

# Cadmium accumulation in the snow crab *Chionoecetes opilio*

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**ABSTRACT:** The aim of this work is to evaluate the significance of Cd uptake via food in the snow crab *Chionoecetes opilio*. We used *in vivo* gamma counting to measure the retention efficiency of a single dietary dose of <sup>109</sup>Cd(II) and follow elimination kinetics of the metal retained over a 5 mo period. Whole-body autoradiography was used to determine the fine-scale tissue distribution. The biological half-life of Cd retained in the body after the first 15 d, which represented 26 to 52% of the ingested dose, ranged from 141 to 346 d. Whole-body autoradiography showed that <sup>109</sup>Cd was distributed to all the tissues, highest concentrations being found in the hepatopancreas and gut, followed in decreasing order by antennal glands ≥ hemolymph > gonads > gills ≈ hypodermis ≈ muscle > eye. We used these data and a reasonable estimate of Cd levels in benthic organisms of lower trophic levels living in the Estuary and Gulf of St. Lawrence to model the long-term dietary uptake of Cd in snow crab. Predicted concentrations in hepatopancreas and muscle agreed quite well with field data (predicted range 13.0 to 39.2 nmolCd g<sup>-1</sup> and field range 3.5 to 24.1 nmolCd g<sup>-1</sup> for hepatopancreas; predicted range 0.20 to 1.33 nmolCd g<sup>-1</sup> and field range 0.52 to 2.03 nmolCd g<sup>-1</sup> in muscle). The similarity between experimentally based predictions and field data strongly suggests that diet is a major transfer route of Cd towards snow crab in the St. Lawrence.

**KEY WORDS:** Uptake · Food · Snow crab · Cadmium · Pharmacokinetics · Distribution · Whole-body autoradiography · *In vivo* gamma counting

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## INTRODUCTION

Cadmium is one of the trace metals for which spatial distribution in marine sediments exhibits the largest variations, both horizontally and vertically, as a result of natural diagenetic processes (Rosenthal et al. 1995, Gobeil et al. 1997) or pollution. The extent of the accumulation of sedimentary Cd in benthic sediment-dwellers and deposit-feeders has been shown to be related to the amount of labile Cd in the sediments (Lee et al. 2000a,b,c), which can then be transferred

via food towards benthic predators of higher trophic levels, such as flatfish and decapod crustaceans.

Decapod crustaceans are well known accumulators of cadmium (Rainbow 1988, Ozretic et al. 1990, Everaarts & Nieuwenhuize 1995, Kannan et al. 1995). Most of the research work published to date about Cd accumulation routes in large marine crustaceans, such as shrimp and crabs, has targeted the direct uptake of the metal from water (Bjerregaard 1985, 1990, 1991, Bjerregaard & Depledge 1994, Martin & Rainbow 1998, Rainbow et al. 1999). But uptake via food may also be an important route (Fowler 1982). Jennings & Rainbow (1979) found that the crab *Carcinus maenas* accumulated 10% of the Cd available from its food, but could not estimate the relative importance of the dietary uptake route to the total Cd accumulation. In

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another study, Davies et al. (1981) determined the distribution of Cd within captive crabs (*Cancer pagurus*) exposed experimentally to Cd-contaminated food and water. They compared these experimental results with the distribution of Cd within field-collected crabs and concluded that the dominant uptake route of cadmium *in situ* was through the diet. However, in order to be able to assess Cd concentration in the tissues of a given decapod crustacean as a function of varying Cd levels in its food, a quantitative knowledge of the relationship existing between the two is required (Landrum et al. 1992).

The snow crab *Chionoecetes opilio* (Brachyura, Majidae), is a common predator on mud and sand-mud substratum in cold waters along the Atlantic and Pacific Canadian coasts (Bailey & Elner 1989, Lovrich & Sainte-Marie 1997), whose diet mainly consists of benthic crustaceans, echinoderms, polychaetes, molluscs (70 to 80%), and demersal fish (10 to 15%) (Br thes et al. 1994, Lovrich & Sainte-Marie 1997). In the present work, we studied the pharmacokinetics and tissue distribution of a single dietary dose of radio-labelled  $^{109}\text{Cd}(\text{II})$  in the snow crab over a 5 mo period, under environmental conditions similar to those encountered in the cold coastal waters this species inhabits. Retention efficiency and elimination rate were quantified with *in vivo* gamma counting, a technique allowing repeated monitoring of the activity of radioactive metal isotopes in the same individuals (Rouleau et al. 2000). The fine-scale tissue distribution of  $^{109}\text{Cd}$  was determined by whole-body autoradiography (Ullberg et al. 1982). Data obtained from this laboratory study were used to model the trophic transfer of Cd in snow crab. Modeling results were then compared to Cd concentrations in snow crabs sampled in the Estuary and Gulf of St. Lawrence and in the Saguenay Fjord.

## MATERIALS AND METHODS

**Laboratory experiments.** Mature female snow crabs were caught in October 1996 in the Estuary and Gulf of St. Lawrence and kept in large tanks supplied with aerated filtered flowing seawater under conditions of natural photoperiod and ambient water temperature. They were fed twice a week with shrimp and/or chopped capelin. This feeding frequency has proven successful in maintaining crabs in our laboratory for up to 2 yr.

Six of these crabs (50 to 80 g, 50 to 60 mm shell width) were transferred to 60 l aquaria provided with running seawater (3 to 3.5°C) 1 wk before the beginning of experiments in November 1996. Two crabs were housed in each aquarium. The day before

the experiment started, supplemented fish food (Provencher et al. 1995) was spiked with  $^{109}\text{Cd}(\text{II})$  (New England Nuclear, 10.5 MBq  $\mu\text{mol}^{-1}$ ,  $t_{1/2} = 462.6$  d), thoroughly homogenised for 5 min, and moulded in small balls weighing 0.3 g. Spiked food was kept at 4°C overnight to allow for the binding of Cd to food components, such as proteins.

On Day 0, crabs were given 1 ball of  $^{109}\text{Cd}$ -spiked food each (0.46 MBq, 44 nmol) that they ate almost completely within 10 min. Water in the aquarium was then changed immediately to avoid its contamination by radioactive Cd leaching from food particles. The radioactivity of the crabs was first monitored 1 h after feeding (see below). The animals were then fed with uncontaminated food as mentioned above from Day 3 until the end of the experiments. Weight of organisms during the experiment did not vary.

The rate of water renewal in the aquaria was kept high enough (1.5 to 2.0 l  $\text{min}^{-1}$ ) to approximate an open system. Water samples (3 ml) were collected at least twice a week throughout the experiments and their radioactivity was monitored for 10 min with an LKB Clinigamma counter. Radioactivity in all water samples was below the detection limit of 0.05 Bq  $\text{ml}^{-1}$ .

Whole-body  $^{109}\text{Cd}$  activity was repeatedly monitored over 154 d, using an *in vivo* gamma counting system consisting in a 3 inch (ca 76 mm) NaI(Tl) gamma detector (Cannberra-Packard, Meriden, CT) fitted into a 2 cm thick cylindrical Pb shield with a 0.6 cm thick copper-brass inside lining (Rouleau et al. 1998, Rouleau et al. 2000). The detector-shield assembly was fixed onto a specially designed stand and placed at  $40 \pm 0.5$  mm above the crabs, which were placed upside down, motionless, and positioned on a target drawn on plastic plate cut to the exact size of the base of the stand. The 88 keV  $\gamma$ -ray emission of  $^{109}\text{Cd}$  was monitored for 1 to 3 min daily during the first 10 d and every 2 to 4 d thereafter. Crabs did not appear to be affected by the repeated manipulations needed for gamma counting, since they fed normally, behaved normally (no unusual activity that may have been indicative of stress), and their physical aspect did not change.

$^{109}\text{Cd}$  activity was quantified with Genie-PC Gamma Analysis software (Cannberra-Packard). After corrections for background and decay, activity data were standardised by expressing them as a percentage of the activity measured at the beginning of the experiment. The average statistical counting error was 1.3%. The error due to variations in the positioning of animals under the detector, expressed as a coefficient of variation of 5 replicate measurements, was 2%.

At the end of the experiments, all animals were sacrificed and dissected. The hepatopancreas was collected, weighed, uniformly spread over the surface of a

60 mm Petri dish, and its radioactivity measured. Legs were separated from the body and radioactivity measured as above. Radioactivity of the body, of which the size and shape was similar to that of the Petri dish used, was counted and this value added to the value obtained from the legs. Data obtained were used to calculate the percentage of the Cd body burden contained in the hepatopancreas and in the rest of the body (body + legs). The concentration index,  $I_C$ , was then calculated as:

$$I_C = \frac{\% \text{ of body burden } ({}^{109}\text{Cd activity in tissue}/{}^{109}\text{Cd activity in whole body} \times 100)}{\% \text{ of body weight (g tissue/g whole body} \times 100)} \quad (1)$$

which is equivalent to the ratio of the concentration of  ${}^{109}\text{Cd}$  in the tissue over the whole-body averaged:

$$I_C = \frac{[{}^{109}\text{Cd}] \text{ in tissue}}{[{}^{109}\text{Cd}] \text{ in whole body}} \quad (2)$$

Values of  $I_C > 1$  indicate that a tissue is enriched in  ${}^{109}\text{Cd}$  compared to the average whole-body metal concentration.

Additional snow crabs were used for whole-body autoradiography (Ullberg et al. 1982). Four snow crabs were fed as mentioned above and 2 of them were sampled at each of Days 3 and 14. Immediately after

removal from their aquaria, the animals were embedded in a carboxymethylcellulose gel and quickly frozen in a slurry of hexane and dry ice. Resulting blocks were sectioned on tape (50  $\mu\text{m}$  thick sections) at  $-20^\circ\text{C}$  with a specially designed cryomicrotome (Jung Cryomacrocut, Leica). Tissue sections were then freeze-dried and applied to an X-ray film ( ${}^3\text{H}$ -Hyperfilm, Amersham) for 40 d, at  $-20^\circ\text{C}$ .

**Field measurements.** Mature adult female ( $n = 32$ , body weight =  $82 \pm 13$  g, carapace width =  $62 \pm 3$  mm) and male ( $n = 60$ , body weight =  $580 \pm 169$  g, carapace width =  $112 \pm 10$  mm) snow crabs were caught in 1998 and 1999 in the Saguenay Fjord, the St. Lawrence Estuary, and the northeastern part of the Gulf of St. Lawrence (Fig. 1). The size difference reflects the important sexual dimorphism in this species. The organisms were immediately measured, weighed, and stored at  $-20^\circ\text{C}$  in clean plastic bags. In the laboratory, muscle and hepatopancreas were collected, homogenized, freeze-dried, and digested in a microwave oven with nitric acid (Nakashima et al. 1988). Cd concentration in the tissues was determined by atomic absorption spectroscopy using a graphite furnace. The detection limit, estimated as 3 times the standard deviation of measures made on analytical blanks, was  $0.02 \text{ nmol Cd g}^{-1}$  wet wt. Biological reference material DORM-1 from

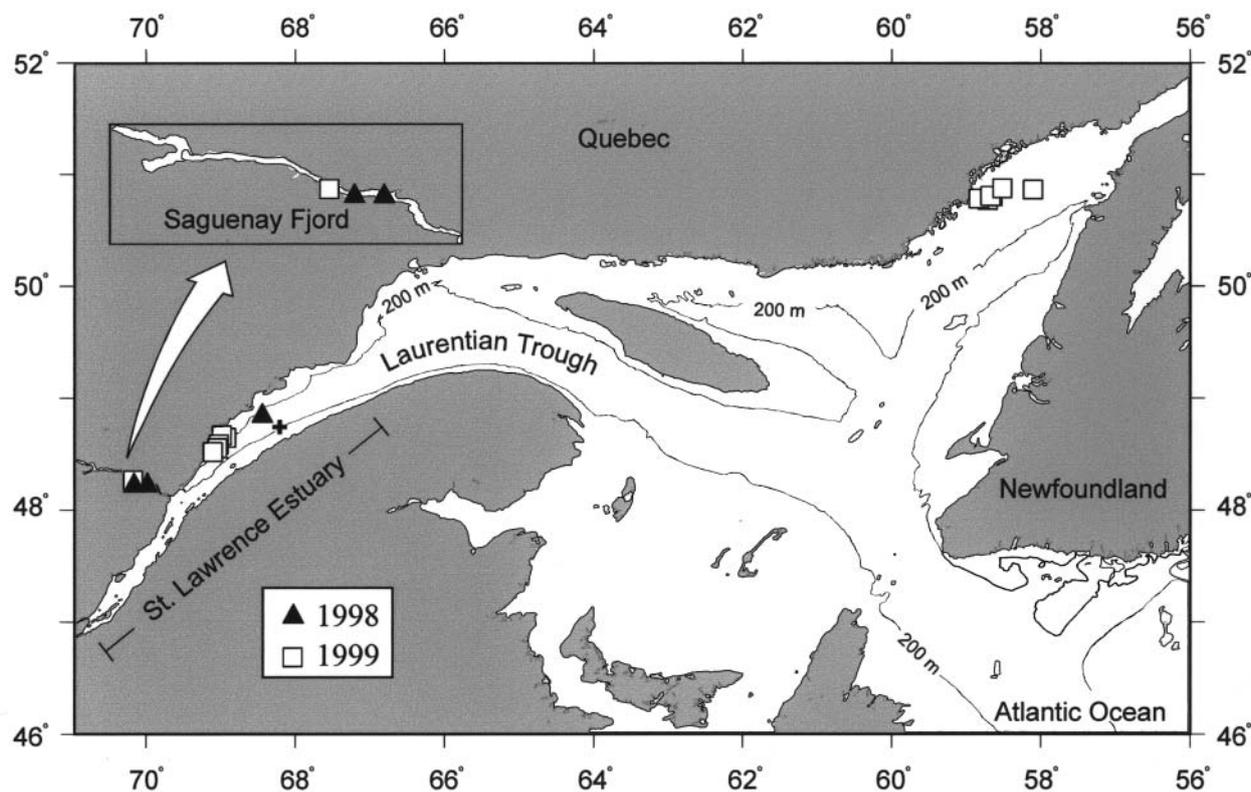


Fig. 1. Location of sites of snow crab captures for the field survey. + indicates the site of capture of crabs used in the laboratory experiment

the National Research Council of Canada having a certified Cd concentration of  $0.77 \pm 0.11 \text{ nmol g}^{-1}$  dry wt was used to assess the quality of our analyses. The precision was 6% and analytical accuracy was always within prescribed limits ( $n = 15$ ).

Cd concentrations were compared according to sampling location and sex. Values of standard skewness and standard kurtosis were outside the prescribed range for some of the groups. Also, variance significantly differed from one group to another, even for log-transformed data. Thus, we use the Kruskal-Wallis test to compare median Cd levels between sexes for a given sampling site and between sampling sites.

## RESULTS

### Pharmacokinetics

The temporal change of  $^{109}\text{Cd}$  activity in the snow crabs is illustrated in Fig. 2. Activity sharply decreased at Days 2 to 3 as non-retained Cd was eliminated with faeces. After defecation,  $^{109}\text{Cd}$  elimination proceeded at a slower rate. Linear regression analysis of  $^{109}\text{Cd}$

activity,  $\text{Cd}_t$ , plotted as a function of time ( $t$ ) was first used to calculate the retention efficiency,  $\text{RE}_0$  (i.e. the percentage of the dose ingested that was retained within the body after the elimination of non-retained Cd with faeces;  $0 \leq \text{RE}_0 \leq 100\%$ ), and the first order elimination rate constant,  $\beta$ , using:

$$\ln \text{Cd}_t = \ln \text{RE}_0 - \beta t \quad (3)$$

However, elimination kinetic was biexponential for Crabs 2, 3, and 6; activity decreased faster in the first 20 to 25 d than during the interval between Days 25 and 154. In these cases, non-linear regression analysis was used with the following biexponential equation:

$$\text{Cd}_t = A_0 e^{-\alpha t} + B_0 e^{-\beta t} \quad (4)$$

where  $\alpha$  and  $\beta$  are first order rate constants characterising the fast and slow eliminating pools respectively, whereas  $A_0$  and  $B_0$  are the initial amounts of radioactivity in each pool ( $A_0 + B_0 = \text{RE}_0$ ).

On average, Crabs 2, 3, and 6 retained 75% of the Cd dose ingested (Table 1). About half of the dose retained was eliminated rapidly within 8 to 20 d, as calculated from the value of  $\alpha$  (Table 1). The propor-

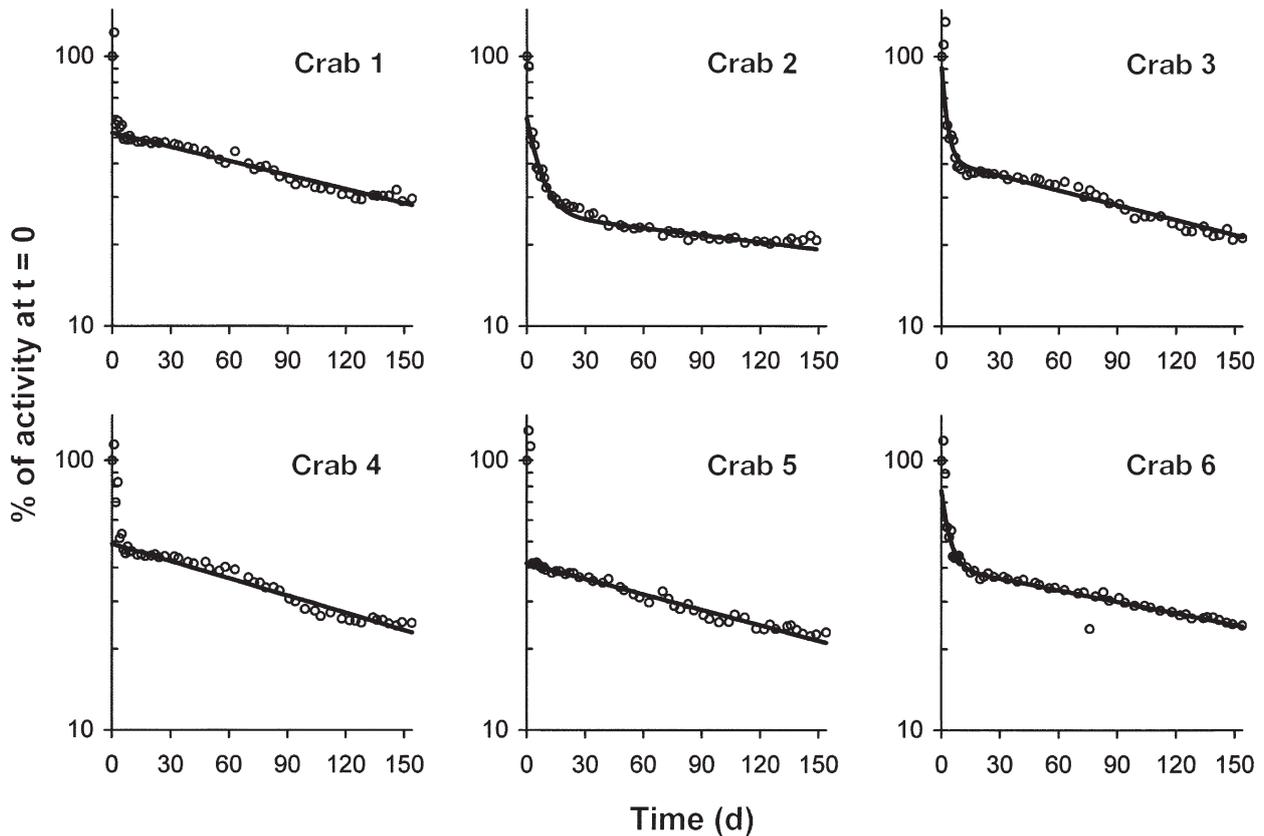


Fig. 2. *Chionoecetes opilio*. Change of  $^{109}\text{Cd}$  activity measured over 154 d in female crabs. Data are standardised as a percentage of the radioactivity measured immediately after feeding

Table 1. *Chionoecetes opilio*. Kinetic parameters of dietary  $^{109}\text{Cd}(\text{II})$ . Values ( $\pm\text{SE}$ ) of  $A_0$ ,  $B_0$ ,  $\text{RE}_0$ , and rate constants  $\alpha$  and  $\beta$  were calculated from experimental data, excluding data from Days 0 to 3. Time to eliminate 95% of pool A and B,  $t_{0.95\alpha}$  and  $t_{0.95\beta}$ , and biological half-life of pool B,  $t_{0.5\beta}$ , were calculated with 2.996/rate constant and 0.693/rate constant respectively

	$A_0$ (%)	$B_0$ (%)	$\text{RE}_0$ (%)	$\alpha$ ( $\text{d}^{-1}$ )	$t_{0.95\alpha}$ (d)	$\beta$ ( $\text{d}^{-1}$ )	$t_{0.5\beta}$ (d)	$t_{0.95\beta}$ (d)	$r^2$
Crab 1	–	–	$52.1 \pm 0.4$	–	–	$0.0040 \pm 0.0001$	173	750	0.97
Crab 2	$33.3 \pm 1.8$	$26.4 \pm 0.7$	$59.7 \pm 2.5$	$0.148 \pm 0.014$	20	$0.0020 \pm 0.0003$	346	1500	0.97
Crab 3	$50.2 \pm 13.0$	$40.9 \pm 0.7$	$90.7 \pm 13.7$	$0.379 \pm 0.070$	8	$0.0042 \pm 0.0002$	165	710	0.96
Crab 4	–	–	$49.2 \pm 0.5$	–	–	$0.0049 \pm 0.0001$	141	610	0.97
Crab 5	–	–	$41.5 \pm 0.3$	–	–	$0.0044 \pm 0.0001$	158	680	0.98
Crab 6	$37.4 \pm 6.6$	$40.3 \pm 0.7$	$77.7 \pm 7.3$	$0.259 \pm 0.044$	12	$0.0033 \pm 0.0001$	210	910	0.96

tion of the initial metal dose then retained in the slow pool,  $B_0$ , was similar to the values of  $\text{RE}_0$  found for the three other crabs (26 to 52%). Biological half-life values ( $t_{0.5\beta}$ ) of Cd varied from 141 to 346 d.

### Tissue distribution.

Whole-body autoradiograms are shown in Fig. 3. To compare the radiolabelling of the various tissues semi-quantitatively, the autoradiograms were digitised as black and white images with 256 values of grey, and average grey value (AGV) of the various areas was determined (Table 2).

Three days after the administration of  $^{109}\text{Cd}$  with food, most of the radioactive metal was located in the hepatopancreas (Fig. 3). The AGV of 247 (Table 2) corresponds to a complete saturation of the X-ray film. The very high level of radioactivity in the hepatopancreas resulted in some fogging of the X-ray film that renders difficult the evaluation of the relative labelling of other tissues. This is well illustrated by the fact that the AGV for the respiratory chambers, which contain only water and should not be labelled, was higher than

that of background. Nevertheless, it can be seen that some of the radiolabel has already been transferred toward the other tissues, e.g. the antennal glands, the gills, and the hypodermis. However, areas corresponding to the foregut, muscle, and gonads had AGV values similar to those of tissues mentioned above (Table 2), although there was no evident labelling visible in the autoradiogram (Fig. 3). There was no labelling noticeable for the spermathecae and the exoskeleton.

The distribution picture is clearer in the autoradiograms obtained 14 d after feeding. Fogging of the film was less important and the AGV for the respiratory chambers was similar to background. This allows a better discrimination of the radioactivity level in tissues other than gut and hepatopancreas, for which the concentration of  $^{109}\text{Cd}$  decreased in the order antennal glands  $\geq$  hemolymph  $>$  gonads  $>$  gills  $\approx$  hypodermis  $\approx$  muscle  $>$  eye. The spermathecae and the exoskeleton were not noticeably labelled. The 40 d exposure of tissue sections to film still resulted in a saturation of the film in areas corresponding to hepatopancreas. Such saturation was also seen for the foregut, midgut, and hindgut.

Table 2. *Chionoecetes opilio*. Average grey value (AGV)  $\pm$  SD (1 = black, 256 = white) and tissue/background ratio for various areas of the digitised whole-body autoradiograms shown in Fig. 3. Values within brackets are the number of pixels quantified

	$t = 3 \text{ d}$		$t = 14 \text{ d}$		
	AGV	Tissue/background	AGV	Tissue/background	
Background	$34 \pm 17$ [139700]	–	Background	$2 \pm 4$ [47500]	–
Respiratory chambers	$60 \pm 8$ [2840]	1.8	Respiratory chambers	$2 \pm 3$ [1790]	1
Foregut	$94 \pm 13$ [4350]	2.8	Foregut	$245 \pm 14$ [7050]	122
Hepatopancreas	$247 \pm 3$ [33830]	7.3	Hepatopancreas	$246 \pm 13$ [18100]	123
Gonads	$90 \pm 6$ [5970]	2.6	Gonads	$61 \pm 19$ [8790]	30
Gills	$66 \pm 12$ [11070]	1.9	Gills	$34 \pm 11$ [2960]	17
Hypodermis	$69 \pm 11$ [2220]	2.0	Hypodermis	$24 \pm 8$ [1020]	12
Antennal gland	$100 \pm 12$ [2120]	2.9	Antennal gland	$119 \pm 17$ [1210]	59
Muscle	$82 \pm 10$ [3760]	2.4	Muscle	$21 \pm 9$ [2750]	10
Hemolymph	–	–	Hemolymph	$91 \pm 20$ [380]	45
Eye	–	–	Eye	$13 \pm 7$ [2230]	6

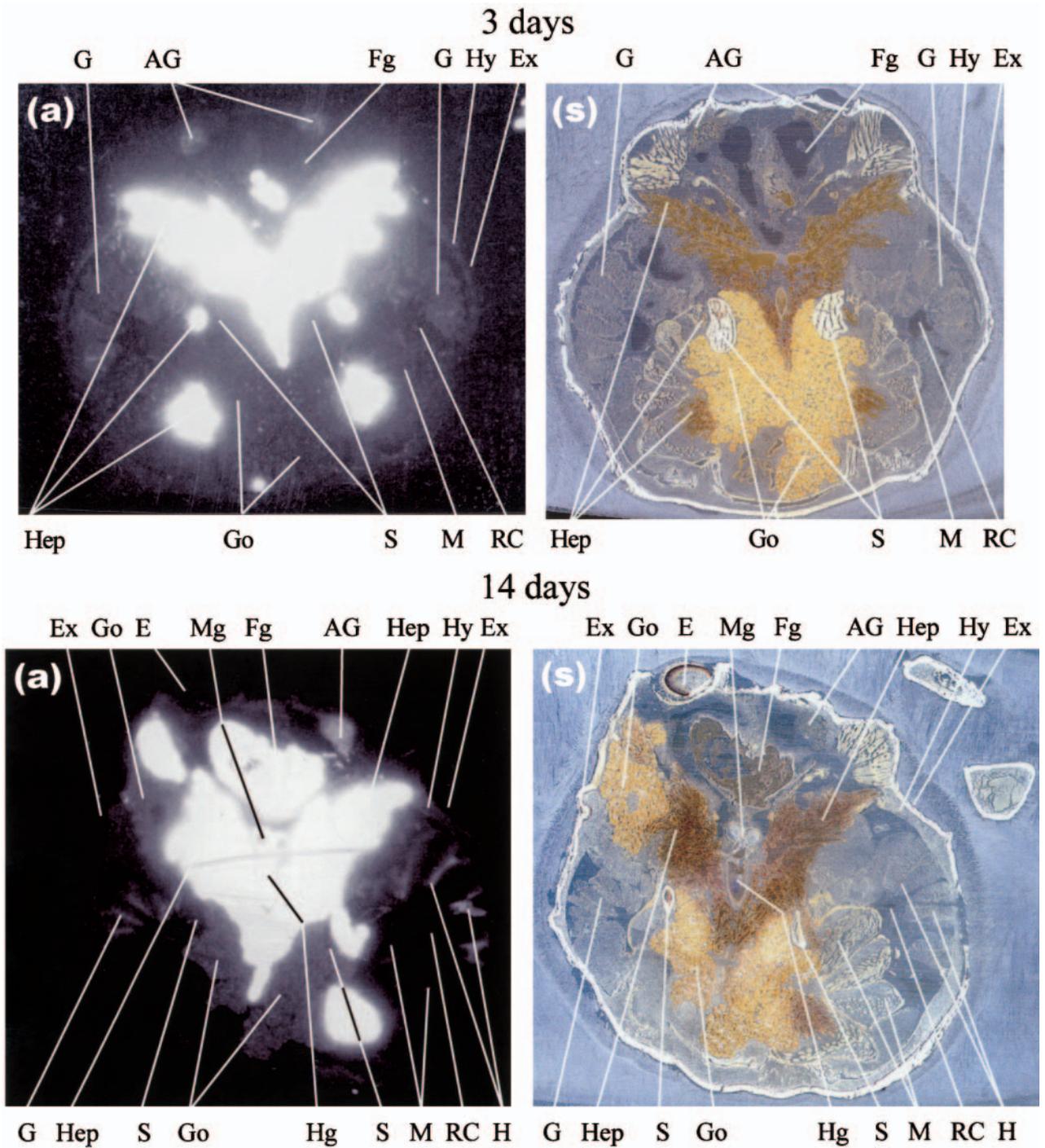
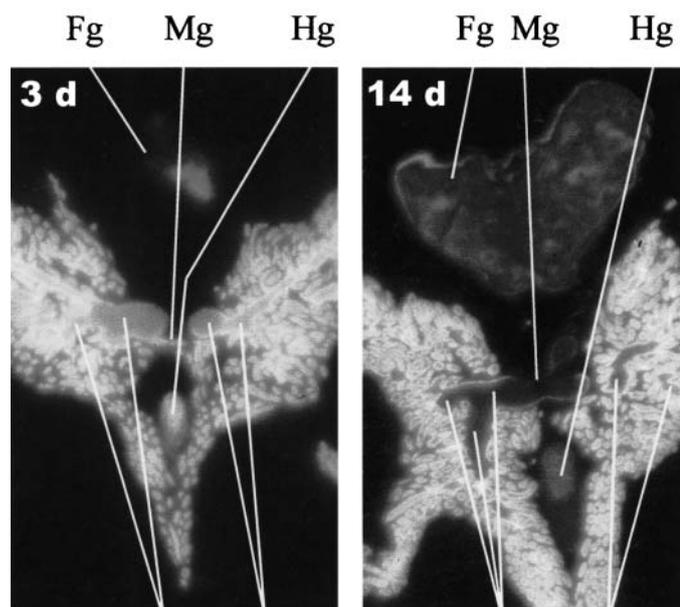


Fig. 3. *Chionoecetes opilio*. Whole-body autoradiograms from female snow crab sampled 3 and 14 d after feeding with a single dietary dose of  $^{109}\text{Cd}$ . (a) Autoradiogram, (s) corresponding tissue section. White areas correspond to high concentrations of the radiolabel. Exposure time: 40 d. AG = antennal gland, E = eye, Ex = exoskeleton, Fg = foregut, G = gills, Go = gonads, H = hemolymph, Hep = hepatopancreas, Hg = hindgut, Hy = hypodermis, Mg = midgut, M = muscle, RC = respiratory chamber, S = spermathecae

To have a better view of the relative labelling of gut and hepatopancreatic tissues, some autoradiograms were applied again to film for 1 d only (Fig. 4). This allows visual confirmation that  $^{109}\text{Cd}$  concentrations

were the highest in hepatopancreatic tissues at Day 14. The labelling of all parts of the gut was less intense. It is noteworthy that the labelling of hepatopancreatic diverticula wall is higher than that of the lumen.



### Lumen of hepatopancreas diverticulae

Fig. 4. *Chionoecetes opilio*. Detail of whole-body autoradiograms from female snow crab sampled 3 and 14 d after feeding with a single dietary dose of  $^{109}\text{Cd}$ . Exposure time: 1 d. Fg = foregut, Hg = hindgut, Mg = midgut

For crabs sampled at Day 3, the labelling of hindgut lumen and hepatopancreatic diverticula lumen relative to hepatopancreatic tissues was higher than at Day 14.

At Day 154 (Fig. 5), the hepatopancreas contained almost 70% of the body burden of  $^{109}\text{Cd}$ , whereas the rest of body accounted for the remaining 30%. Because of its small size compared to the rest of the body, average value of  $I_C$  for hepatopancreas was an order of magnitude higher ( $I_C = 7.5 \pm 0.8$  and  $0.36 \pm 0.07$ , respectively). This distribution is similar to that observed in autoradiograms at Day 14, indicating that the distribution of dietary  $^{109}\text{Cd}$  within the body of the snow crabs rapidly reached equilibrium.

### Cd in snow crabs from the St. Lawrence and the Saguenay fjord

Field data are shown in Fig. 6. Similar cadmium concentrations were found in the hepatopancreas of snow crabs of both sexes sampled in the Estuary and Gulf of St. Lawrence (range 3.5 to 32.9 nmolCd  $\text{g}^{-1}$  wet wt). Cadmium levels in the hepatopancreas of male crabs from the Saguenay (range 6.0–88.2 nmolCd  $\text{g}^{-1}$  wet wt) were significantly higher compared to the other groups (Kruskal-Wallis test,  $p < 10^{-5}$ ). In general, Cd concen-

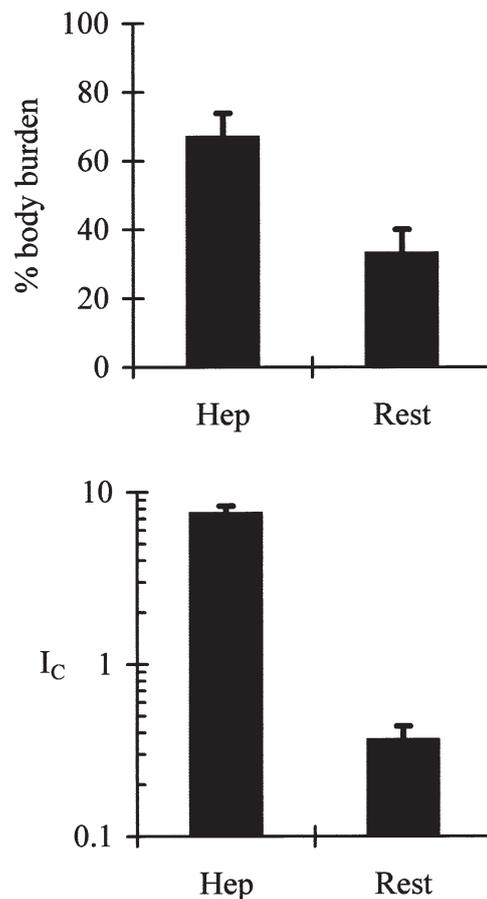


Fig. 5. *Chionoecetes opilio*. Percentage of  $^{109}\text{Cd}$  body burden and concentration index,  $I_C$ , of hepatopancreas and rest of body of snow crabs ( $n = 6$ ) dissected 154 d after feeding with  $^{109}\text{Cd}$ -contaminated food. Values are means  $\pm$  SD. Hep = hepatopancreas, Rest = rest of body

trations measured in the muscle were an order of magnitude lower compared to the hepatopancreas. However, Cd levels measured in the muscle of female crabs from the Gulf of St. Lawrence (range 0.5 to 5.4 nmolCd  $\text{g}^{-1}$  wet wt) were significantly higher than for other groups (range 0.1 to 1.9 nmolCd  $\text{g}^{-1}$  wet wt; Kruskal-Wallis test,  $p < 0.01$ ).

## DISCUSSION

### Pharmacokinetics and distribution

Autoradiograms give some clues about the processing of dietary  $^{109}\text{Cd}$  in the snow crab. The low labelling of foregut in autoradiograms taken at Day 3 shows that the radiolabel was efficiently transferred to the hepatopancreas. The relatively high labelling of hindgut and hepatopancreatic diverticula lumen may be related to the presence of non-assimilated  $^{109}\text{Cd}$ . The

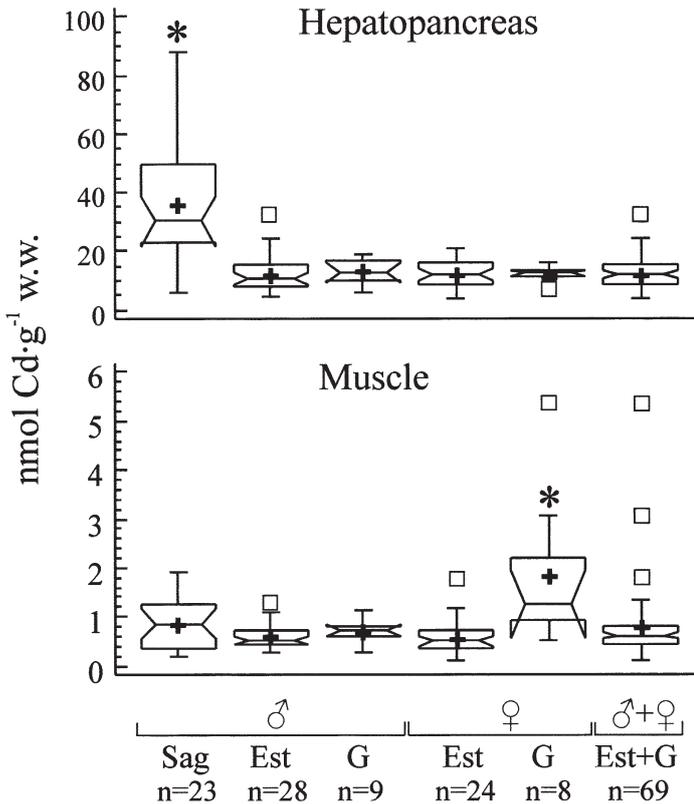
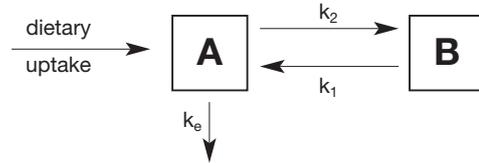


Fig. 6. *Chionoecetes opilio*. Box-and-Whisker plot of Cd concentration in the hepatopancreas and muscle of snow crabs sampled in the Estuary and Gulf of St. Lawrence and in the Saguenay Fjord. Horizontal bar in box is median concentration, +: average concentration, □: outlier data, and \*: significantly higher Cd levels for a given group (Kruskal-Wallis test, see text)

labelling of all parts of the gut, including the foregut, at Day 14 indicates that small quantities of the radioactive metal initially stored in the hepatopancreatic tissues were transferred back into the luminal space, transported to the foregut and midgut with the digestive juices (Dall & Moriarty 1983), and subsequently eliminated via the hindgut. This distribution picture is likely typical of the slow long-term elimination of assimilated Cd.

The mechanism underlying the bi-exponential elimination kinetics observed for 3 of the snow crabs is not clear. It might be due to interindividual variations of the rate of the initial processing of contaminated food, which would have been slower in these cases. The occurrence of two kinetically distinct metal pools may have an impact on the choice of a long-term kinetic model of the fate of dietary Cd in the snow crab, e.g. choosing a 1- or a 2-compartment model. Although the proportion of the metal dose retained in the fast pool A was similar to that of the slow pool B, it is noteworthy that the former was eliminated some 70 times faster, as

shown by the values of  $t_{0.95\alpha}$  and  $t_{0.95\beta}$  (Table 1). It is thus reasonable to expect that the long-term kinetics of



Cd in the snow crab can be well described by the slow compartment only (Trudel & Rasmussen 1997). As a precaution, we used the data from Crabs 2, 3, and 6 to compute the long-term kinetics of dietary Cd with the classical 2-compartment model shown below, in which uptake and elimination (characterised by the rate constant  $k_e$ ) take place in the same compartment, whereas exchange between compartments are characterised by rate constants  $k_1$  and  $k_2$ .

Results suggest that Pool A is indeed negligible, since it would account for  $\leq 5\%$  of the total Cd burden. Thus, for Crabs 2, 3, and 6, only the slow pool B will be considered in further discussion, e.g. the value of  $RE_0$  for these individuals will be considered equal to the value of  $B_0$  only (Table 1).

Compared to Ag(I) and inorganic Hg(II) (Rouleau et al. 1999, 2000), values of  $RE_0$  observed for dietary Cd(II) are 2 times lower, whereas elimination rate is slower than for inorganic Hg ( $\beta$  range = 0.011 to 0.022  $d^{-1}$ ) and faster than for Ag ( $\beta < 0.0007 d^{-1}$ ). Like Ag, dietary Cd was rapidly distributed to all internal tissues. Both autoradiographic and quantitative data correspond to the general distribution observed for wild crabs, with Cd concentrations in muscle and/or gonads that are lower compared to hepatopancreas (Davies et al. 1981, Kannan et al. 1995, this work). An interesting observation is the labelling of the antennal glands (Fig. 3), which was not observed for the other metals and organometals we studied previously (Rouleau et al. 1999, 2000). Antennal glands are the main excretory organs of decapod crustaceans (McLaughlin 1983). Their role is similar to that of the kidney in fish and mammals, which is a known target organ for Cd (Tjälve et al. 1986, Beresford et al. 1999). The autoradiographic evidence presented here suggest that antennal glands may be implicated in the storage, sequestration, or excretion of Cd. They might also be a specific target for Cd toxicity. Additional research work will be necessary to further investigate the possible chronic effects of Cd on the excretory processes of the snow crab and other decapod crustaceans.

#### Field data

Cd concentrations measured in the hepatopancreas and muscle of the snow crab (Fig. 6) are similar to those

reported for other crab species from non-polluted areas (Davies et al. 1981, Kannan et al. 1995). Our data do not reveal any consistent location- or sex-related difference of Cd levels in snow crab. Though Cd levels measured in the hepatopancreas of male snow crabs from the Saguenay Fjord were higher, no difference could be found between male and female crabs from the Estuary and Gulf of St. Lawrence. Furthermore, Cd levels in muscle of crabs from the Saguenay were similar to those measured in male snow crabs from the St. Lawrence. The same kind of discrepancy was observed for female snow crab from the Gulf, Cd level being higher in their muscle compared to the other groups, but not the hepatopancreatic concentration. These differences are difficult to explain. They do not reflect a significant spatial difference of sedimentary Cd levels, since surface sediment Cd concentrations in different sampling areas are similar, ca 1.5 nmol Cd g<sup>-1</sup> dry sediment (Pelletier & Canuel 1988, Gobeil et al. 1997). These differences may be related to the relatively small number of animals sampled and the occurrence of some very high individual values for Cd concentration that tend to positively skew the distribution of the data.

### Modelling of Cd trophic uptake

To assess the potential significance of Cd trophic transfer for the snow crab, we modelled the long-term accumulation of Cd in their tissues with a simple one-compartment model (Rouleau et al. 2000) expressed as:

$$C_p = C_f RE_0 I_C (k_{in}/k_e) (1 - e^{-k_e t}) \quad (5)$$

where  $C_p$  and  $C_f$  represent the concentrations of Cd in the predator's tissues and its food, respectively, and  $k_{in}$

is the food consumption rate (g food g<sup>-1</sup> body weight d<sup>-1</sup>).  $C_p$  was calculated with experimental average values of  $RE_0$ ,  $I_C$ , and  $k_e$ , assumed to be the same for both female and male. Value of  $k_{in}$  was taken from the literature (Table 3). Cd concentration measured in the benthic worm *Maldane sarsi* in the St. Lawrence Estuary (7.4 ± 2.8 nmol g<sup>-1</sup>, n = 5) was considered a reasonable approximation of  $C_f$ . Finally, considering that  $t_{0.95\beta}$  values (1.4 to 4.1 yr) are smaller than the age of adult snow crab (6 to 12 yr for female, 9 to 15 yr for male; Sainte-Marie et al. 1995, Alunno-Bruscia & Sainte-Marie 1998), equilibrium was assumed. Thus, for  $t = \infty$ , Eq. (5) becomes:

$$C_p = C_f RE_0 I_C k_{in}/k_e \quad (6)$$

or

$$C_p = C_f BMF \quad (7)$$

where BMF is the biomagnification factor.

Since metal concentrations measured in the tissues of organisms from a given location often show important interindividual variations because of differences in accumulation and elimination capacities, we also modeled a concentration range for Cd levels in snow crab tissues that would comprise 99% of the individuals from a given population. We calculated the minimum ( $C_{p_{min}}$ ) and maximum ( $C_{p_{max}}$ ) accumulation with values of  $RE_0$ ,  $I_C$ , and  $k_e$  that were 1 SD below or above average values (Table 3), assuming that values of  $RE_0$ ,  $I_C$ , and  $k_e$  were normally distributed and that they were independent from each other.

Comparison of predicted values of  $C_p$  (average and range) and pooled field data of all snow crabs collected in the Estuary and Gulf of St. Lawrence (minus outliers, see Fig. 6) is shown in Fig. 7. Considering that model predictions were strictly generated from our

Table 3. *Chionoecetes opilio*. Values of parameters used with Eq. (6) to model the long-term trophic accumulation of Cd in snow crab in the Estuary and Gulf of St. Lawrence. Values of  $RE_0$ ,  $I_C$ , and  $k_e$  for  $C_{p_{min}}$  and  $C_{p_{max}}$  are 1 SD below or above average values

	Average	$C_{p_{min}}$	$C_{p_{max}}$
$RE_0^a$	0.42	0.33	0.51
$I_C^b$ : hepatopancreas	7.5	6.7	8.3
muscle	0.36	0.29	0.43
$k_e^c$	0.0038	0.0048	0.0028
$k_{in}^d$	0.0035	0.0035	0.0035
$C_f^e$	7.4	7.4	7.4

<sup>a</sup>Retention efficiency  
<sup>b</sup>Concentration index; experimental value for the rest of body (Fig. 4) was used for muscle  
<sup>c</sup>Elimination rate constant (d<sup>-1</sup>)  
<sup>d</sup>Rate of food consumption (g food g<sup>-1</sup> body weight d<sup>-1</sup>) (Thompson & Hawryluk 1990)  
<sup>e</sup>Estimated Cd concentration in food (nmol g<sup>-1</sup>)

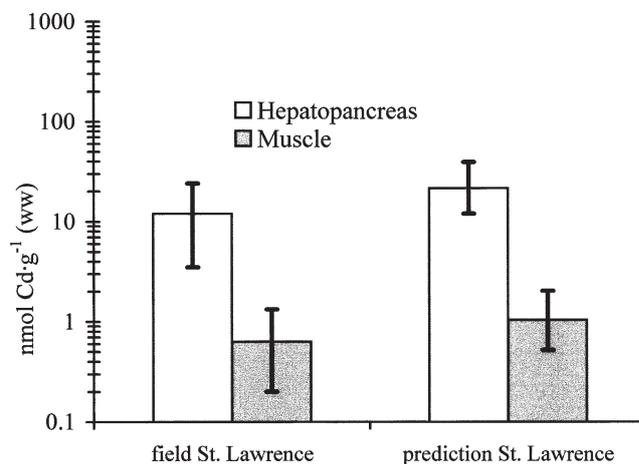


Fig. 7. *Chionoecetes opilio*. Results of Cd accumulation modelling for the St. Lawrence, using Eq. (6) with parameter values shown in Table 3. Bars represent  $C_{p_{min}}$  and  $C_{p_{max}}$  for predicted values and range for field data (all males and females from the Estuary and the Gulf)

laboratory data and estimate of Cd levels in food, the agreement between modelled and field Cd concentrations is quite good. Predicted average concentrations were only 1.6 to 1.8 times higher than field data, whereas predicted and field range (expressed as:  $\log_{10} [\text{Cd}]_{\text{max}} - \log_{10} [\text{Cd}]_{\text{min}}$ ) differed by no more than 0.3 log unit.

In order to refine the model, more data will be needed about Cd levels in snow crab prey. Another factor that will need to be considered is the effect of the biological incorporation of Cd on retention efficiency. Wallace et al. (1998) showed that the bioavailability of the cytosolic fraction of Cd in the oligochaete *Limnodrilus hoffmeisteri* was much higher than that of Cd in metal-rich granules, and that the absorption of Cd by the grass shrimp *Palaemonetes pugio* fed with these worms was directly related to the proportion of bioavailable Cd (e.g. the cytosolic fraction) in the latter. The food to which we added  $^{109}\text{Cd}$  for our laboratory experiment contained 40% water and, on a dry weight basis, 47% protein, 24% carbohydrates, and 8% lipid. Proteins, to which bioavailable Cd atoms are bound in the cytosolic fraction of natural prey, were in large excess relative to the  $^{109}\text{Cd}$  added. It is thus reasonable to assume that  $^{109}\text{Cd}$  in the food given to snow crabs was mostly bound to proteins and had a behaviour similar to that of the metal contained within the cytosolic fraction of naturally contaminated living prey. Thus, the use of spiked food to feed the snow crabs, instead of live prey that had previously accumulated Cd, though technically convenient, may have resulted in an overestimation of  $\text{RE}_0$ . This would explain the somewhat higher predicted values of  $C_p$  compared to field data.

## CONCLUSION

Although direct uptake of Cd by snow crab from either the sediment or water cannot be excluded at present, the similarity between our laboratory-based predictions and field data strongly suggests that trophic uptake is an important accumulation route of Cd in snow crab living in the St. Lawrence. Based on experimental data, the quantitative relationships between exposure to Cd in food and equilibrium Cd levels in snow crab tissues (Eq. 7) are:

$$[\text{Cd}]_{\text{hepatopancreas}} = C_F \cdot 2.9 \text{ (range 1.6 to 5.3)} \quad (8)$$

$$[\text{Cd}]_{\text{muscle}} = C_F \cdot 0.14 \text{ (range 0.07 to 0.23)} \quad (9)$$

Improvement of these relationships would eventually be possible through a better knowledge of the Cd concentrations in the prey organisms of the snow crab.

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