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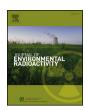
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# Interspecific comparison of radiocesium trophic transfer in two tropical fish species

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#### ABSTRACT

The trophic transfer of radiocesium ( $^{134}$ Cs) was investigated in two tropical fish, the silver moony *Monodactylus argenteus* and the spotted scat *Scatophagus argus*. Juveniles of both species were exposed to dietary  $^{134}$ Cs using the pulse-chase feeding methodology. The food was brine shrimp (*Artemia salina*) previously exposed to the dissolved radiotracer. Depuration kinetics of  $^{134}$ Cs were followed for 45 d. Results showed that Cs was similarly efficiently assimilated by both species (AE > 50%). The estimated trophic transfer factors in the two species ranked from 1 to 2, suggesting that  $^{134}$ Cs could be biomagnified in both omnivorous species. In complement, dissections of 7 body compartments were carried out at three different times in order to highlight  $^{134}$ Cs organotropism.  $^{134}$ Cs organotropism was similar in both species: more than 50% of  $^{134}$ Cs was quickly distributed in the muscles and skeleton (after 3 days of depuration), which is likely related to the analogous behavior between Cs and K. an essential element for muscle contractions and bone formation.

#### 1. Introduction

Anthropogenic activities have resulted in various degrees of contamination of the world's seas and oceans with radionuclides (Friedlander et al., 2005). Radionuclides such as <sup>134</sup>Cs are waste products from industrial activities that can enter aquatic systems (Twining et al., 1996). Scientific literature generally reports relatively low bioaccumulation of radiocesium (<sup>134</sup>Cs and <sup>137</sup>Cs) in fish (e.g. Heldal et al., 2003; Kasamatsu and Ishikawa, 1997; Topcuoğlu, 2001). However, after the accident at the civilian Fukushima Daichii NPP, several field investigations have shown the ability of different ecological groups of fish (such as pelagic or benthic fish) to accumulate the released radiocesium (Iwata et al., 2013; Wada et al., 2013, 2016). The highest <sup>134</sup>Cs concentrations in marine fish (338 Bq kg<sup>-1</sup>) were reported in seabass caught in the surrounding of Hitachi, Japan (Chen, 2013). Until now, mechanisms for the unusually high accumulation capacities reported have yet to be explained.

The determination of radionuclide bioaccumulation parameters under controlled laboratory conditions can be key to better understanding the significance of field measurements (Warnau and Bustamante, 2007). Indeed, an experimental radiotracer approach can provide information about contamination pathways or uptake and

depuration capacities of exposed fish (e.g. Pan and Wang, 2016; Zhao et al., 2001). Such experiments allow accurately assessing kinetic parameters in living fish, such as assimilation efficiency or uptake and depuration rates that are crucial to understand Cs bioaccumulation patterns and useful for modelling approaches (Doi et al., 2012; Thomann, 1981; Wang et al., 2000).

Previous experimental works have clearly demonstrated the importance of the food pathway in the bioaccumulation of Cs by fish (e.g. Mathews and Fisher, 2009; Zhao et al., 2001). Some studies have highlighted that concentration factors in prey and ingestion rate of the predator are the dominant factors influencing the bioaccumulation of Cs in piscivorous fish (Mathews and Fisher, 2009; Zhao et al., 2001). More recently, Pan and Wang (2016) have shown that Cs bioaccumulation is affected by the trophic ecology of fish. However, there is still a lack of knowledge regarding the ability of omnivorous tropical fish to accumulate Cs from their food.

In this context, the present work investigated the trophic transfer of <sup>134</sup>Cs in two omnivorous tropical fish, the silver moony *Monodactylus argenteus* and the spotted scat *Scatophagus argus*. Although both species are omnivorous, their food habits are slightly different: the silver moony feeds on plankton and detritus while the spotted scat feeds on worms, crustaceans, insects and plant matter (Froese and Pauly, 2017).

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The choice of these species has been driven by the need for more data regarding the radionuclide transfer in species from tropical areas (e.g. Fowler and Fisher, 2005), where previous radioactive contamination events have occurred (e.g. Mittelstaedt et al., 1999) and the assumption that tropical organisms have a greater ability to accumulate radionuclides (e.g. Ke et al., 2000; Metian et al., 2016). In this study, two levels of biological organization were considered; i.e. the whole organism and the different organs and tissues, in order to determine the biokinetic parameters of <sup>134</sup>Cs depuration from the fish and to characterize transfer dynamics of this radionuclide to, among, and from the body compartments throughout the depuration phase.

#### 2. Materials and methods

#### 2.1. Acclimation of fish

Wild juveniles of silver moony *M. argenteus* and spotted scat *S. argus* from Southeast Asia were purchased from a French fish wholesaler and shipped to the IAEA-Environment Laboratories premises in the Principality of Monaco. Fish were acclimated for 3 months to laboratory conditions (700-L aquarium for juveniles; open circuit:  $200\,\mathrm{L\,h^{-1}}$  in each tank; 0.45-µm filtered seawater; salinity: 35 p.s.u.; temperature:  $25\pm0.3\,^\circ\mathrm{C}$ ; pH:  $8.1\pm0.1$ ; light/dark:  $12\,\mathrm{h/12\,h}$ ). During the acclimation phase, fish were fed 3 to 4 times per day with adult brine shrimp (*Artemia salina*) at a daily ration of 0.1– $0.3\,\mathrm{g}$  wet weight (wwt) per individual. No mortality was observed during the acclimation period.

#### 2.2. Experimental procedure

#### 2.2.1. Radiolabelling of brine shrimp

In order to investigate the trophic transfer of  $^{134}\text{Cs}$  in *M. argenteus* and *S. argus*, radiolabelling of brine shrimp was carried out as described by Pouil et al. (2017). Radiotracer of high specific activity in aqueous solution was purchased from Amersham, UK ( $^{134}\text{Cs}$ , [ $t_{1/2}$ ] = 2.1 years, specific activity: 37–370 MBq mg Cs $^{-1}$ ). The experimental seawater was spiked with  $^{134}\text{Cs}$  (nominal activity of 5 kBq L $^{-1}$ ), no change in pH was detectable after the tracer addition. At the end of the exposure period, average  $^{134}\text{Cs}$  activity in the brine shrimp was 9 Bq g $^{-1}$  wwt. Briefly, preparation of the radiolabelled brine shrimp was carried out by exposing them for 18 h in aerated 20-L aquaria (approx. 300 g wwt of adult brine shrimp per aquarium). At the end of the exposure period, radiolabelled brine shrimp were stored at  $-20\,^{\circ}\text{C}$  before their use.

#### 2.2.2. Exposure of tropical fish

Thirty individuals of each species (8.4  $\pm$  1.4 g wwt for silver moony and 4.8  $\pm$  1.4 g wwt for spotted scat) were transferred into two 70-L aquaria (open circuit:  $100\,L\,h^{-1}$ ; aerated, 0.45-µm filtered seawater; salinity: 35 psµ; temperature: 25  $\pm$  0.3 °C; pH: 8.1  $\pm$  0.1; light/dark: 12 h/12 h) prior to the experiment.

The experiment consisted in a single-feeding exposure with thawed  $^{134}\text{Cs}\text{-radiolabelled}$  brine shrimp (i.e. 1–12 Bq/individual; see Pouil et al., 2016, 2017 for details). Two hours after the single feeding, all fish were whole-body  $\gamma\text{-counted}$  alive. After counting, 21 individuals of each species were transferred into two new 20-L aquaria containing clean, flowing seawater (parameters as previously described). The remaining 9 fish of each species were returned into the initial 70-L aquaria. No regurgitation of the ingested radiolabelled brine shrimp was observed.

The 21 individuals were  $\gamma$ -counted alive (see Section 2.3) at different time intervals over 45 d to follow the whole-body  $^{134}Cs$  depuration kinetics. The batch of 9 fish were sampled at different times (after 3, 7 and 14 days of depuration, n = 3) during the depuration phase, anesthetized, dissected in 7 body compartments (digestive tract, gills, kidney, liver, muscles, skeleton and all remaining tissues), and then  $\gamma$ -counted to determine the body distribution of  $^{134}Cs$ .

#### 2.3. Gamma counting

 $^{134}$ Cs activity was counted in living fish and dissected tissues and organs using a high-resolution  $\gamma$ -spectrometer system composed of three Germanium (N- or P-type) detectors (EGNC 33–195-R, Canberra $^*$  and Eurysis $^*$ ) connected to a multi-channel analyzer and a computer equipped with a spectra analysis software (Interwinner $^*$  6). The radioactivity was determined by comparison with standards of known activity and of appropriate geometry (Cresswell et al., 2017). Measurements were corrected for counting efficiency and physical radioactive decay. The counting time was adjusted to obtain a propagated counting error less than 5% (Rodriguez y Baena et al., 2006) and to ensure well-being of counted fish (see Pouil et al., 2017).

#### 2.4. Data treatment

Whole-body depuration kinetics were fitted using nonlinear regression routines with iterative adjustment (Statistica\* 7) and statistical methods described by Warnau et al. (1996). Briefly, the depuration kinetics of the <sup>134</sup>Cs were best fitted using a two-component exponential model:

$$A_t = A_{0s}. e^{-k_{es}t} + A_{0l}. e^{-k_{el}t}$$
(1)

where  $A_t$  and  $A_0$  are the remaining activities (%) at time t (d) and 0, respectively;  $k_e$  is the depuration rate constant (d<sup>-1</sup>); 's' and 'l' are the subscripts for the 'short-lived' and 'long-lived' components, respectively. The short-lived component represents the depuration kinetics of the radiotracer fraction that is weakly associated with the organisms and rapidly eliminated (e.g. fraction in faeces), whereas the long-lived component describes the depuration kinetics of the radiotracer fraction that is assimilated by and tightly bound to the organism (Warnau et al., 1996). The long-lived component allows assessing the assimilation efficiency (AE) of the radiotracer ingested with food (AE =  $A_{01}$ ). For each exponential component (s and l), a biological half-life can be calculated ( $T_{b \nmid s_0}$  and  $T_{b \mid s_0}$ ) from the corresponding depuration rate constants ( $k_{es}$  and  $k_{el}$ , respectively) according to the relation  $T_{b \mid s_0} = \ln 2/k_e$ .

and  $k_{el}$ , respectively) according to the relation  $T_{b\frac{1}{2}} = ln2/k_e$ . To assess the biomagnification potential of  $^{134}$ Cs following dietary exposure, trophic transfer factors (TTFs) were also calculated for a specific link in the food chain in which a predator consumes metal in prey as follows:

$$TTF = \frac{AE \times IR}{k_{el}} \tag{2}$$

Where AE is the assimilation efficiency of the ingested metal in the fish, IR is the weight-specific ingestion rate of prey (g g  $^{-1}$  d  $^{-1}$ ) and  $k_{el}$  is the depuration rate constant (d  $^{-1}$ ) of the radionuclide out of the predator (see Mathews et al., 2008; Zhao et al., 2001). A TTF >1 suggests that biomagnification is possible, and TTF <1 suggests that biomagnification is unlikely (Reinfelder et al., 1998). For these TTF calculations we considered a range of ingestion rates (IR) by fish likely to be encountered under natural conditions (0.02–0.10 g g  $^{-1}$  d  $^{-1}$ ; Zhao et al., 2001). Significant differences for remaining activities in the two species were tested using a Student t-test. The data were arcsin-transformed, and tested for homogeneity of variance and normal distribution before the statistical analysis (Zar, 1996). For  $^{134}\mathrm{Cs}$  organotropism, significant differences between the two species were tested using Wilcoxon-Mann-Whitney non-parametric test (Zar, 1996). The level of significance was always set at  $\alpha=0.05$ .

#### 3. Results and discussion

Experimental works have provided evidence that the food pathway can be the main uptake route of radiocesium for different taxa (Metian et al., 2016; Pouil et al., 2015; Zhao et al., 2001). Surprisingly, compared to other radionuclides, limited information is available on the trophic transfer of cesium in tropical areas (Metian et al., 2016), and

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