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Accumulation of ^{137}Cs in the European Sea Bass *Dicentrarchus Labrax* (L.) in a salinity gradient: Importance of uptake via gills, diet and ingested water*

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Abstract. Radio-caesium is an important radionuclide released considering food and feed products. In aquatic environments caesium tends to accumulate in fish, both through its diet and its gills. This presentation discusses the caesium accumulation in fish living in estuaries. The aim of this work is to conclude on the importance of potential uptake routes: via the gills, diet or ingested with water. It is suggested that the magnitude of caesium accumulation in fish is related to waterborne potassium concentrations. Based on this theory, fish living in seawater should show a suppressed caesium accumulation compared to fish that stays in freshwater. However, to compensate for water losses, seawater fish are constantly drinking, adding an alternative route of entry for caesium. Apart from these two possible uptake routes of waterborne caesium, fish may take up caesium via their diet. Separate uptake-and-elimination studies were performed to measure the waterborne and dietary caesium uptake and elimination rate constants in fully acclimated Sea Bass at six different salinities (ranging from 1‰ to 35‰). Short-term uptake studies were performed as well to evaluate the effect of the waterborne potassium concentration. A toxicokinetic model evaluated several scenarios to conclude on the importance of each uptake route.

1. INTRODUCTION

Radio-caesium is an important radionuclide released considering food and feed products. In aquatic environments caesium tends to accumulate in (predatory) fish, both via its diet and through its gills. This presentation discusses the caesium accumulation in fish living in estuaries. The aim of this work is to conclude on the importance of potential uptake routes: via the gills, diet or ingested with water. European Sea Bass, a fish of particular (commercial) interest in estuaries, is able to adapt to both freshwater and sea water conditions.

The magnitude of the caesium accumulation in (freshwater) fish seems to be related to potassium concentrations [1]. In estuaries steep potassium gradients are found. This is due to the graded mixing of fresh water, low in potassium concentration, and sea water, high in potassium. Due to this steep gradient and consequent competition between potassium and caesium, it is to be expected that fish living in sea water show a suppressed caesium accumulation compared to fish that stays in the more freshwater side of the estuary. However, physiological differences between both marine and freshwater adapted Sea Bass might counteract this competitive uptake kinetics [2]. Living in freshwater, fish experience water inflow and loss of electrolytes. Chloride cells compensate for this loss by taking up electrolytes from the water. The excess of water is eliminated via urine. In contrast to freshwater fish, fish living in sea water are faced with a water loss and a possible surplus of electrolytes, normally eliminated via the chloride cells in the gills. To compensate for water losses, sea water fish are constantly drinking, adding

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an alternative route of entry for caesium. Apart from these two possible uptake routes for waterborne caesium, fish, either in freshwater or sea water, may take up caesium via their diet [2].

In order to investigate the importance of potential uptake routes, we performed separate uptake-and-elimination studies over a two month experimental period. These experiments form the basis of a toxicokinetic model. Both waterborne and dietary caesium accumulation dynamics were measured in acclimated Sea Bass at six different salinities (ranging from 1‰ to 35‰). Short-term uptake studies were also performed to evaluate the correlation between the individual ^{42}K-influx and ^{137}Cs-influx and the effect of the waterborne potassium concentration on these fluxes.

2. MATERIAL AND METHODS

2.1 Fish, fish maintenance, radiotracers, and detection

European Sea bass *Dicentrarchus labrax* (L.) were kept in artificial sea water and acclimated to the desired salinity, i.e. 1‰, 5‰, 10‰, 20‰, 28‰, and 35‰, by adjusting it with 1‰ per day, and kept at the desired salinity for 150 d before experiments started. Fish were fed a commercial pellet on a 1% ration. At start of the experiments, fish weighed between 4.7 and 9.9 g. ^{137}Cs was obtained commercially with a specific activity of 790 GBq/mol. ^{42}K was produced with a specific activity of 3,7 TBq/mol via irradiation of KNO_3 in the research reactor of the Delft University of Technology, The Netherlands. A 3″ well-type NaI-detector was used for ^{137}Cs and ^{42}K detection.

2.2 Accumulation dynamics ^{137}Cs as function of salinity

The uptake-and-elimination experiment of waterborne ^{137}Cs proceeded in two stages. First Sea bass were placed in sea water containing 10 kBq/L ^{137}Cs for 17 d and daily measured on radioactivity. After this 17 d uptake period, Sea bass were transferred to ^{137}Cs free sea water and the release of radioactivity followed by live counting Sea bass regularly for 50 d. At termination of the experiment, fish were killed by decapitation. To study the dietary uptake kinetics, mussels *Mytilus edulis* (L.) served as food source and were loaded with ^{137}Cs by placing them at 20‰ sea water and 20 kBq/l ^{137}Cs for 32 days. The final ^{137}Cs concentration in the mussels was 1,3 kBq/g fresh wt. Each individual kept fish, was fed with 0.7 g mussel. Food was directly taken and fish were live counted on a regular base after ingestion of the ^{137}Cs loaded mussel. Data were scaled to a waterborne ^{137}Cs concentration of 10 kBq/l, corresponding to a dietary ^{137}Cs concentration of 700 Bq/g to compare the data with the accumulation of waterborne ^{137}Cs.

2.3 ^{137}Cs and ^{42}K uptake rates as function of salinity

To measure net uptake rates of waterborne ^{137}Cs and ^{42}K, fish were placed at 9215 kBq/l ^{42}K and 182 kBq/l ^{137}Cs for 14 h. Fish were killed by decapitation and analysed for ^{137}Cs and ^{42}K. Uptake rates were measured at the salinity at which fish were acclimated ^{137}Cs uptake rates were scaled to a waterborne ^{137}Cs concentration of 10 kBq/l to compare with fluxes derived from long-term experiments. Potassium net uptake was expressed as μmol/g/d.

2.4 Modelling the ^{137}Cs accumulation

As a first approach, the accumulation dynamics were described by a general two-compartment model:

$$C(t) = A \cdot e^{-a \cdot t} + B \cdot e^{-b \cdot t} \quad (1)$$

in which $C(t)$ is the radiocaesium concentration in the fish at time at t, A the concentration in the first compartment and B the concentration in the second compartment. All concentrations are related to the total weight of the fish (i.e. in Bq/g fish on a wet wt basis). The overall rate constants a and b determine the biological half-life of radiocaesium for each compartment.

The above model is very useful to describe the experimental results, but is often not very versatile; i.e. it applies only to the experimental conditions regarding the accumulation period and uptake route. Therefore, the above model was described in physiological based rate constants and two models tested (see Figure 1). The first model tested was the central compartment model, in which radiocaesium enters in the central (first) compartment and subsequently distributed over the peripheral compartments. The second model implemented different entrance routes for waterborne and dietary uptake, respectively. The differential equations describing both models were solved using a Laplace transformation and the analytical solution expressed in a similar form as equation (1), now the parameters A, B, a, and b being expressed in rate constants (see Figure 1).

Models parameters were fitted for each individual fish using the software package Scientist (Micromath Scientific Software Inc).

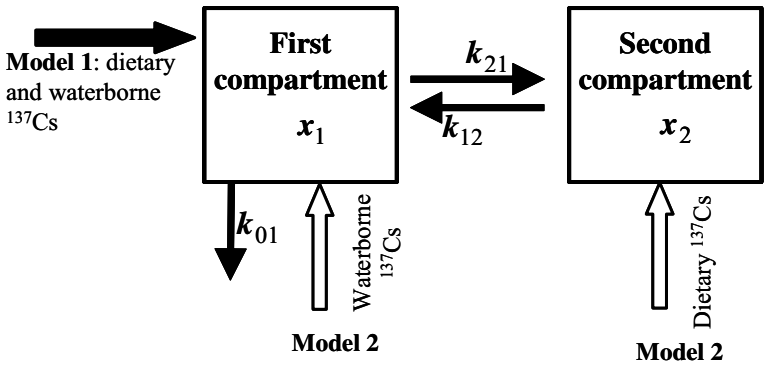

Figure 1. Physiological compartment models used to describe the accumulation dynamics in Sea bass. Model 1 assumes entrance of radiocaesium in the first, central compartment, from which it is further distributed over the peripheral compartment(s)/organs (here compartment 2). The second model assumes two different uptake routes. When radiocaesium is taken up from the water, it enters the fish through the gills, from where it is distributed over the two compartments. However, when radiocaesium is taken up via the diet by the intestine, it is first filtered by the liver, from which it is eventually distributed over the other organs.

3. RESULT AND DISCUSSION

3.1 Accumulation dynamics of ^{137}Cs as function of salinity

Figure 2 shows the results from ^{137}Cs accumulation studies. Panel A displays the uptake of waterborne ^{137}Cs and its subsequent elimination, Panel B focuses on the accumulation of dietary ^{137}Cs (note the difference in axis scale). The accumulation dynamics of waterborne ^{137}Cs was similar at most salinities (from 5‰ to 35‰) with an uptake rate of 2.6 ± 0.5 Bq/g/d; only at 1‰ the accumulation seemed to be higher with an uptake rate of 3.6 ± 1.0 Bq/g/d. This higher uptake rate was not confirmed by direct measurements of the influx (see below). Elimination of waterborne ^{137}Cs occurred in a bi-phasic fashion, indicating that ^{137}Cs was located in two biological (i.e. organs) and/or chemical compartments (i.e. binding, biotransformation). One compartment, containing $29 \pm 4\%$ of the accumulated activity, showed relative fast kinetics, characterised with an overall biological half-life of 5.7 ± 1.7 d independent of salinity. The second compartment, containing $71 \pm 6\%$ of the accumulated activity, showed relative slow kinetics characterised by an overall biological half-life of 95 ± 23 d, also independent of salinity.

The accumulation dynamics of ingested ^{137}Cs showed a slightly different pattern. After an initial ingestion of ^{137}Cs labelled mussel, a sharp release of about half of the ingested activity can be seen (see Figure 2, Panel B). This release is egestion of unassimilated ^{137}Cs in the faeces, as was confirmed qualitatively in a separate experiment. On the average, and independent of salinity, $43 \pm 7\%$ of the

initially ingested ^{137}Cs activity was assimilated (thus taken up by the fish). The assimilated activity was distributed over two compartments with similar biological half-lives as observed for accumulated waterborne ^{137}Cs, namely 5 ± 2 d and 350 ± 220 d for the fast and slow compartment, respectively. However, the distribution of the assimilated activity over these compartments was different: $46 \pm 22\%$ was located in the fast compartment, while $54 \pm 9\%$ was located in the slow compartment.

Apart from taken ^{137}Cs from the diet, fish might also assimilate ^{137}Cs from ingested water. Drinking rates were determined in a separate experiment as function of salinity (not shown) and combined with the above determined assimilation efficiency of the intestine for ^{137}Cs (i.e. $43 \pm 7\%$) in order to estimate the contribution of drinking. A maximum contribution of 10% to the uptake of waterborne ^{137}Cs was calculated in full sea water, but this contribution decreased to 3% at a salinity of 1‰ because fish drink less.

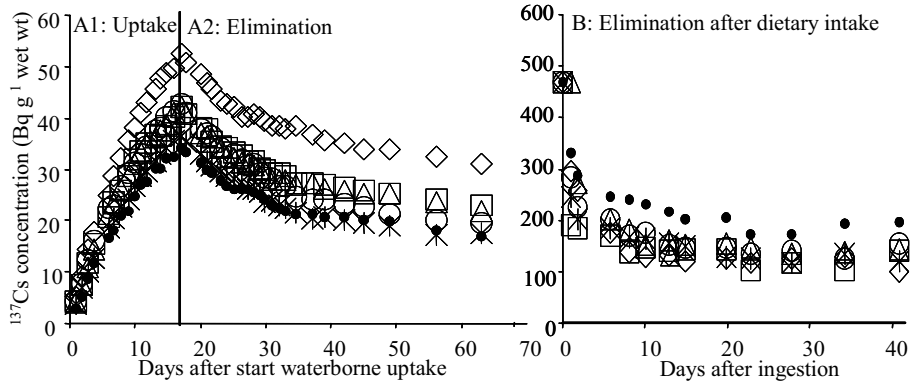

Figure 2. Accumulation dynamics of radiocaesium in Sea bass at different salinities (◇ 1‰, □ 5‰, △ 10‰, • 20‰ × 28‰, ○ 35‰) via water exposure (A) and diet (B). Data points are average concentrations of 4 to 5 fish, for which the standard deviation was 25%.

3.2 Modelling the accumulation dynamics: a physiological approach

The overall accumulation parameters (size of the compartments A and B, and the elimination rate constants a and b), as discussed in section 3.1, are a result of the underlying physiological processes and the distribution of radiocaesium over the different organs. Figure 1 is a very simplistic picture of these processes. It lumps together all organs and/or chemical forms that show an (approximate) similar behaviour. Radiocaesium can move from one compartment to the other and is released through one of the compartments. The rate constants were fitted from the elimination study followed on the uptake of waterborne radiocaesium (see Table 1).

Table 1. Model parameters (/d/g) for the two compartmental model fitted against the elimination of radiocaesium after exposure of Sea bass to waterborne radiocaesium (10 kBq/l) in.

	Salinity					
parameter	1‰	5‰	10‰	20‰	28‰	35‰
k_{01}	0.10 ± 0.02	0.18 ± 0.03	0.33 ± 0.05	0.28 ± 0.04	0.13 ± 0.02	0.11 ± 0.02
k_{21}	0.009 ± 0.001	0.064 ± 0.01	0.145 ± 0.022	0.158 ± 0.024	0.036 ± 0.005	0.026 ± 0.004
k_{12}	0.009 ± 0.001	0.014 ± 0.002	0.018 ± 0.003	0.022 ± 0.003	0.016 ± 0.002	0.016 ± 0.002

There are several physiological interpretations of this model. A very common one is the "central compartment model" [3]. This model assumes a central compartment (here compartment 1, see Figure 1) which distribute the material, here radiocaesium, over the peripheral compartments (here only one

peripheral compartment, compartment 2, is defined). Entrance of material is in the central compartment, irrespective of the source (dietary or waterborne). This model could well explain uptake-and elimination study, but failed to describe the dietary uptake experiment, using the same model parameters.

Therefore a second model was fitted that allowed the uptake of dietary radiocaesium in the second compartment. These dynamics might find a physiological explanation, since nutrients taken up from food will be first absorbed in the liver before distributed over the organs, while salts taken up by the gills are directly accessible for all organs [2]. In this case, the slow second compartment could represent the liver, while the other fast compartment is the rest of the fish. A complete entry of all available radiocaesium in the second compartment was, however not satisfactory. To merge both experiments in one model, dietary ^{137}Cs should be initially distributed over both the fast and slow compartment, with about 37% distributed over the first (fast) compartment, independent of salinity. This might be explained by the limited capacity of the liver and the uptake capacity of the other organs. The liver only accounts for 1 to 2% of the total fish weight and estimated concentrations are about 10 times higher compared to concentrations in (mainly) flesh.

3.3 Analogy with potassium metabolism: ^{137}Cs and ^{42}K uptake rates as function of salinity

The accumulation experiments conclude that ^{137}Cs accumulation dynamics in Sea bass is independent of salinity. These experiments are however not conclusive on the analogy between ^{137}Cs and potassium accumulation, although carried out in a potassium gradient resulting from the salinity range. Adaptation might counteract the competitive effects resulting in an apparent independence of the ^{137}Cs accumulation with salinity (and thus potassium concentration). Therefore potassium influxes were determined simultaneously with ^{137}Cs uptake rates in individual, acclimated Sea bass over a salinity range from 1‰ to 35‰. Figure 3 shows the ^{137}Cs and ^{42}K-traced potassium influx as function of salinity and corresponding potassium concentration (Panel A), and the correlation between these two fluxes (Panel B). The potassium influx increased with potassium concentration, tended to approach a plateau at higher potassium concentrations. ^{137}Cs influx didn't display this behaviour, but tended to be independent of the potassium concentration. No correlation exists between the potassium influx and the ^{137}Cs influx of an individual fish (Figure 3, Panel B), indicating different uptake kinetics.

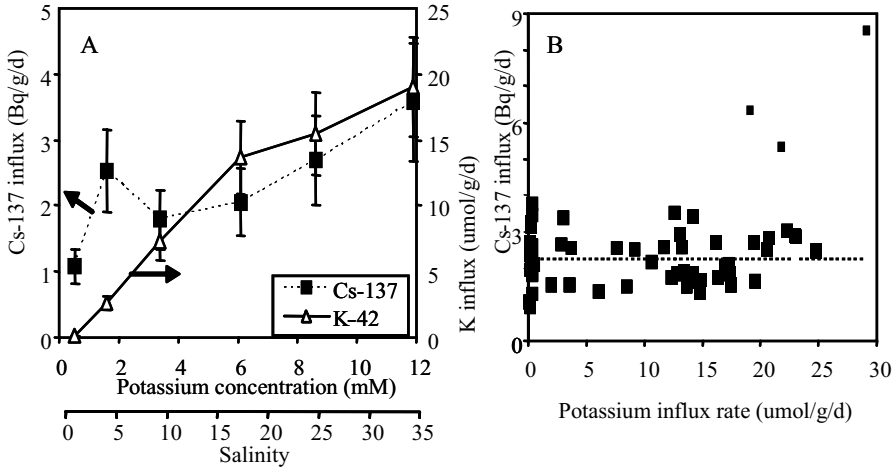

Figure 3. ^{137}Cs and ^{42}K influx as function of potassium concentration (Panel A). The corresponding salinity is given as reference. Panel B: Correlation between the individual ^{42}K influx and ^{137}Cs influx. Caesium data are scaled to a waterborne ^{137}Cs concentration of 10 kBq/l.

4. CONCLUSIONS

This study concludes that ^{137}Cs accumulation dynamics in the Sea Bass is independent of the ambient potassium concentration and potassium uptake, regardless of salinity and adaptation. Dietary uptake is the major contributor to the ^{137}Cs accumulation dynamics, although uptake of waterborne ^{137}Cs might partly account for the overall accumulation. When caesium concentrations in prey are in (semi)equilibrium with waterborne caesium concentrations, uptake via gills is of equal importance as dietary uptake. When waterborne caesium levels drop, dietary uptake becomes the major entry. Salinity might increase caesium prey concentration due to competitive effects with potassium, and the importance of dietary uptake increase, confirmed by the model.

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