Journal of Environmental Radioactivity 190–191 (2018) 141–148

Contents lists available at ScienceDirect

Journal of Environmental Radioactivity

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

A double-tracer radioisotope approach to assess simultaneous bioaccumulation of caesium in the olive flounder *Paralichthys olivaceus*

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ARTICLE INFO

Keywords:
Cesium
Radionuclides
Bioaccumulation
Olive flounder
Double-tracer

ABSTRACT

To better understand bioaccumulation of radiocaesium in the commercially important Japanese flatfish, *Paralichthys olivaceus*, the uptake and depuration kinetics of caesium via both seawater and food were assessed simultaneously using controlled aquaria. The pre-conditioned fish were exposed to radionuclides via the two different pathways (aqueous versus dietary) concurrently using two isotopes of caesium, ^{137}Cs and ^{134}Cs, respectively. Dissolved caesium uptake was linear and did not reach a steady state over the course of the 8-day exposure period. Consumption of ^{134}Cs-labelled food led to higher bioaccumulation rates of radioactive Cs than via seawater exposure of ^{137}Cs during uptake and following depuration, though the model-derived long-lived biological half-lives of both pathways was approximately 66 d. Further development of this method for assessing multiple radiocaesium bioaccumulation pathways simultaneously could lead to a promising new approach for studying Cs contamination in marine organisms.

1. Introduction

As a consequence of the accident at the Tokyo Electric Power Company (TEPCO) Fukushima Dai-ichi Nuclear Power Plant (FDNPP; IAEA, 2015), large amounts of radioactive caesium [estimates for ^{137}Cs vary from 3.5 PBq according to Tsumune et al. (2012) to 27 PBq reported by Bailly du Bois et al. (2012)] had been released into the ocean. This radioactive release was predominantly transported southward (Aoyama et al., 2012; Tsumune et al., 2012), and relatively high concentrations of radioactive caesium [both ^{134}Cs (half-life of 2.065 y) and ^{137}Cs (30.167 y)] were detected in a variety of marine organisms around the southern coast of Fukushima Prefecture after the accident (Arakawa et al., 2015; Shigenobu et al., 2014). Approximately 6 years have passed since the accident occurred, and the radioactive caesium concentrations in seawater off the coast of Fukushima Prefecture have now dropped so that they are close to pre-accident levels (0.001–0.002 Bq L^{-1}) (Kusakabe et al., 2013; Oikawa et al., 2013). Concentration reductions have also been observed in seaweed, cephalopods, shellfish, and crustaceans; however, the rates of reduction have varied among taxonomic groups. Radiocaesium concentrations have also declined in fish species that were significantly contaminated [e.g., Japanese rockfish (*Sebastes cheni*), fat greenling (*Hexagrammos otakii*), and marbled sole (*Pleuronectes yokohamae*)] (Iwata et al., 2013; Sohtome et al., 2014; Wada et al., 2013).

The Japanese government banned landings of many marine species in the vicinity of Fukushima, including *Paralichthys olivaceus,* after the accident due to the presence of high levels of radioactive Cs (Wada et al., 2013). The olive flounder *P. olivaceus* is a demersal fish native to the subtropical and temperate western Pacific Ocean and widely distributed in the coastal waters around Japan. An economically important aquaculture species in East Asia since the 1990s (Kikuchi and Takeda, 2001), the olive flounder was a target species of a stock enhancement program that released around one million hatchery-raised juveniles annually in Fukushima Prefecture (Tomiyama et al., 2008). Several studies have monitored the radiocaesium contamination in *P. olivaceus* following the accident, including modelling the uptake and depuration biokinetics of this fish or assessing the depuration biokinetics using naturally exposed fish (Kurita et al., 2015; Tateda et al., 2015, 2016, 2017).

Studies have focused on the differences in the bioaccumulation of radionuclides in marine organisms depending on the particular contaminant pathway, be it through aqueous, dietary, sedimentary, or maternal exposure routes. The uptake and depuration of radionuclides by marine organisms is variable depending on species, element, and environmental conditions. Some studies have been able to demonstrate that radiocaesium concentrations increase with increasing trophic levels (Kasamatsu and Ishikawa, 1997; Mathews and Fisher, 2008), providing evidence for bioaccumulation and suggesting

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https://doi.org/10.1016/j.jenvrad.2018.05.014
Received 28 February 2018; Received in revised form 16 May 2018; Accepted 16 May 2018

biomagnification (Mathews and Fisher, 2008; Pan and Wang, 2016; Zhao et al., 2001).

While these pathways have previously been evaluated separately in the laboratory for many species of marine organisms exposed to a suite of radioisotopes and metals including Cs (e.g., Bustamante et al., 2006; Metian et al., 2011, 2016; Warnau et al., 1996a, 1996b), to our knowledge no such experiments have yet been performed to quantify the simultaneous uptake and depuration of caesium radionuclides via both seawater exposure and diet. The advantages of analysing these exposure pathways concurrently are both practical and scientific. From a practical standpoint, experimental resources including time may be much reduced. Scientifically, the compounding effects of two exposure pathways can be evaluated, as contamination in the marine environment will always involve multiple concurrent sources of exposure. We were able to measure the effects of these two exposure pathways simultaneously through the use of two different radioisotopes of Cs, ^{134}Cs and ^{137}Cs.

Here we demonstrate the concurrent bioaccumulation and depuration of radioactive Cs in the Japanese flatfish *Paralichthys olivaceus*, commonly known as olive flounder, via both food and seawater exposure pathways. We also evaluate the utility of this double-tracer radioisotope approach in assessing these processes simultaneously in the laboratory and explore possible future applications of this methodology.

2. Material and methods

2.1. Experimental organisms

Japanese aquaculture juvenile fish *Paralichthys olivaceus* were obtained from a fish wholesaler (Tropic Nguyen, France). They were acclimated to laboratory conditions for 4 weeks in an open circuit 500-L aquarium; flux: 50 L h^{-1} of 1-μm filtered seawater; salinity: 38 g L^{-1}; temperature: 20.5 ± 0.5 °C; pH: 8.0 ± 0.1; light/dark cycle: 12 h/12 h. During this period fish were fed daily with frozen *Artemia salina* and *Euphasia pacifica*.

2.2. Radiotracers and counting

The uptake and depuration of radiocaesium in *P. olivaceus* were determined using radiotracers purchased from Polatom (^{134}CsCl in aqueous solution) and Areva Cerva Lee (^{137}CsCl in 0.1 N HCl). ^{134}Cs and ^{137}Cs were counted using a high-resolution γ-spectrometer system composed of four high-purity germanium (HPGe) detectors (efficiency = 50%) connected to a multi-channel analyzer and a computer equipped with spectra analysis software Interwinner 6. Precise activities of ^{134}Cs (605, 796 keV) and ^{137}Cs (662 keV) were determined using standards (i.e., phantoms, as described in Cresswell et al., 2017) of known activity and appropriate geometries, and measurements were subsequently corrected for counting efficiencies and radioactive decay (Cresswell et al., 2017). Counting times ranged from 20 to 73 min with an average of 50 min. The counting times were adjusted to obtain propagated counting errors generally less than 5%, although a few samples with very low activities had counting errors up to 15%.

2.3. Experimental procedure

A single experiment was conducted to investigate Cs bioaccumulation in the Japanese flatfish simultaneously through seawater and dietary exposure pathways over a long period (87 d total consisting of 8 d of uptake followed by 79 d of depuration). The experiment was conducted using eleven *P. olivaceus* fish (mean initial weight 5.19 ± 1.85 g) in 70-L closed-circuit aquaria constantly aerated with an aquarium water pump under the following conditions: salinity = 38 g L^{-1}, temperature = 20.5 ± 0.5 °C, pH = 8.0 ± 0.1, light/dark cycle = 12 h/12 h. All 11 organisms were exposed for 8 d to seawater spiked with ^{137}Cs dissolved in 1 μm-filtered seawater (1 Bq mL^{-1}), and 10 of these were fed food labelled with ^{134}Cs to allow for one single-exposed (^{137}Cs via seawater) control.

Radiolabelled food was prepared by growing *Artemia salina* in seawater containing 220 kBq ^{134}Cs, with *Isochrysis galbana* to keep the prey fed and healthy over 8 d, leading to labelled *A. salina*. Fish were fed this ^{134}Cs-labelled *A. salina* (mean daily weight 2.7 ± 0.2 g; mean daily activity = 232 ± 13 Bq) for six morning feedings (days 0, 1, 2, 3, 4, and 7) and supplemented with unlabelled krill every afternoon. With regards to the multiple feeding approach used here, ^{134}Cs activity also reflects prior feedings, as well as any depuration that occurred during the following day. During depuration, the same daily feeding schedule was kept using both unlabelled *A. salina* and krill. For seawater exposure, a daily spike of ^{137}Cs accompanied six daily water changes (days 0, 1, 2, 3, 4, and 7) for an average seawater ^{137}Cs activity of 1.066 ± 0.063 Bq g^{-1} over the exposure period (^{137}Cs radioactivity in the water was measured before and after each seawater renewal; i.e., time-integrated activity). This concentration is a fraction of the maximum ^{137}Cs concentrations in the discharge following the accident and comparable in magnitude to values observed in surface seawater near Fukushima (Buesseler et al., 2011).

During the 79-day depuration period, 7 fish were placed under uncontaminated conditions (constantly aerated, open-circuit aquarium; flow = 50 L h^{-1}; salinity = 38 g L^{-1}, temperature = 20.5 ± 0.5 °C, pH = 8.0 ± 0.1, light/dark cycle = 12 h/12 h), collected at different time intervals, and whole-body radioanalyzed alive.

2.4. Data analyses

The uptake kinetics of dissolved ^{137}Cs was expressed in terms of change in concentration factor (CF, ratio of whole-body fish ^{137}Cs activity in Bq g^{-1} wet weight as a function of the time-integrated seawater ^{137}Cs activity in Bq g^{-1}) over time for the seawater exposure. Kinetics were best described using a linear model (Eq. (1))

$$CF_t = k_u t \quad (1)$$

where CF_t is the concentration factor at time t (d) and k_u is the biological uptake rate constant (d^{-1}; e.g., Whicker and Schultz, 1982).

Depuration kinetics for ^{134}Cs and ^{137}Cs were fit to a simple, two-component exponential loss model (Eq. (2)):

$$A_t = A_{0s}e^{-k_{es}t} + A_{0l}e^{-k_{el}t} \quad (2)$$

where k_e is the depuration rate constant (d^{-1}), and A_t and A_0 are the total activities (Bq) at time t (d) and 0, respectively; 's' and 'l' subscripts denote the short- and long-lived exponential components. Biological half-lives ($T_{b½s}$ and $T_{b½l}$) were calculated from the corresponding depuration rate constant (k_{es} and k_{el}, respectively) according to the relation $T_{b½} = \ln 2/k_e$ as in Whicker and Schultz (1982). Model constants and statistics were estimated by iterative adjustment of the model using the non-linear curve fitting routines in the Statistica software package (StatSoft, Inc., 2004) and statistical methods as in Warnau et al. (1996a, 1996b) and Metian et al. (2011). Additional statistical analyses were performed using R (R Core Team, 2016).

The percentage of ^{134}Cs food activity assimilated was calculated by dividing the total ^{134}Cs activity measured in the fish each day during the uptake phase by the total cumulative ^{134}Cs activity in the food given (as ^{134}Cs-labelled *A. salina*). The relative contribution of ^{134}Cs (food) and ^{137}Cs (seawater) to total activity was calculated as the proportion of the mean activity of each radioisotope (^{134}Cs or ^{137}Cs in Bq) to the mean total activity (i.e., ^{134}Cs + ^{137}Cs in Bq) in the fish each day measurements were taken during the experiment.