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

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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

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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