In *Daphnia magna* also interaction effects of metals were successfully studied using stable isotopes (Komjarova & Blust, 2008, 2009). Strady et al. (2011) have further shown the potential of using stable isotopes to simultaneously trace aqueous and dietary uptake in the same animals in the case of oysters. A prerequisite for simultaneous tracing is that there is no interaction between the uptakes from the different routes. In crab, Cd is mainly present in HP and almost entirely bound to metallothionein (MT) (Pedersen et al., 1994, 1998). As the binding capacity for Cd ions in MT is limited, expression is induced at a certain exposure level (Pedersen et al., 2014) and overload could lead to an interaction of the different uptake routes.

One challenge when using stable isotope tracing lies within the chemical analysis. Stable isotopes are part of the natural isotope distribution of an element and are therefore abundant wherever natural Cd is present in the experiment. Therefore, high background concentration is expected. Another analytical issue when using ICP-MS is polyatomic interference on all Cd masses in the different tissues. These challenges need to be addressed to enable the detection of Cd in tissues of animals exposed to low naturally relevant concentrations.

Aqueous uptake of Cd in branchuryan crabs has been studied closely in the green crab *Carcinus meanas*, a species partly sharing the habitat with brown crab. Various factors such as temperature, salinity, exposure concentration, calcium concentration, molting stage, ovarian stage and feeding status influencing the uptake of Cd from water, have been identified (see Bjerregaard et al. (2005)). The dietary uptake route has not been studied equally well (Pedersen et al., 2014), although a comparative study indicated that the uptake from feed contributes most to the overall Cd accumulation in green crab (Bjerregaard et al., 2005). A recent study has quantified the Cd concentrations in green crabs along the Norwegian coast and found a different pattern between green and brown crab. For green crab, there was no clear difference in Cd concentrations between crabs from North and South (Knutsen et al., 2018), as seen in brown crab (Julshamn et al., 2012). This indicates that there might be differences in uptake and elimination processes in the two species, as already known for other crab species (Rainbow & Black, 2005a, 2005b). The accumulation of Cd in brown crab, although commercially important, has not gotten much attention. To our knowledge, only Davies et al. (1981) investigated the uptake of Cd from feed and water in brown crab and concluded that dietary uptake exceeds aqueous uptake. However, deep-freezing of crabs before dissection make the results uncertain, as this can have a significant influence on the Cd concentrations in the different organs and can mask the actual distribution of Cd (Wiech et al., 2017). In general, the importance of the different uptake routes in brown and green crab have been estimated based on assimilation efficiencies for dietary uptake and concentration factors for aqueous uptake, often only considering data from the end of the exposure phase (Bjerregaard et al., 2005). Further, concentrations of Cd in prey and seawater, and ingestion rates under natural conditions were not taken into account (Davies et al., 1981) adding uncertainty to the results and making a direct comparison of uptake routes difficult.

In the present study, we wanted (1) to determine accumulation parameters of Cd in brown crab from aqueous and dietary route at the same time in the same animal, by (2) applying the method of stable isotope tracing. To address the observation in Northern Norway, (3) the importance of the different uptake routes was estimated using a modelling approach.

2. Material and methods

2.1. Experimental animals

Female, intermoult brown crabs (*Cancer pagurus*) (n = 156) with a carapace width of 131 ± 5 mm (mean ± SD), caught with baited traps in September 2016 around the southern tip of Sotra, Norway, were used in the experiment. Prior to the experimental period, crabs were acclimated to the laboratory conditions at Austevoll Research Station, Institute of Marine Research, Norway, for minimum five days, before the controlled feeding regime was established. The claws of the crabs were tied with a rubber band to avoid cannibalism and provide safety for the personnel handling the animals. Each of the rubber bands was carrying a number for identification of individual crabs. The animal handling and experimental protocols were approved by the Norwegian Food Safety Authority (FOTS ID 8845) and performed in accordance with the Norwegian and European law for the use of animals in experiments.

2.2. Experimental setup

During the experimental period of 96 days starting 04 Oct 2016, the crabs were maintained in two 900 L tanks (control and exposure) in two levels of plastic baskets (34 × 25 × 16 cm) at a maximum density of 32 crabs/m^2. Crabs were mainly kept in darkness with only slight exposure to the natural light regime. Seawater was taken from 160 m water depth, sand-filtered and continuously exchanged at least ten times daily, and the pressure regulated using valves with flow-meters. Water temperature was measured daily and ranged from 7.2 to 9.0 °C during the experimental period. Salinity was measured to 35 ppt and pH to 8.0 at start and end of the experiment. Aeration with air stones was used to obtain a sufficient air saturation (> 88%) and a homogenous mixture of the water. To minimize potential desorption of Cd from feces to the water, the tank was flushed and cleaned two to three times a week.

2.3. Feeding

Gavage feeding was applied in order to know the exact amount of feed ingested. Crabs from control and exposure tank (see 2.4 for exposure) were taken out of the water and fed individually with 6 mL feed per week, by feeding them two or three times with 2 or 3 mL, respectively (Ingestion rate *I*: 2.36 $mg_{feed}\ g_{crab}^{-1}\ day^{-1}$ ww or 9.39 $mg_{feed}\ g_{HP}^{-1}\ day^{-1}$dw) using a disposable plastic syringe with gavage needle (15 G, 1.8 × 80 mm, Jørgen Kruuse A/S, Denmark). The feed was a slurry prepared from codfish powder (cooked, dried and micro milled cod fillet, Seagarden AS, Norway) sieved through 200 µm and mixed with deionized water in a blender to a dry weight content of 22.5%. Gavage feeding is only possible when crabs are moving their mouth parts voluntarily, which can take minutes. A few drops of deionized water flavored with shrimp powder (Seagarden AS) was an effective stimuli for the crabs to open their mouth parts and the feeding time could be shortened to approximately under one minute per crab. To impede crabs from spitting out the feed, they were kept out of the water for minimum 30 s after feeding.

2.4. Exposure

Crabs in the exposure tank (n = 78) were exposed to Cd in seawater (0.5 µg ^{106}Cd/L) and in feed (1 mg ^{108}Cd/kg wet weight) (Fig. 1) for 42 days, followed by a depuration phase of 56 days. To obtain an accurate concentration of ^{108}Cd in feed, a stock solution was prepared by dissolving metallic Cd enriched in ^{108}Cd (69.9%, Neonest AB/BuyIsotope.com, Stockholm, Sweden) in nitric acid and dissolving it in deionised water to the desired concentration. Stock solution was added to the feed and the mixture homogenized by stirring. To spike the sea water with the desired level of ^{106}Cd, a stock solution was prepared by dissolving

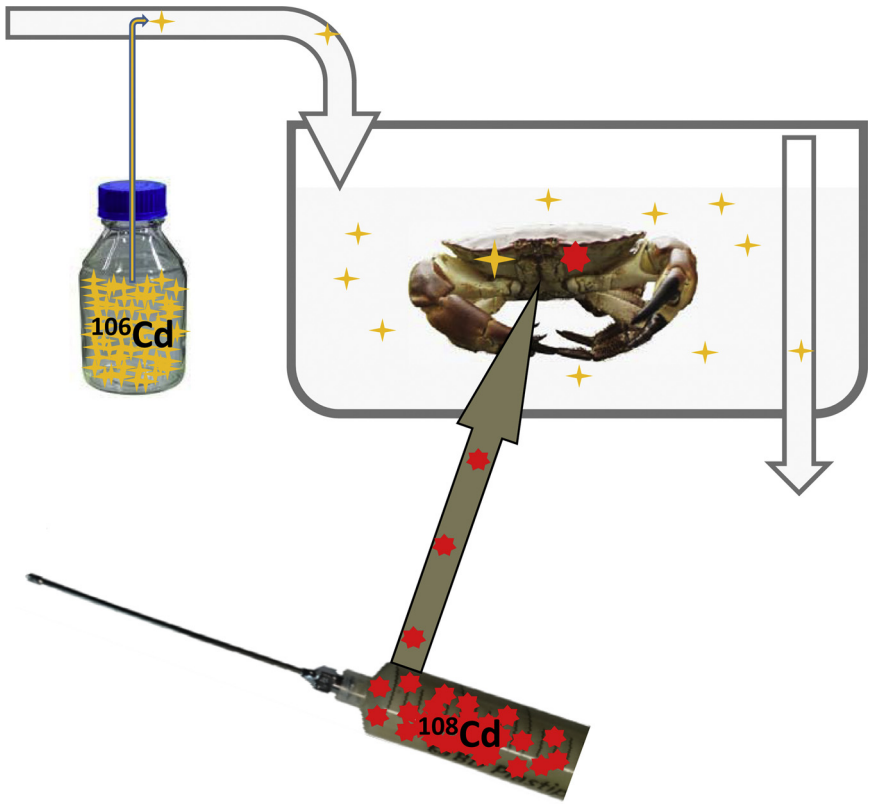

Fig. 1. Schematic illustration of the methodological approach, where Cd accumulation from water and feed was traced simultaneously in the same animal using two different stable Cd isotopes.

$CdCl_2$ enriched in ^{106}Cd (73.1%, Neonest AB/BuyIsotope.com, Stockholm, Sweden) in deionised water and dosed using a peristaltic pump (Watson-Marlow). Flow rate was checked daily. In addition, during the exposure phase, weekly water samples from the exposure tank were measured in the exposure phase and a concentration of 0.518 ± 0.010 µg ^{106}Cd/L, corresponding to 0.708 ± 0.014 µg Cd/L (mean ± SD, n = 7) and a maximum of 0.002 µg ^{108}Cd/L (n = 7) was found. The seawater in the control group during exposure contained 0.033 ± 0.016 µg total Cd/L (mean ± SD, n = 7). During the depuration phase the highest measured concentration of ^{106}Cd and ^{108}Cd in water was 0.002 µg/L (n = 6). In the control tank, the highest measured concentration of ^{106}Cd and ^{108}Cd concentration was 0.001 µg/L (n = 4). The enriched feed contained 1.01 ± 0.03 mg ^{108}Cd /kg ww, corresponding to 1.44 ± 0.04 mg Cd /kg ww (mean ± SD, n = 5). The control feed contained 0.010 ± 0.004 mg Cd /kg ww (mean ± SD, n = 11).

2.5. Sampling

Samples of HP, gills, hemolymph, claw meat and gonad were collected from five individuals per treatment on day 0, 2, 7, 14, 21, 30 and 42 in the exposure phase and on day 2, 4, 7, 21, 35 and 56 in the depuration phase. Hemolymph was drawn through the arthodial membrane of the posterior pereiopod using a disposible syringe. Then, crabs were humanely sacrificed (WHO/FAO, 2012) piercing the two main nerve ganglia according to Baker (1955). Crabs were dissected fresh. Gills were squeezed to remove the contained liquid and blotted dry using tissue paper. All samples were kept on ice during sampling and frozen as soon as possible after and kept at −20 °C until ICP-MS analysis. For HP a subsample was kept at −80 °C for the measurement of MT. Total weight and carapace width (CW) before and after the experiment, gonad maturation stage according to Haig et al. (2016), and gonad and HP weight was determined for each crab. Further, gonadosomatic index (weight of gonad/CW^2 ·100) and hepatosomatoc index (weight of HP/ CW^2 ·100) were calculated. To assess if there were statistically significant physiological differences between crabs sampled at the different sampling days, data were analyzed using ANOVA. Data were checked for homoscedasticity (Levene`s test) and log-transformed, if necessary. For categorical parameters (gonad maturation stage), non-parametric statistics was applied. The significance level was p = 0.05. The analysis was done using STATISTICA 12 (©Statsoft, Tulsa, USA).

2.6. Chemical analysis

2.6.1. ICP-MS analysis

All isotopes of Cd were measured with a tandem quadrupole Agilent 8800 ICP-MS with collision/reaction cell (CRC). The use of NH_3 as reaction gas was found to be the most efficient for removing polyatomic interferences on all Cd isotopes. ^{103}Rh was used as online internal standard. A control standard was analyzed every ten samples as drift check, both for concentration and mass bias. Masses 106, 108, 110, 112, 113, 114 and 116 were corrected from isobar overlapping from Pd, In and Sn. All isotope ratios were measured in pulse detector mode. Samples were diluted if concentrations exceeded the pulse mode limit (< 1.2 Mcps/s) due to nonlinear calibration between pulse and analog mode. The instrumental setup is shown in the Supplementary material A.

2.6.2. Sample preparation

Tissue samples were freeze-dried (Freezone, Labconco, US) before being homogenized. Approximately 0.2 g (dry weight) of the homogenized sample and certified reference material (CRM) were microwave digested (Ethos, Milestone, Italy) with 2 mL HNO_3 and 0.5 mL 30% H_2O_2. After digestion, samples were diluted to 50 mL with deionized water. The CRM were Lobster HP (TORT 3, National Research Council Canada) and Oyster Tissue (1566b, National Institute of Standards and

Technology, USA). All HP, gill and hemolymph samples were analyzed while for claw meat and gonad only samples taken at day 42 were analyzed.

2.6.3. Calibration

A Cd standard with a naturally abundant isotopic composition (Inorganic Ventures, Christiansburg, USA) was used for calibration and the Cd concentration (w/V) for each isotope was calculated from the natural mass percent abundance. Using this calibration, mass bias corrected isotope ratio (IR) cps_1/cps_2 equals C_1/C_2, where cps denotes counts per second on the detector and C denotes the isotope concentration (w/V) of isotope 1 and 2. Cadmium concentrations for all isotopes measured in TORT-3 (n = 7) and NIST 1566b (n = 6) were within the certified ranges.

2.6.4. Isotope corrections

The natural total Cd concentration in samples was calculated from the natural background concentration of ^{114}Cd (the naturally most abundant Cd isotope) divided by its natural abundance. This concentration ($^{114}Cd_n$) had to be corrected for the natural contribution of other Cd isotopes, such as ^{106}Cd and ^{108}Cd. In the same way, the concentration of ^{106}Cd in the water ($^{106}Cd_w$) and ^{108}Cd in the feed ($^{108}Cd_f$) had to be corrected for the contribution of other Cd isotopes found in the enriched ^{106}Cd (73.1 mass %) and ^{108}Cd (69.9 mass %) isotope standards used to spike water and feed. Therefore, the total measured concentrations (C; in w/V) of each isotope can be defined as the sum contribution of natural background (C_n) and the isotope in water (C_w) and feed (C_f):

$$C_{114} = C_{w,114} + C_{n,114} + C_{f,114} \quad (1)$$

$$C_{106} = C_{w,106} + C_{n,106} + C_{f,106} \quad (2)$$

$$C_{108} = C_{w,108} + C_{n,108} + C_{f,108} \quad (3)$$

The mass fraction isotopic ratios (IR_n, IR_w, IR_f) for ^{106}Cd and ^{108}Cd were calculated in respect to ^{114}Cd present in the initial background, water and feed before exposure, as follows:

$$IR_{f,108} = C_{f,108} / C_{f,114} \quad (4)$$

$$IR_{w,106} = C_{w,106} / C_{w,114} \quad (5)$$

$$IR_{n,106} = C_{n,106} / C_{n,114} \quad (6)$$

$$IR_{n,108} = C_{n,108} / C_{n,114} \quad (7)$$

$$IR_{f,106} = C_{f,106} / C_{f,114} \quad (8)$$

$$IR_{w,108} = C_{w,108} / C_{w,114} \quad (9)$$

The corrected contributions of ^{114}Cd natural background ($^{114}Cd_n$), ^{106}Cd in water ($^{106}Cd_w$), and ^{108}Cd in feed ($^{108}Cd_f$) were calculated by simultaneously solving Eqs. (1) to (9) using an equation solver software (wxMaxima 16.12.0; http://andrejv.github.io/wxmaxima). This resulted in the following equations, which depend on the total measured concentrations and IRs:

$$^{106}Cd_w = -\frac{\begin{array}{l} C_{114}(IR_{f,108}IR_{n,106}IR_{w,106} - IR_{f,106}IR_{n,108}IR_{w,106}) \\ + C_{106}(IR_{n,108}R_{w,106} - IR_{f,108}IR_{w,106}) \\ + C_{108}(IR_{f,106} - IR_{n,106})IR_{w,106} \end{array}}{\begin{array}{l} IR_{n,106}IR_{w,108} - IR_{f,106}IR_{w,108} + IR_{f,108}(IR_{w,106} - IR_{n,106}) \\ - IR_{n,108}IR_{w,106} + IR_{f,106}IR_{n,108} \end{array}} \quad (10)$$

$$^{108}Cd_f = -\frac{\begin{array}{l} C_{114}IR_{f,108}(IR_{n,108}IR_{w,106} - IR_{n,106}IR_{w,108}) \\ + C_{106}IR_{f,108}(IR_{w,108} - IR_{n,108}) + C_{108}IR_{f,108}(IR_{n,106} - IR_{w,106}) \end{array}}{\begin{array}{l} IR_{n,106}IR_{w,108} - IR_{f,106}IR_{w,108} + IR_{f,108}(IR_{w,106} - IR_{n,106}) \\ - IR_{n,108}IR_{w,106} + IR_{f,106}IR_{n,108} \end{array}} \quad (11)$$

$$^{114}Cd_n = \frac{\begin{array}{l} C_{114}(IR_{f,108}IR_{w,106} - IR_{f,106}IR_{w,108}) + C_{106}(IR_{w,108} - IR_{f,108}) \\ + C_{108}(IR_{f,106} - IR_{w,106}) \end{array}}{\begin{array}{l} IR_{n,106}IR_{w,108} - IR_{f,106}IR_{w,108} + IR_{f,108}(IR_{w,106} - IR_{n,106}) \\ - IR_{n,108}IR_{w,106} + IR_{f,106}IR_{n,108} \end{array}} \quad (12)$$

The natural total concentration of Cd in samples are calculated from $^{114}Cd_n$ multiplied by 1/natural abundance of ^{114}Cd. Limits of detection (LOD) and limits of quantification (LOQ) were calculated based on control group concentrations using Eqs. (10) and (11). LOQs increased with increasing concentration and range of natural Cd in the control group (Supplementary material B). Concentrations of naturally abundant Cd from the samples had no significant effect on the results as they were subtracted by using the equations for the added enriched isotopes on the treatment group.

2.7. Determination of metallothionein

The concentration of MT in HP was examined in five crabs of the exposure group at start (t = 0d) and end (t = 42d) of the exposure phase. Metallothionein was measured spectrophotometrically at wavelength 412 nm after extraction and derivatization of thiols with Ellman`s reagent DTNB as described by Viarengo et al. (1997). The method was shown to be suitable for green crab (Pedersen et al., 1997). A standard curve was made using glutathione. Assuming a fixed ratio of thiol groups between glutathione and MT of 1:19 and a molecular weight for MT of 5800 (Overnell, 1986), the concentration of MT could be calculated.

2.8. Modelling the accumulation parameters

Crabs accumulate about 90% of the total Cd body burden in HP, considering the whole animal including carapace (Bjerregaard & Depledge, 2002). Therefore, the accumulation in HP was used as a proxy for the accumulation of Cd in the whole crab. All modelling was based on data of Cd concentrations in HP.

To estimate the parameters describing the accumulation of Cd in HP for both uptake routes (aqueous and dietary), data were fitted to standard bioaccumulation equations derived from OECD TG 305 (2012) and related guidance document (OECD, 2017):

$$Cd_{crab}(t) = \begin{cases} C_{input} \cdot \frac{k_1}{k_2} \cdot (1 - \exp(-k_2 \cdot t)) & \text{for } 0 \le t < t_{dep} \\ Cd_{crab}(t_{dep}) \cdot \exp(-k_2 \cdot (t - t_{dep})) & \text{for } t \ge t_{dep} \end{cases} \quad (13)$$

We neglected growth of the crab, since it was zero during the course of the experiment. To apply the generic model for either exposure route, we adapted the exposure concentration and uptake rate constant to the respective experimental condition as follows:

$Cd_{crab}(t)$ (aqueous): Cd^{w}_{crab}, Cd concentration in HP of the crab over time [µg ^{106}Cd kg crab^{-1}],
(dietary): Cd^{f}_{crab}, Cd concentration in HP of the crab over time [µg ^{108}Cd kg crab^{-1}],

C_{input} (aqueous): C_w, water exposure concentration [µg ^{106}Cd L^{-1}],
(dietary): C_f, feed exposure concentration [µg ^{108}Cd kg feed $^{-1}$],

k_1	(aqueous): k_w, uptake rate constant from water [L kg crab^{-1} d^{-1}], (dietary): k_f, uptake rate constant from feed [kg feed kg crab^{-1} d^{-1}],
k_2	(both aqueous and dietary): k_e, elimination rate constant [d^{-1}],
t	independent variable time [d],
t_{dep}	onset of the depuration phase [d].

At $t = 0$, the initial concentration in the crab equals zero:

$$Cd_{crab}(0) = 0,$$

while at the onset of the depuration phase, i.e. $t = t_{dep}$, one has, in the generic form:

$$Cd_{crab}(t_{dep}) = C_{input} \cdot \frac{k_1}{k_2} \cdot (1 - \exp(-k_2 \cdot t_{dep})) \quad (14)$$

which follows from Eq. (13), first part, when t approaches t_{dep}. Generally, when $k_2 > 0$, the accumulation curve of Cd in the crab during the uptake phase will be concave and increasing, while the depuration curve of Cd in the crab over time will be convex and decreasing. However, because of the shape of our data in the depuration phase (Fig. 3) and the statistical analysis of the applied models (Supplementary material C), two versions of the model were applied: one with k_2 being unconstrained, i.e. allowed to have any value, and one with k_2 constrained to be zero. For the case of a constrained elimination rate, i.e. $k_2 \to 0$, elimination is assumed to be negligible. Since, with k_2 very small, we have $(1 - \exp(-k_2 \cdot t)) \approx (1 - (1 - k_2 \cdot t)) = k_2 \cdot t$, the approximate model equations (generic form) become:

$$Cd_{crab}(t) = \begin{cases} C_{input} \cdot k_1 \cdot t & \text{for } 0 \le t < t_{dep} \\ C_{input} \cdot k_1 \cdot t_{dep} & \text{for } t \ge t_{dep} \end{cases} \quad (15)$$

In this case, the uptake curve will essentially be linear over time, with the level of accumulation nearly constant from the onset of the depuration phase onwards.

For the dietary uptake route, we additionally considered the sub-model

$$k_f = \alpha \cdot I \quad (16)$$

with I the ingestion rate of feed in the experiment [kg feed kg crab^{-1} d^{-1}], and α the assimilation efficiency, as a dimensionless constant.

2.8.1. Model fitting and statistical analysis

The model Eq. (13) define Cd concentration in crab as a function of time, with separate branches relating to the respective uptake and elimination phases. The model is nonlinear in the unknown parameters, k_1 (k_w or k_f, respectively) and k_2 (k_e in both cases), hence, fitting the models to the measured time series basically is a problem of nonlinear regression. Initially, we allowed k_e to be fitted separately for each of the routes, before constraining it to zero.

We used the R-package bcmfR_0.3-2.zip, as distributed by OECD (Aldenberg, 2017) with additional enhancements for the negligible elimination rate case, and supplementary routines for summarizing regression output, as well as estimating parameter and prediction uncertainty. The predictive limits of the model fits were calculated with the Bayesian bootstrap (Rubin, 1981, 1987).

The fitting procedure used was the nonlinear least squares regression function nls from the base R-package stats. Both untransformed Cd accumulation/depuration data were fitted, as well as $\log_{10}$-transformed accumulation/depuration data with the method of 'transform-both-sides' (Ritz & Streibig, 2008).

The regression quality was assessed through the Shapiro-Wilk test for normality of the regression residuals and Q-Q plots that compares the distribution of the standardized residuals to a standard Normal distribution. For both assessments implementations from the R-package nlstools were used (Baty et al., 2015).

2.9. Modelling the relative importance of the uptake routes

To compare the relative importance of the aqueous route RI_{water} and dietary route RI_{feed} to the overall accumulation of Cd in crab at different feed and water concentrations, we consider $k_e = 0$ and defined them as:

$$RI_{water}(t) = \frac{Cd^{w}_{crab}(t)}{Cd^{w}_{crab}(t) + Cd^{f}_{crab}(t)} \quad (17)$$

and

$$RI_{feed}(t) = \frac{Cd^{f}_{crab}(t)}{Cd^{w}_{crab}(t) + Cd^{f}_{crab}(t)}, \text{ respectively.} \quad (18)$$

Adapting the generic Eq. (15) to the respective uptake route and substituting into Eqs. (17) and (18) for the respective route, (t) cancels and the relative importance of the aqueous route RI_{water} becomes:

$$RI_{water} = \frac{k_w \cdot C_w}{k_w \cdot C_w + k_f \cdot C_f} \quad (19)$$

and the relative importance of the dietary route RI_{feed} becomes

$$RI_{feed} = \frac{k_f \cdot C_f}{k_w \cdot C_w + k_f \cdot C_f}, \text{ respectively} \quad (20)$$

Considering Eq. (16), we got the final equations used for the calculations of the relative importance of the uptake routes in percent (Fig. 4):

$$RI_{water} = \frac{k_w \cdot C_w}{k_w \cdot C_w + \alpha \cdot I \cdot C_f} \cdot 100 \quad (21)$$

and

$$RI_{feed} = \frac{\alpha \cdot I \cdot C_f}{k_w \cdot C_w + \alpha \cdot I \cdot C_f} \cdot 100 \quad (22)$$

with I being adapted to a more natural feeding rate of 79.4 $mg_{feed}\ g_{HP}^{-1}\ day^{-1}$ dw according to Woll et al. (2006) and adjusted to HP and dry weight according to the ratio of total crab weight and HP weight and average dry weight content from crabs used in the present study. To illustrate the relative importance of the uptake routes of Cd for brown crab at different concentrations found in feed [µg/kg dw] and seawater [µg/L], the concentrations were illustrated as cadmium concentration ratio (CCR) being C_f/C_w.

3. Results and discussion

Although low and environmentally relevant concentrations were used in seawater (0.5 µg ^{106}Cd/L) and feed (1 mg ^{108}Cd/kg wet weight) and the background Cd concentrations in the wild-caught crabs were high and strongly varying, it was possible to reliably detect and quantify even low contributions of Cd from both uptake routes in all measured tissues except claw meat (Fig. 2). This was made possible by using mathematical corrections preventing the natural concentrations of Cd to outweigh the signal from spiked and accumulated Cd isotopes. The issue of plasma-formed polyatomic mass interferences on all Cd isotopes was overcome using NH_3 as reaction gas in the CRC.

At the end of the exposure phase, most Cd from feed was accumulated in HP followed by gills, gonad and hemolymph with concentrations of 2850 ± 1870 µg/kg dw, 15.3 ± 8.0 µg/kg dw, 5.47 ± 4.96 µg/kg dw, and 0.14 ± 0.08 µg/kg dw (2 values < LOQ) (mean ± SD, n = 5), respectively. The measured claw meat concentrations were all below LOQ. A similar tissue distribution was found in green crab fed six meals of ^{109}Cd labelled blue mussel soft parts over 11 days (Bjerregaard et al., 2005). Considering the total body burden, HP was by far the organ accumulating most Cd with 91 ± 4% of the

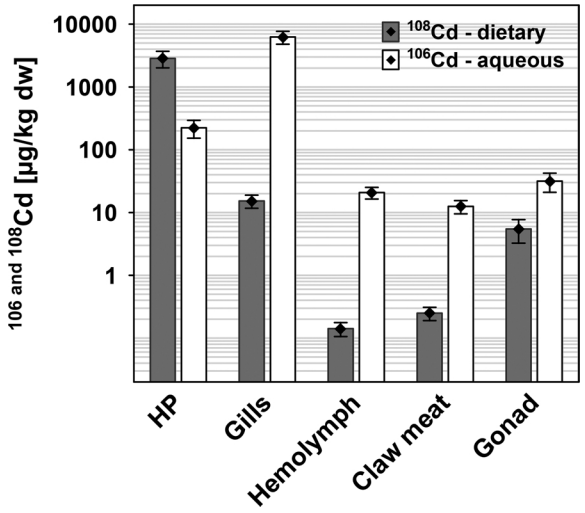

Fig. 2. Distribution of Cd taken up from feed and water in the different tissues of brown crab after 42 days of exposure. Filled columns show mean concentrations of ^{108}Cd taken up from feed and the clear columns concentrations of ^{106}Cd taken up from water. Whiskers represent standard errors (n = 5). Concentrations below LOQ (^{108}Cd in hemolymph and claw meat) are illustrated with the actual measured concentrations.

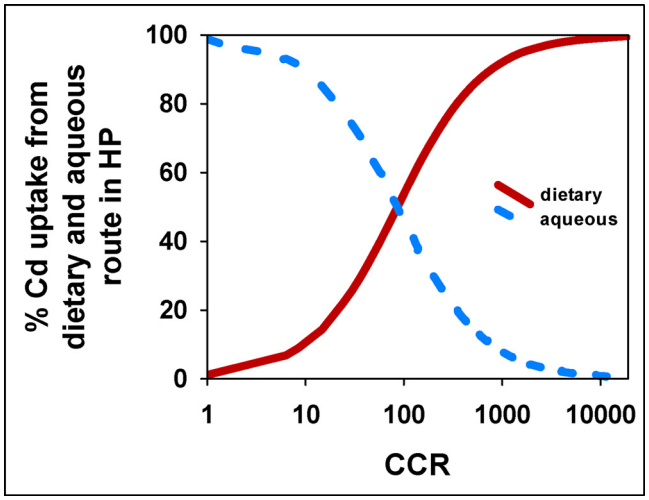

Fig. 4. Prediction of the relative importance of dietary and aqueous uptake route to the total Cd hepatopancreas burden of brown crab using modelling. The Cd concentration ratio (CCR) is the ratio of Cd concentration in diet [µg/kg dw] to seawater [µg/L].

total body burden of the traced Cd in green crab (Bjerregaard et al., 2005). Much less Cd was found in the other organs with on average 0.03 to 4.6% (Bjerregaard et al., 2005). Cadmium accumulated from water, was mainly found in gills at the end of the exposure phase, followed by HP, gonad, hemolymph and claw meat with concentrations of 6235 ± 3240 µg/kg dw, 224 ± 159 µg/kg dw, 31.6 ± 23.8 µg/kg dw, 20.8 ± 9.8 µg/kg dw and 12.5 ± 6.7 µg/kg dw (n = 5) (mean ± SD), respectively. Gills were also found to have higher Cd concentrations than HP in green crabs after aqueous exposure to 100 pm ^{109}Cd/mL for 27 d (Bjerregaard & Depledge, 1994). In that study, the concentration in muscle was much higher than in hemolymph (Bjerregaard and Depledge, 1994). The difference to our studies, might be due to the fact that we only analyzed muscle meat from claw, which might contain lower Cd concentrations than muscle meat from other parts of the crab like the thoracic sternum, as the proximity to the HP might lead to higher Cd concentrations. Norway lobster, *Nephrops*

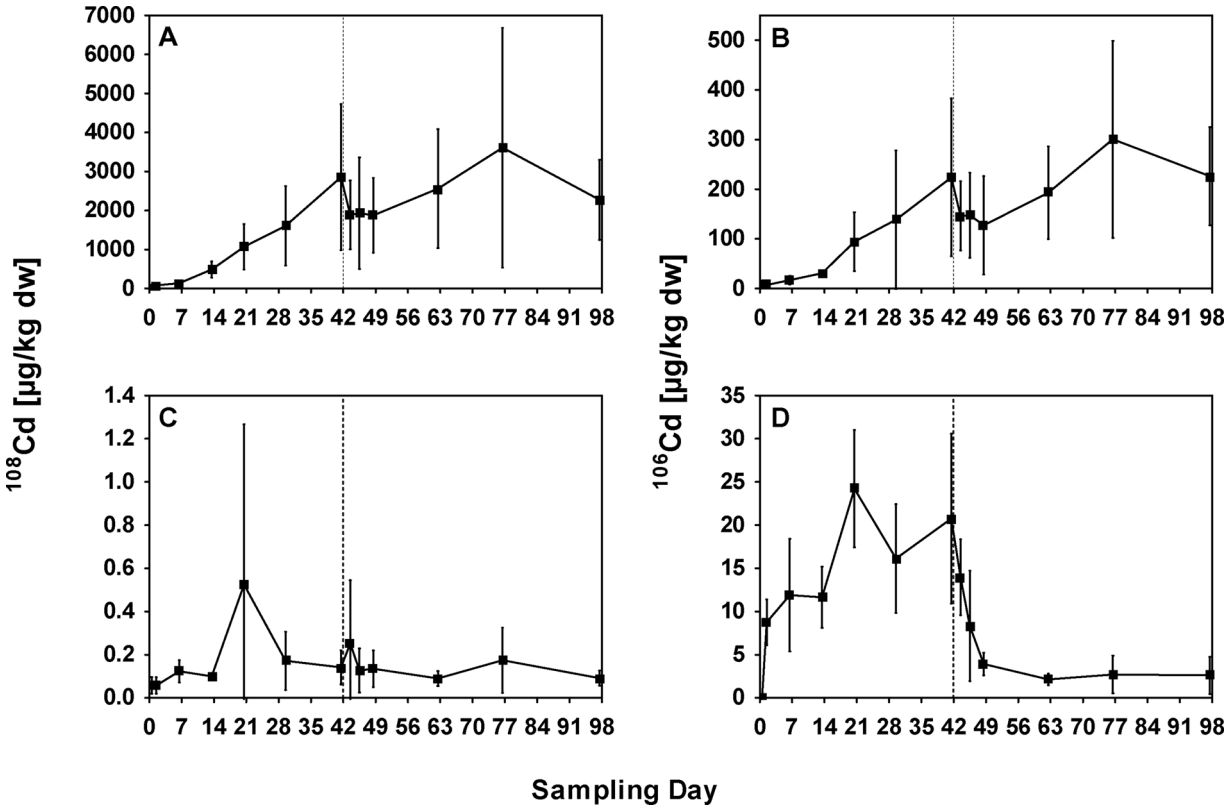

Fig. 3. Accumulation curve of mean concentrations of Cd in HP (A,B) and hemolymph (C,D) of brown crab taken up from feed (A,C) and seawater (B,D) after exposure to 0.5 µg ^{106}Cd/L in seawater and 1 mg ^{108}Cd/kg ww in feed. The vertical dashed line marks the end of the exposure phase. Error bars indicate the standard deviation of the five samples taken per sampling day.

norvegicus, also accumulated Cd from feed mainly in HP, however when exposed to Cd in seawater, the concentration in gills were not higher than in HP (Canli & Furness, 1995).

The concentration factor (C_w/C_{HP}) for the aqueous uptake of Cd in HP was 10.6 ± 7.6 (mean ± SD, n = 5) at the end of the exposure phase, which is comparable to findings in green crab using similar exposure conditions with factors of 6.9 ± 7.5 and 6.5 ± 5.1 at concentrations of 173 and 800 ng/L respectively (Bjerregaard et al., 2005).

The ^{106}Cd concentration in hemolymph increased until day 21 and remained stable until the end of the exposure phase, where it rapidly drops (Fig. 3). In contrast, the ^{108}Cd concentration coming from feed is very low during the whole experiment and 64% of the measurements were below LOQ. This indicates that Cd from feed is directly taken up from the mid-gut into HP through ducts arising ventrally on either side of the mid-gut together with nutrients from feed (Warner, 1977). Cd from water will mainly be taken up over the gills and will be present in hemolymph before it is taken up into the HP (Bjerregaard, 1990). Due to the 'open' circulatory system of crabs, Cd in hemolymph comes into contact with all internal tissues. This explains why the concentrations of ^{108}Cd from feed at day 42 are rather low in all tissues except HP, while ^{106}Cd is found in higher amounts in all tissues (Fig. 2). However, the concentrations of Cd in the different tissues taken up from water and feed are also concentration dependent and different exposure concentrations might result in different patterns. Therefore, kinetic modelling was applied to compare the importance of the different uptake routes in HP.

The Cd concentration in HP increased continuously during the exposure phase of 42 days and steady state was not reached. During the depuration phase, no clear pattern of decrease in Cd concentration was seen. A short decrease was followed by a temporary increase. However, none of the concentrations from the sampling days of the depuration phase were significantly different ($P > 0.32$), which might be due to high inter-individual variation. High inter-individual variation in Cd concentrations is common in brown crab sampled in the field (Wiech et al., 2017). In contrast to the decrease in the beginning of the depuration phase, the latter increase was rather unexpected. However, similar patterns with increasing concentrations of Cd during the depuration phase have also been observed earlier. After an exposure of seven days to Cd in water, the concentration of Cd in HP of the freshwater prawn *Macrobrachium australiense*, was first rapidly decreasing within two days and subsequently was increasing again until day 7, where it was stable until day 21 (Cresswell et al., 2017). In fish, Cd concentrations in liver and kidney increased and concentrations in the gut and white muscle decreased simultaneously, representing a redistribution of Cd within the organism (Harrison & Klaverkamp, 1989; Wicklund Glynn et al., 1992). In the present study, an internal redistribution of Cd from other organs into HP is not very likely. The only considerable amount of Cd at the end of the exposure phase was seen in gills and these concentrations also increased during the depuration phase (Supplementary material D). The hemolymph concentrations were rather low and rapidly decreased to a negligible level after exposure. If a redistribution would have taken place, it should have been visible as an increase in the hemolymph concentrations, acting as intermediate organ for Cd transported between for example gills and HP (Bjerregaard, 1990; Cresswell et al., 2017). It cannot be ruled out that Cd was accumulated in tissues not analyzed and a redistribution from these tissues occurred. However, it is not likely, as it has been shown for green crab that Cd does not accumulate in considerable amounts in other tissues than those measured here (Bjerregaard et al., 2005). The high assimilation efficiency of 98% in HP calculated from eq.16 for the present study confirms this, as it means that most of the Cd from feed was accumulated in HP. Reinfelder et al (1998) discussed that in accumulation experiments with long-term exposures, as the present study, especially for metals, a substantial elimination can occur already during the exposure phase. This can result in a lower elimination rate after exposure ended and might partly explain the low depuration observed in the present experiment. In freshwater prawn *Macrobrachium australiense* it was recently shown, that the depuration rate of Cd from HP was much lower after long-term compared to short-term exposure (Cresswell et al., 2017). The transport of Cd from hemolymph to HP is strongly dependent on the physiological condition in green crab (Bjerregaard, 1990). We found no physiological differences in crabs at the different sampling days. However, inter-individual differences were present in crabs from the same sampling day, which might be connected to the feeding stage of the animals. As all crabs had the same feeding regime while being in the lab, and no weight change during the experiment was seen, it is possible that the differences in the physiological state of the crabs at the time of their collection persisted throughout the course of the experiment and contributed to the inter-individual variation.

To examine if there was an interaction between the uptake of Cd from the two uptake routes at the concentration levels used, the concentration of MT in HP was measured to investigate if the standing pool of MT was sufficient to accommodate the influx of Cd from both sources. Cadmium in HP is mainly present in the soluble cytosolic fraction and almost entirely bound to MT. In green crab, a dietary exposure to 5.1 mg Cd/kg ww for 18 days led to an induction of MT, while 1.1 and 3.1 mg Cd/kg ww did not (Pedersen et al., 2014). This indicates that the binding capacity of present MT for Cd was reached at the highest concentration. Exceeding this capacity could lead to a compensatory response affecting the Cd uptake and distribution of the two uptake routes differently, as their route of uptake is different. Cadmium from diet will enter the HP directly, while Cd from water is first taken up into hemolymph and subsequently into MT in HP. A difference in the uptake mechanism into HP between Cd from diet and water at high exposure concentrations in crab was suggested earlier (Pedersen et al., 2014). That was based on their observation in green crab that the fraction of Cd bound in the soluble fraction in HP decreased with increasing aqueous exposure, while this was not the case for Cd taken up from diet to a comparable concentration in HP (Pedersen et al., 2014). Consequently, the importance of the two routes could be prone to under- or overestimation at high exposure concentrations. In our study, there were no statistically significant difference between the MT concentrations in HP at start and end of the exposure phase with 20.2 ± 2.1 nmnol/g ww (mean ± SD, n = 5) at day 0 and 16.1 ± 3.7 nmol/g ww (mean ± SD, n = 5) at day 42, respectively ($P > 0.05$). Levels are comparable to findings in green crab (Pedersen et al., 2014). Therefore, there was no interaction between the uptake of Cd from the two routes in HP and the measured Cd concentrations reflect the real uptake for both uptake routes, although traced simultaneously in the same animals.

The present study demonstrates that the use of stable isotopes when studying trace metal uptake has several advantages compared to radiotracers, as already discussed by Croteau et al (2004). Advantages, such as low costs for the tracer and low handling hazard and less restrictions, become especially important when using large laboratory animals such as fish or crab, with high water and space demand. Further advantages of the method relate to the correction for background Cd. This makes it possible to use wild-caught animals in laboratory experiments, which might be necessary when larger animals or species difficult to raise in captivity are studied. Wild-caught organisms or parts of organisms enriched with a stable isotope can in this way be used as feed. This is especially useful when using wild-caught filter feeders, such as blue mussels enriched with stable isotopes as feed to study trophic transfer. Although still being a benefit, is it no longer necessary to use pure stable isotope standards for enrichment, which often are expensive and difficult to obtain, as we can correct for the content of the other isotopes. The correction also enables the use of natural water with its possible background contamination and further, laboratory equipment does not have to undergo laborious cleaning to avoid background contamination. However, as the accumulation of metals often is concentration dependent, the total exposure has to be

considered when interpreting results. Also acclimation effects can occur. Further, restrictions could also arise when total element exposures are too high, as an interaction of the different uptake routes might occur if tracing different routes simultaneously. Also toxic effects might influence the accumulation and the LOQ increases with ascending background concentrations. Therefore, the introduction of unrecognized contamination and high impurities in isotopic stock solutions should be avoided wherever possible. Since there is no need for a control group to correct the background concentrations against, experimental animals in the control group can be reduced to the number necessary to control for other effects, as for example to study if possible toxic effects observed in exposed animals are due to the exposure. Both uptake routes can also be studied in the same animals simultaneously, making two different treatment groups redundant. In conclusion, the introduced methodology makes accumulation studies using stable isotopes a robust alternative to radiotracers.

The modelling based on the standard bioaccumulation equations with additional adaptations to estimate the accumulation parameters, delivered reasonable results for both routes. The case with constrained k_e to zero and $\log_{10}$ transformed data resulted in a better model fit for both uptake routes (Supplementary material C) and k_w and k_f were determined to be 6.721 ± 0.567 L kg crab^{-1} d^{-1} (mean ± SE) and 0.0092 ± 0.0008 kg feed kg crab^{-1} d^{-1} (mean ± SE). Using Eq. (16), we calculated the mean assimilation efficiency α in HP to 98% for the dietary route. This corresponds to similarly high values reported for green crab with 91 ± 4% (Bjerregaard et al., 2005) and 81–96% (Pedersen et al., 2014). This means that almost all Cd administered in the feed was accumulated in HP. One factor facilitating the uptake from feed in the present study might be the chemical form of Cd. It has been shown earlier that trophic availability of Cd in crustaceans depends on the chemical form (Rainbow et al., 2011) and as the Cd in our study was spiked to the processed feed as watery solution, it was probably easier accessible than Cd in natural prey. However, as the assimilation efficiency in green crab fed with blue mussels exposed to Cd for spiking, was equally high (Bjerregaard et al., 2005), crab seems to have a high digestive power making it efficient in taking up Cd from diet.

To predict the relative importance of the uptake routes to the total accumulation of Cd in brown crab at different concentrations in diet and water in the field, we used a modelling approach based on estimated accumulation parameters for the two uptake routes. To be able to make a prediction for brown crab along the Norwegian coast, the respective CCR was estimated. Knowledge on the feeding habits of brown crab is limited and stomach analysis difficult due to the fact that prey items are masticated and ground in the gastric mill when entering the stomach. Therefore, analysis is prone to overestimation of animals holding parts difficult to grind and digest (Woll, 1995). Nevertheless, the two most frequently found feed items in the stomachs were blue mussel (*Mytilus edulis*) and horse mussel (*Modiolus modiolus*) (Woll, 1995), with mean Cd values along the Norwegian coast of 0.12 mg Cd/kg ww and 2.3 mg Cd/kg ww (Duinker et al., 2016) corresponding to about 0.75 and 11.4 mg/kg dw, respectively. In a recent investigation on Cd in seawater in the North of the Norwegian coastline, concentrations were measured to 0.05 ± 0.07 µg/L (mean ± SD, n = 18)) (Falk, 2015). Considering these data, an average CCR of 15 000 for blue mussels 228 000 for horse mussel can be expected, corresponding to an importance of diet to more than 99% for both cases according to the modelling (Fig. 4). An attempt to map the total range of Cd concentrations in the potential feed organisms for crabs in Northern Norway, found concentrations between 0.4 and 11 mg/kg dw (Ness, 2014) resulting in CCRs between 8000 and 220 000. This corresponds to a relative importance of the dietary route of at least 98% for all the considered CCRs. Therefore, we suggest, based on the output of our model, that the large difference in Cd between crabs from the South and North of the Norwegian coast, can rather be explained by differences in foraging than differences in water concentrations. The CCR for Cd between a crab's diet and seawater can be considered to be equally high in other regions of the brown crab's distribution. It is therefore reasonable to assume that the dietary route contributes most to the overall Cd uptake in brown crab in general. When investigating differences in Cd between different locations in decapods, it should therefore be focused on factors connected to foraging and feed preference, potentially connected to migratory patterns. Our findings suggest that dietary uptake of Cd in decapods should be investigated further and that knowledge on dietary exposure is crucial to understand Cd accumulation in crab.

Most studies comparing the relative importance of dietary and aqueous uptake assume steady state conditions (Lee & Fisher, 2016; Thomann, 1981). However, our modelling approach showed that the prediction of relative importance (Eq. (20)) is the same regardless of whether it is derived considering steady state conditions or not (k_e being zero). The assumption being that k_e is the same for both uptake routes, which is reasonable in crab, as Cd accumulated in HP will be tightly bound to MT regardless of origin (Pedersen et al., 2014; Pedersen et al., 1994).

When interpreting model outputs, uncertainty connected to the used input parameters and underlying assumptions has to be taken into account. The used ingestion rate was determined for crabs in captivity and feeding *ad libitum* on constantly present feed, not necessarily being representative *in situ* and also other factors like physiological state and temperature can influence *I* (Woll et al., 2006). For fish, there is evidence that dietary Cd uptake is regulated and the increase in uptake non-proportional to feed concentrations with a saturation at high concentrations (Douben, 1989; Reinfelder et al., 1998). The importance of the dietary route will then be over-estimated with increasing concentrations (Reinfelder et al., 1998). However, in green crab no sign of saturation in uptake was seen at Cd concentrations up to 5.1 mg/kg ww at a high feeding rate (Pedersen et al., 2014) and the aqueous uptake was increasing proportional over a wide range of exposure concentrations (Bjerregaard et al., 2005). The ingestion rate in itself might as well influence the α, although this seems not to be important in crab, as a similarly high α was found in crab exposed to Cd at a low *I* in this study, in comparison to crab feed at a much higher *I* (Bjerregaard et al., 2005; Pedersen et al., 2014). Also developmental stage and organism size were suggested to influence metal accumulation (Reinfelder et al., 1998). In the present study, the size of the experimental animals was determined by practical issues such as availability, demand of space and large enough size for gavage feeding. However, no clear relationship between size and Cd concentration was found in crabs of commercial size (Julshamn et al., 2012). Further accumulation might be influenced by other environmental and also physiological conditions of the crab.

4. Conclusion

Tracing stable isotopes is a suitable method to investigate the accumulation of trace metals in the same organism at the same time. Analytical challenges with background concentrations of natural isotope distribution and polyatomic interferences in the different matrices can be overcome with the right analytical setup and modern mathematical corrections using a computer software helping to solve equations. For the brown crab, we have shown that the dietary route is more important for the uptake of Cd in HP than aquatic route. The accumulation parameters, uptake rate constant from feed k_f and water k_w and assimilation efficiency α from feed were determined from the data of the conducted laboratory study using non-linear regression modelling. We applied the estimated parameters in a further modelling approach combined with naturally relevant concentrations in diet and seawater to determine the importance of the uptake routes. Considering naturally relevant concentrations, it is clear that the dietary pathway is far more important for the uptake of Cd into HP and thereby the whole body burden of brown crab.

Conflict of interests

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

Acknowledgements

The authors thank Dr. Knut Ladegaard Pedersen, University of Southern Denmark, Denmark for the technical support regarding the metallothionein measurements and Dr. Gro van der Meeren and Dr. Marc Berntssen, Institute of Marine Research, Norway for fruitful discussions regarding study planning. Further Seagarden AS, Avaldsnes, Norway is acknowledged for the donation of shrimp and fish powder. This work has been supported by the Norwegian Food Safety Authority.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.aquatox.2018.05.015.

References

Aldenberg, T., 2017. bcmfR_0.3-2.zip. OECD (Accessed 26 January 2018). http://www.oecd.org/chemicalsafety/testing/section-3-environmental-fate-behaviour-software-tg-305.htm.
Baker, J.R., 1955. Experiments on the humane killing of crabs. J. Mar. Biol. Assoc. United Kingdom 34 (01), 15–24.
Barrento, S., Marques, A., Teixeira, B., Carvalho, M.L., Vaz-Pires, P., Nunes, M.L., 2009. Accumulation of elements (S, As, Br, Sr, Cd, Hg, Pb) in two populations of *Cancer pagurus*: ecological implications to human consumption. Food Chem. Toxicol. 47 (1), 150–156.
Baty, F., Ritz, C., Charles, S., Brutsche, M., Flandrois, J.-P., Delignette-Muller, M.-L., 2015. A toolbox for nonlinear regression in R: the package nlstools. J. Statistical Softw. 66 (5), 1–21.
Bjerregaard, P., 1990. Influence of physiological condition on cadmium transport from haemolymph to hepatopancreas in *Carcinus maenas*. Mar. Biol. 106 (2), 199–209.
Bjerregaard, P., Bjørn, L., Nørum, U., Pedersen, K.L., 2005. Cadmium in the shore crab *Carcinus maenas*: seasonal variation in cadmium content and uptake and elimination of cadmium after administration via food. Aquat. Toxicol. 72 (1), 5–15.
Bjerregaard, P., Depledge, M., 1994. Cadmium accumulation in *Littorina littorea, Mytilus edulis* and *Carcinus maenas*: the influence of salinity and calcium ion concentrations. Mar. Biol. 119 (3), 385–395.
Bjerregaard, P., Depledge, M., 2002. Trace metal concentrations and contents in the tissues of the shore crab *Carcinus maenas*: effects of size and tissue hydration. Mar. Biol. 141 (4), 741–752.
Canli, M., Furness, R.W., 1995. Mercury and cadmium uptake from seawater and from food by the Norway lobster *Nephrops norvegicus*. Environ. Toxicol. Chem. 14 (5), 819–828.
Cresswell, T., Mazumder, D., Callaghan, P.D., Nguyen, A., Corry, M., Simpson, S.L., 2017. Metal transfer among organs following short-and long-term exposures using autoradiography: cadmium bioaccumulation by the Freshwater prawn *Macrobrachium australiense*. Environ. Sci. Technol. 51 (7), 4054–4060.
Croteau, M.-N., Luoma, S.N., Topping, B.R., Lopez, C.B., 2004. Stable metal isotopes reveal copper accumulation and loss dynamics in the freshwater bivalve Corbicula. Environ. Sci. Technol. 38 (19), 5002–5009.
Davies, I.M., Topping, G., Graham, W.C., Falconer, C.R., McIntosh, A.D., Saward, D., 1981. Field and experimental studies on cadmium in the edible crab *Cancer pagurus*. Mar. Biol. 64 (3), 291–297.
Douben, P.E., 1989. Metabolic rate and uptake and loss of cadmium from food by the fish *Noemacheilus barbatulus* L. (stone loach). Environ. Pollut. 59 (3), 177–202.
Duinker, A., Lunestad, B.T., I.S, R., Maage, A., 2016. Nasjonalt tilsynsprogram for produksjon av skjell og andre bløtdyr – prøver analysert i 2015: Kjemiske forurensende stoffer og mikroorganismer. National Institute of Nutrition and Seafood Research (NIFES), Bergen Norwegian Report, pp. 39.
Falk, A.H., 2015. "Heavy metal crabs"; Dyphavsvann - en kilde til Cd i taskekrabbe fra Nordland? Akvaplan-niva rapport, vol. 6819-01 Akvaplan-niva Norwegian Report, pp. 40.
FAO, 2018. Species Fact Sheets: Cancer pagurus (Linnaeus, 1758). FAO Fisheries and Aquaculture Department. http://www.fao.org/fishery/species/2627/en.
Fisher, N.S., Reinfelder, J.R., 1995. The trophic transfer of metals in marine systems. In: Tessier, A., Turner, D.R. (Eds.), Metal Speciation and Bioavailability in Aquatic Systems. John Wiley & Sons, London, UK, pp. 363–406.
Haig, J.A., Bakke, S., Bell, M.C., Bloor, I.S., Cohen, M., Coleman, M., Dignan, S., Kaiser, M.J., Pantin, J.R., Roach, M., 2016. Reproductive traits and factors affecting the size at maturity of *Cancer pagurus* across Northern Europe. ICES J. Mar. Sci. 73 (10), 2572–2585.
Harrison, S., Klaverkamp, J., 1989. Uptake, elimination and tissue distribution of dietary and aqueous cadmium by rainbow trout (*Salmo gairdneri* Richardson) and lake whitefish (*Coregonus clupeaformis* Mitchill). Environ. Toxicol. Chem. 8 (1), 87–97.
Julshamn, K., Nilsen, B.M., Valdersnes, S., Frantzen, S., 2012. Årsrapport 2011. Mattilsynets program: Fremmedstoffer i villfisk med vekt på kystnære farvann: Delrapport I: Undersøkelser av miljøgifter i taskekrabbe. National Institute of Nutrition and Seafood Research (NIFES), Bergen Norwegian Report.
Knutsen, H., Wiech, M., Duinker, A., Maage, A., 2018. Cadmium in the shore crab *Carcinus maenas* along the Norwegian coast: geographical and seasonal variation and correlation to physiological parameters. Environ. Monitor. Assess. 190 (4), 253.
Komjarova, I., Blust, R., 2008. Multi-metal interactions between Cd, Cu, Ni, Pb and Zn in water flea *Daphnia magna*, a stable isotope experiment. Aquat. Toxicol. 90 (2), 138–144.
Komjarova, I., Blust, R., 2009. Effect of Na, Ca and pH on simultaneous uptake of Cd, Cu, Ni, Pb, and Zn in the water flea *Daphnia magna* measured using stable isotopes. Aquat. Toxicol. 94 (2), 81–86.
Lee, C.-S., Fisher, N.S., 2016. Bioaccumulation of methylmercury in a marine copepod. Environ. Toxicol. Chem. 36 (5), 1287–1293.
Luoma, S.N., Rainbow, P.S., 2005. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. Environ. Sci. Technol. 39 (7), 1921–1931.
Maulvault, A.L., Anacleto, P., Lourenço, H.M., Carvalho, M.L., Nunes, M.L., Marques, A., 2012. Nutritional quality and safety of cooked edible crab (*Cancer pagurus*). Food Chem. 133 (2), 277–283.
Ness, J., 2014. Cadmium Concentrations of Macrofauna in the Salten Region, Northern Norway. Unpublished MSc thesis. University of Nordland, Tromsø, Norway.
Noël, L., Chafey, C., Testu, C., Pinte, J., Velge, P., Guérin, T., 2011. Contamination levels of lead, cadmium and mercury in imported and domestic lobsters and large crab species consumed in France: differences between white and brown meat. J. Food Compos. Anal. 24 (3), 368–375.
OECD, 2012. Test No. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure. OECD Publishing, Paris.
OECD, 2017. Guidance document on aspects of OECD TG 305 on Fish bioaccumulation. Environment, Health and Safety Publications—Series on Testing and Assessment. OECD, Paris pp. 132.
Overnell, J., 1986. Occurrence of cadmium in crabs (*Cancer pagurus*) and the isolation and properties of cadmium metallothionein. Environ. Health Perspect. 65, 101.
Pedersen, K.L., Bach, L.T., Bjerregaard, P., 2014. Amount and metal composition of midgut gland metallothionein in shore crabs (*Carcinus maenas*) after exposure to cadmium in the food. Aquat. Toxicol. 150, 182–188.
Pedersen, K.L., Pedersen, S.N., Højrup, P., Andersen, J.S., Roepstorff, P., Knudsen, J., Depledge, M., 1994. Purification and characterization of a cadmium-induced metallothionein from the shore crab *Carcinus maenas* (L.). Biochem. J. 297 (3), 609–614.
Pedersen, S., Lundebye, A.-K., Depledge, M., 1997. Field application of metallothionein and stress protein biomarkers in the shore crab (*Carcinus maenas*) exposed to trace metals. Aquat. Toxicol. 37 (2), 183–200.
Pedersen, S.N., Pedersen, K.L., Højrup, P., Knudsen, J., Depledge, M.H., 1998. Induction and identification of cadmium-, zinc-and copper-metallothioneins in the shore crab *Carcinus maenas* (L.). Comp. Biochem. Physiol. Part. C: Pharmacol., Toxicol. Endocrinol. 120 (2), 251–259.
Rainbow, P., Black, W., 2005a. Cadmium, zinc and the uptake of calcium by two crabs, *Carcinus maenas* and *Eriocheir sinensis*. Aquat. Toxicol. 72 (1), 45–65.
Rainbow, P., Black, W., 2005b. Physicochemistry or physiology: cadmium uptake and effects of salinity and osmolality in three crabs of different ecologies. Mar. Ecol. Prog. Ser. 286, 217–229.
Rainbow, P.S., Luoma, S.N., Wang, W.-X., 2011. Trophically available metal—a variable feast. Environ. Pollut. 159 (10), 2347–2349.
Reinfelder, J.R., Fisher, N.S., Luoma, S.N., Nichols, J.W., Wang, W.-X., 1998. Trace element trophic transfer in aquatic organisms: a critique of the kinetic model approach. Sci. Total Environ. 219 (2), 117–135.
Ritz, C., Streibig, J.C., 2008. Nonlinear Regression With R. Springer Science & Business Media.
Rubin, D.B., 1981. The bayesian bootstrap. Ann. Stat. 9 (1), 130–134.
Rubin, D.B., 1987. Multiple Imputation for Nonresponse in Surveys. John Wiley & Sons.
Strady, E., Schäfer, J., Baudrimont, M., Blanc, G., 2011. Tracing cadmium contamination kinetics and pathways in oysters (*Crassostrea gigas*) by multiple stable Cd isotope spike experiments. Ecotoxicol. Environ. Safe 74 (4), 600–606.
Søvik, G., Jenssen, M., Hjelset, A.M., Krogness, C., 2017. Ressursundersøkelse av taskekrabbe langs norskekysten—Rapport fra fangstregistreringer i 2001-2015. Rapport fra havforskningen, vol. 25-2017 Institute of Marine Research Norwegian Report, pp. 14.
Thomann, R.V., 1981. Equilibrium model of fate of microcontaminants in diverse aquatic food chains. Can. J. Fish. Aquat. Sci. 38 (3), 280–296.
Viarengo, A., Ponzano, E., Dondero, F., Fabbri, R., 1997. A simple spectrophotometric method for metallothionein evaluation in marine organisms: an application to Mediterranean and Antarctic molluscs. Mar. Environ. Res. 44 (1), 69–84.
Wang, W.-X., Fisher, N.S., Luoma, S.N., 1996. Kinetic determinations of trace element bioaccumulation in the mussel *Mytilus edulis*. Mar. Ecol. Prog. Ser. 140, 91–113.
Warner, G.F., 1977. Biology of Crabs: Elek.
WHO/FAO, 2012. CODEX ALIMENTARIUS - Code of Practice for Fish and Fishery Products, vol. 2 WHO/FAO, Rome.
Wicklund Glynn, A., Andersson, L., Gabring, S., Runn, P., 1992. Cadmium turnover in minnows, Phoxinus phoxinus, fed 109Cd-labeled Daphnia magna. Chemosphere 24 (3), 359–368.
Wiech, M., Vik, E., Duinker, A., Frantzen, S., Bakke, S., Maage, A., 2017. Effects of cooking and freezing practices on the distribution of cadmium in different tissues of the brown crab (*Cancer pagurus*). Food Control 75, 14–20.
Woll, A., 1995. Ressursbiologisk undersøkelse av taskekrabben (Cancer pagurus) i Møre og Romsdal, vol. Å9506 Møreforsking Ålesund Norwegian Report, pp. 54.
Woll, A.K., Van Der Meeren, G.I., Tuene, S., 2006. Quality improvement by feeding wild-caught edible crab (*Cancer pagurus* L.): a pilot study. Aquacult. Res. 37 (14), 1487–1496.