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

Roberta Hansman, Marc Metian, Simon Pouil, François Oberhänsli, Jean-Louis Teyssié, Peter Swarzenski

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Roberta L. Hansman, Marc Metian, François Oberhänsli, Jean-Louis Teyssié, and Peter W. Swarzenski

International Atomic Energy Agency – Environment Laboratories, Radioecology Laboratory; 4a Quai Antoine 1er, MC-98000 Principality of Monaco

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, 137Cs and 134Cs, respectively. Dissolved caesium uptake was linear and did not reach a steady state over the course of the 8-day exposure period. Consumption of 134Cs-labelled food led to higher bioaccumulation rates of radioactive Cs than via seawater exposure of 137Cs 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 137Cs 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 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). 134Cs and 137Cs 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.

1.4 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))

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

where CF_t is the concentration factor at time t (d) and k_u are the biological uptake rate constants (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):

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

where k_e is the depuration rate constant (d^{-1}), and At and A0 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 ($Tb_{½s}$ and $Tb_{½l}$) were calculated from the corresponding depuration rate constant (k_{es} and k_{el}, respectively) according to the relation $Tb_{½} = \ln 2/k_e$ 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 + 137Cs in Bq) in the fish each day measurements were taken during the experiment.

3. Results

3.1 Uptake

The simultaneous uptake of ^{134,137}Cs by *P. olivaceus* through both aqueous and dietary exposure pathways is shown in Fig. 1 as total activity (Bq) over time (d). Multiple feedings of 134Cs-labelled food resulted in higher total activities in the fish than through seawater exposure. Over the initial four days, the rate of accumulation was more than double for dietary uptake of Cs than seawater exposure (3.441 Bq d^{-1} vs. 1.216 Bq d^{-1}; $R^2 = 0.992$ and 0.971 for linear regression, respectively). Although some depuration occurred during the two-day pause in ^{134}Cs-labelled feedings, the total activity increased over the entire exposed period and 11.5 ± 1.0% of the total food 134Cs activity given to the fish was assimilated (Fig. 2). As seawater ^{137}Cs exposure continued during the two-day pause in feeding and counting, total activity in fish for ^{137}Cs increased linearly over the entire exposure period (1.225 Bq d^{-1}, $R^2 = 0.996$). While the multiple feeding strategy utilized in this experiment does not allow for the calculation of assimilation efficiency (AE) as in single feeding studies, the calculated concentration factor (CF) for seawater exposure reached a value of 1.61 ± 0.47 at the end of the exposure (day 8) with an uptake rate constant (k_u) of 0.205 d^{-1}.

3.2 Depuration

Depuration of ^{134}Cs and ^{137}Cs over the 79-day experiment is shown in Fig. 3A and B, with total activity plotted in (A) and the percentage of remaining activity in (B). Depuration kinetics were best described by a simple two-component exponential model (Fig. 3A; Table 1). The initial depuration rate was higher for ^{137}Cs than ^{134}Cs (k_{es} = 0.41 and 0.16 d^{-1}, respectively), though both appeared to reach a steady plateau by the end of the experiment. Dietary exposure to Cs through multiple feedings led to a higher total activity of ^{134}Cs at this plateau compared to ^{137}Cs seawater exposure; however, the amount of remaining activity compared to the maximum values reached were similar for both exposure pathways. The remarkable similarities in the derived long-lived biological half-lives ($Tb_{1/2l}$ = 65.63 ± 27.74 d and 65.65 ± 17.79 d for ^{134}Cs and ^{137}Cs, respectively) from the fitted two-component exponential loss models for both food and seawater exposure clearly highlight this observation.

3.3 Global bioaccumulation

The relative contribution of ^{134}Cs vs. ^{137}Cs to total activity over the course of the entire experiment is shown in Fig. 4. The average contribution of Cs activity from seawater exposure over all 87 d was 34.6 ± 2.5% (±one standard deviation), and though slightly more variable during the uptake period, there was no significant difference in relative contribution when compared to the loss phase (33.9 ± 4.6% and 34.7 ± 1.2%, respectively; $p > 0.05$). Approximately two-thirds of the total Cs radioactivity in *P. olivaceus* during both uptake and depuration is due to consumption of Cs-contaminated food.

4. Discussion

The olive flounder is a commercially important fishery that was essentially closed in the waters around Fukushima following the accident due to observed increased levels of radiocaesium contamination above the Japanese standard limit for food safety of 100 Bq kg^{-1} wet weight enforced in April 2012 (Wada et al., 2013). Concentrations of 134Cs + 137Cs in the surrounding seawater immediately after the accident were initially very high but decreased rapidly (Aoyama et al., 2016), yet concentrations in P. olivaceus tissues remained high and could be found in excess of the limit up to 3 years later (up to 230 Bq kg^{-1}; Kurita et al., 2015). By these standards, both dietary and aqueous exposure to radiocaesium at the concentrations used in the present experiment led to contamination levels in *P. olivaceus* within one day. During depuration, concentrations of radiocaesium did not fall below the food safety limit by the end of the experiment 79 d after exposure as final average concentrations were 569 ± 211 and 288 ± 76 Bq kg^{-1} for 134Cs and 137Cs, respectively. This accounted for 19.2% and 16.9% of the maximum 134Cs and 137Cs concentrations at the beginning of the depuration period, respectively. This direct comparison from our laboratory experiment and the field should be taken in context however, as the juvenile fish used here can have different uptake and depuration biokinetics than commercial-sized adult flounder (e.g., Suzuki et al., 1992). Nonetheless, it is still useful to make intermediate connections between laboratory and field measurements with the goal of further understanding contamination pathways in the marine environment.

Delineating Cs bioaccumulation pathways in aquatic organisms contributes to our understanding of Cs measurements reported from the field in biota after a contamination event. In ecotoxicological studies, the contribution of different contamination pathways (water, food, and sediment) is usually estimated using bioenergetic models developed by Thomann (1981) implemented with kinetic data measured in controlled conditions (Reinfelder et al., 1998; Thomann et al., 1995; Wang et al., 1996). One of the main disadvantages of this methodology is that it requires the implementation of difficult and complex experimental protocols (e.g., Hédouin et al., 2010; Metian et al., 2009, 2016). In the present study, we carried out a simple experiment using a double-tracer radioisotope approach to more easily provide the first information regarding the contribution of dietary and aqueous sources of Cs in its global accumulation by P. olivaceus. This approach has some limitations (Table 2), and the relative contribution of dietary versus aqueous exposure pathways to radiocaesium bioaccumulation was over-simplified in this study due to the multiple and partially sporadic feedings as compared to implementing a biodynamic model. Nevertheless, the simultaneous exposure using two radioisotopes of caesium suggests the predominant role of food in the bioaccumulation of Cs in P. olivaceus (approximately two-thirds of the Cs whole-body activity derived from food; Fig. 4). This finding is in agreement with previous studies with other fish species (Mathews et al., 2008; Zhao et al., 2001).

Sediment exposure, which was not tested in this experiment, is expected to be an additional pathway for Cs contamination in P. olivaceus due to their benthic niche. Nevertheless, it could be considered in the feeding pathway (particulate pathway). As seawater Cs concentrations are typically much lower than those of sediment, one might expect bioaccumulation from sediment to be higher than via seawater exposure in the marine environment for demersal species. Limited studies comparing seawater and sediment radiocaesium exposure pathways have shown sediment-bound Cs to be bioavailable (Wang et al., 2016), though its contribution to Cs bioaccumulation compared to seawater exposure is variable (<1–31% and 6–24% for seawater and sediment 134Cs uptake pathways, respectively; Metian et al., 2016). Further investigations are needed to characterize the importance of this Cs bioaccumulation pathway in P. olivaceus and properly confirm our results using a bioenergetic model over a long-term experiment.

In fish, the trophic transfer of radionuclides can be best assessed experimentally by two main methods: (1) the “single-feeding” approach where fish are fed radiolabelled food for a unique pulse-chase feeing [as described by Wang and Fisher (1999)], and (2) the “multi-feeding” approach where fish are regularly exposed to radiolabelled food (e.g., Pouil et al., 2017). The latter has the advantage of tracking more similarly to marine organisms consuming contaminated food over a period of time as would be expected in natural systems with prolonged sources of Cs contamination. However, the “multiple feeding” approach utilized in this experiment does not allow for the calculation of assimilation efficiency (AE; see the review of Pouil et al., 2018). Nevertheless, an analogous parameter to AE is the percentage of remaining 134Cs activity when the data plateau after approximately 60 d of depuration, which was 36.0 ± 17.8% for P. olivaceus in this experiment (Fig. 3B). Comparing this to calculated AEs from other single-feeding studies, juvenile cuttlefish displayed an AE of 29.2 ± 3.6% and a similar long-lived biological half-life Tb½l of 66 d after a single feeding of 134Cs-contaminated A. salina, though depuration biological half-lives following seawater exposure and dietary exposure in adults were much different (Tb½l = 6.1 and 16 d, respectively) than for P. olivaceus (Bustamante et al., 2006). From the same flatfish order as P. olivaceus (Pleuronectiformes), the turbot Psetta maxima had a higher AE of 63 ± 2% and Tb½ of 36.5 d following consumption of 134Cs-contaminated prey (Mathews et al., 2008). An even greater AE of 79.6 ± 8.6% with a Tb½ of 13.9 d was determined in the killifish Fundulus heteroclitus after consuming 137Cs-contaminated blackworms (Wang et al., 2016).

Although the assimilation of Cs is very variable among fish species (from 50 to 95%; Pouil et al., 2018), the remaining activity values in the present study are still considered low compared to AEs of other high predatory species such as the seabass Dicentrarchus labrax (Mathews and Fisher, 2008) and the false kelpfish Sebastiscus marmoratus (Pan and Wang, 2016). It is generally assumed that AEs of Cs are higher in predator fish compared to planktivorous and herbivorous species (Pan and Wang, 2016; Rowan and Rasmussen, 1994). In the present study, the amount of remaining activity suggests that this statement is not always true. In fish, the mechanisms underlying species-dependent AE of radionuclides are unclear though Chan et al. (2003) attributed the differences of radionuclide AEs between the mudskipper Periophthalmus modestus and the rabbitfish Siganus canaliculatus to the gut passage time (GPT), with a longer GPT corresponding to a higher AE.

In many studies considering the trophic transfer of Cs in fish, emphasis is on the potential for biomagnification of this radionuclide in marine food chains (e.g., Pan and Wang, 2016; Zhao et al., 2001). To determine this potential, the most common approach consists of calculating the trophic transfer factor (TTF; Reinfelder et al., 1998) from the kinetic parameters (AE and ke) and the ingestion rate (IR). When TTF >1, it indicates a potential Cs biomagnification; when TTF <1, biomagnification is unlikely (Mathews et al., 2008; Reinfelder et al., 1998). Several studies have concluded that biomagnification of Cs can occur in the marine environment. In our study we cannot calculate the TTF since we have not adopted an approach allowing for the proper measurement of the required kinetic parameters; however, the “multi-feeding” approach carried out here can be used to characterize when biomagnification is effective (i.e., when Cs concentrations are higher in fish than in food). As such, based on the first 4 days of feeding with radiolabelled brine shrimp where concentrations of Cs in fish were multiplied by approximately 4.5 (Fig. 2) and assuming a linear increase in Cs concentrations in P. olivaceus (Fig. 1), biomagnification could occur in less than one month. These preliminary results raise the interest of using the multiple feeding approach to confirm experimentally previous results obtained by modelling.

Our results indicated a limited bioaccumulation of Cs in P. olivaceus from seawater exposure. The concentration factor (CF) calculated for P. olivaceus in this experiment of 1.61 ± 0.47 is generally low compared to other fish species (Jeffree et al., 2010; Zhao et al., 2001), and much lower than invertebrates such as cephalopods and decapods (e.g., Bustamante et al., 2006; Metian et al., 2016). It is nevertheless important to note that contrary to what has occurred in past studies, the radiocaesium uptake kinetics did not reach a plateau during the exposure period; thus, it seems we can expect a high CF value in steady-state conditions for this fish species. However, such results suggest low Cs bioaccumulation capacities from aqueous exposure in P. olivaceus (very low uptake rate constant), and we can assume based on this experiment that bioaccumulation of Cs is mainly derived by dietary intake in this species.

A similar double-tracer method has been used previously to assess dietary versus aqueous exposure pathways in the bioaccumulation of radioactive polonium in decapods and fish (Carvalho and Fowler, 1994). The time and resources saved through use of this technique are significant, yet the technical challenges to source, administer, and analyse multiple radioisotopes of a specific element of interest can be great (Table 2). Furthermore, in such experiments full control of single-tracer exposure is not possible and potential cross-contamination could occur such as seawater adsorbed to food or leaching of radiolabelled food into the seawater. Further improvements and future directions for this methodology include utilizing a single pulse-chase feeding rather than the multiple feedings as in this experiment (Pouil et al., 2017), extending exposure time to reach a steady-state concentration factor (Fig. 1), and incorporating Cs bioaccumulation via exposure to contaminated sediments.

5. Conclusions

To maximize resources, the double-radioisotope approach used in this study allows for a novel assessment of the simultaneous determination of caesium bioaccumulation via both dietary and aqueous exposure pathways. Using this method, the results of this work indicate that food was the predominant uptake pathway for radiocaesium in the olive flounder *P. olivaceus*, relative to seawater exposure. Implications for this work would extend to seafood safety programmes that must examine all vectors for contamination.

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Figure Captions

Figure 1. Uptake of ^{134}Cs via food and ^{137}Cs via seawater in Japanese flatfish (*P. olivaceus*) over 8 d. Values are means ± one standard deviation (n = 9-11).

Figure 2. Daily change and total 134Cs activity (Bq) in Japanese flatfish (P. olivaceus) exposed via food over 8 d. Also plotted is the percentage of total food activity assimilated by the fish over time.

Figure 3. Depuration kinetics of 134Cs and 137Cs in Japanese flatfish (P. olivaceus) over 79 d following food and seawater exposure. Total activity (Bq) and kinetic models are displayed in (A) and the percentage of remaining activity in (B). Values are means ± one standard deviation (n = 5–7).

Figure 4. Relative contribution (%) of the uptake pathways (seawater or food) to the total activity of Cs in Japanese flatfish (P. olivaceus) over the course of the experiment (87 d). The end of exposure is indicated following day 8 by *. The dashed line marked X is the average for the entire experiment.

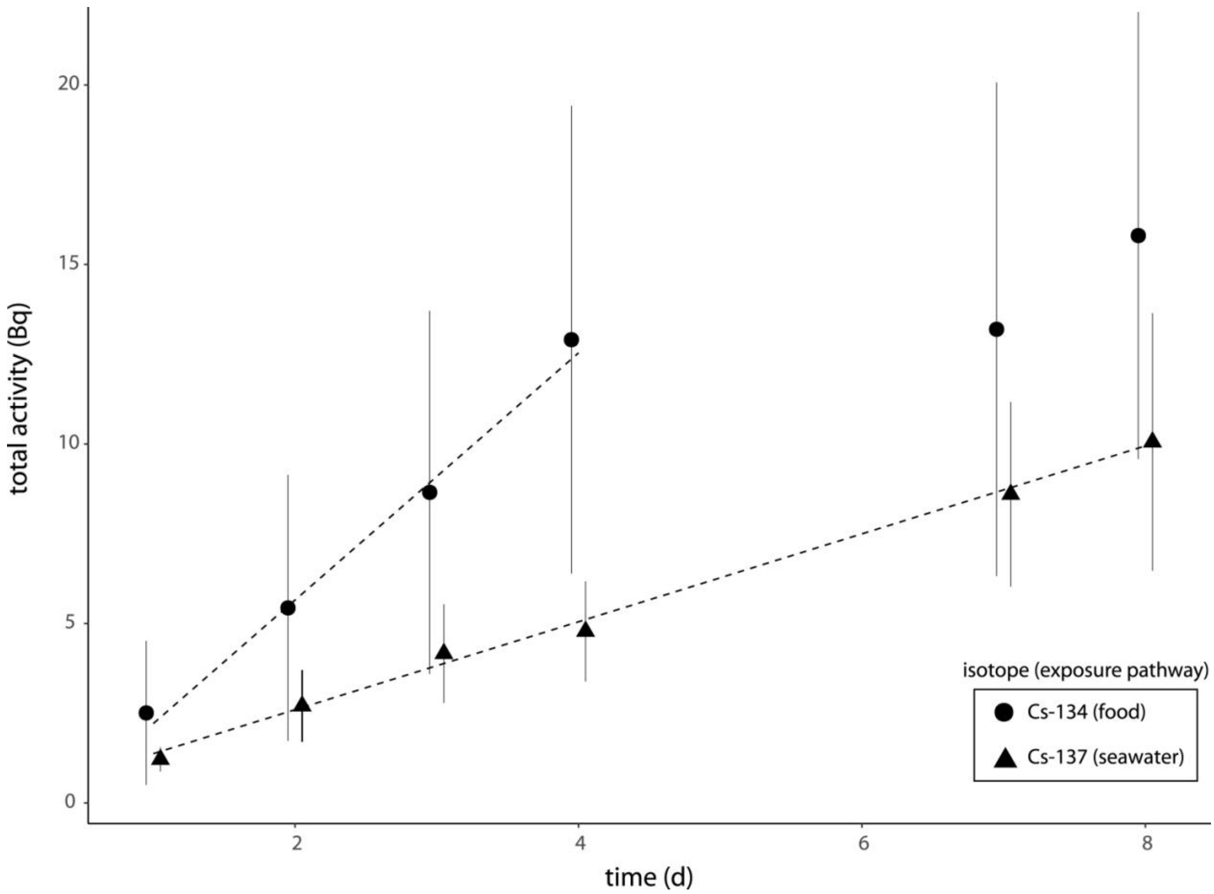

total activity (Bq)
20
15
10
5
0
2
4
6
8
time (d)
isotope (exposure pathway)
Cs-134 (food)
Cs-137 (seawater)

Figure 1

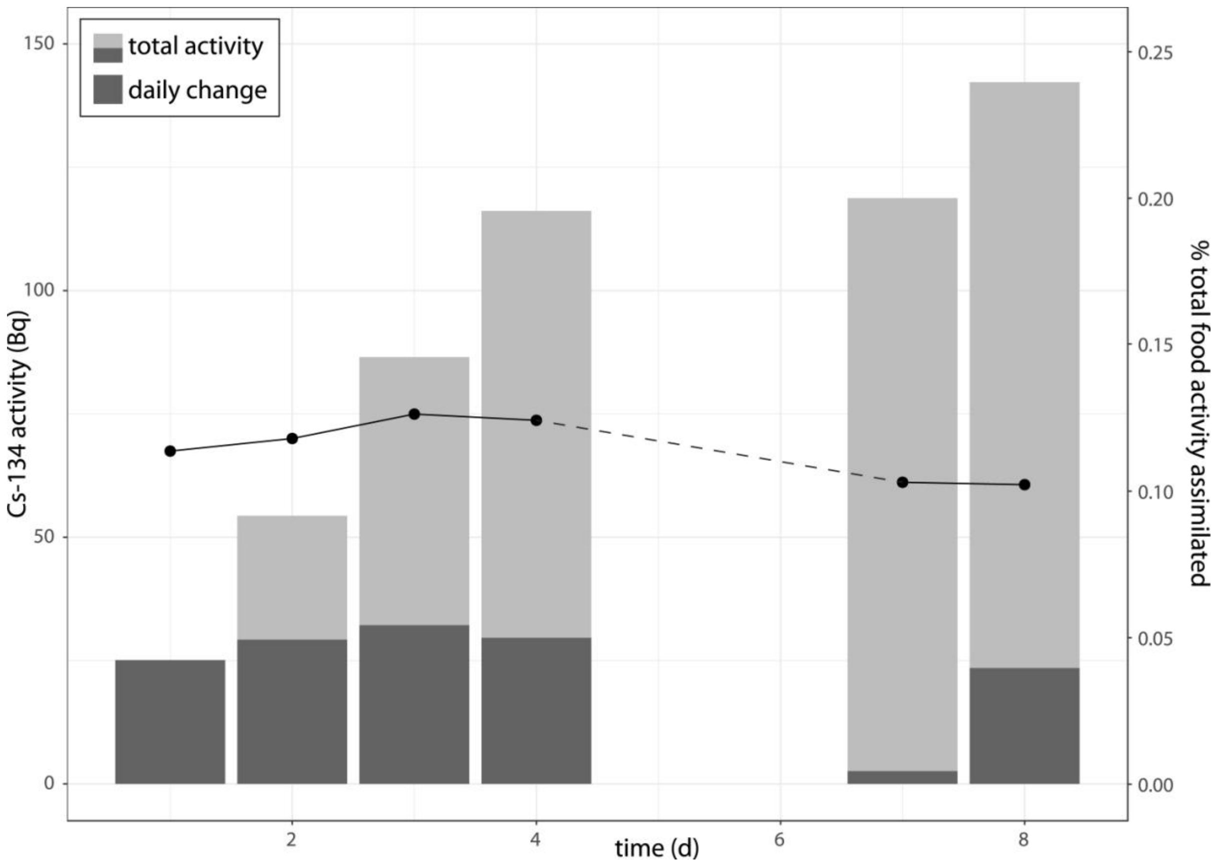

total activity
daily change
150
100
50
0
Cs-134 activity (Bq)
0.25
0.20
0.15
0.10
0.05
0.00
% total food activity assimilated
2
4
6
8
time (d)

Figure 2

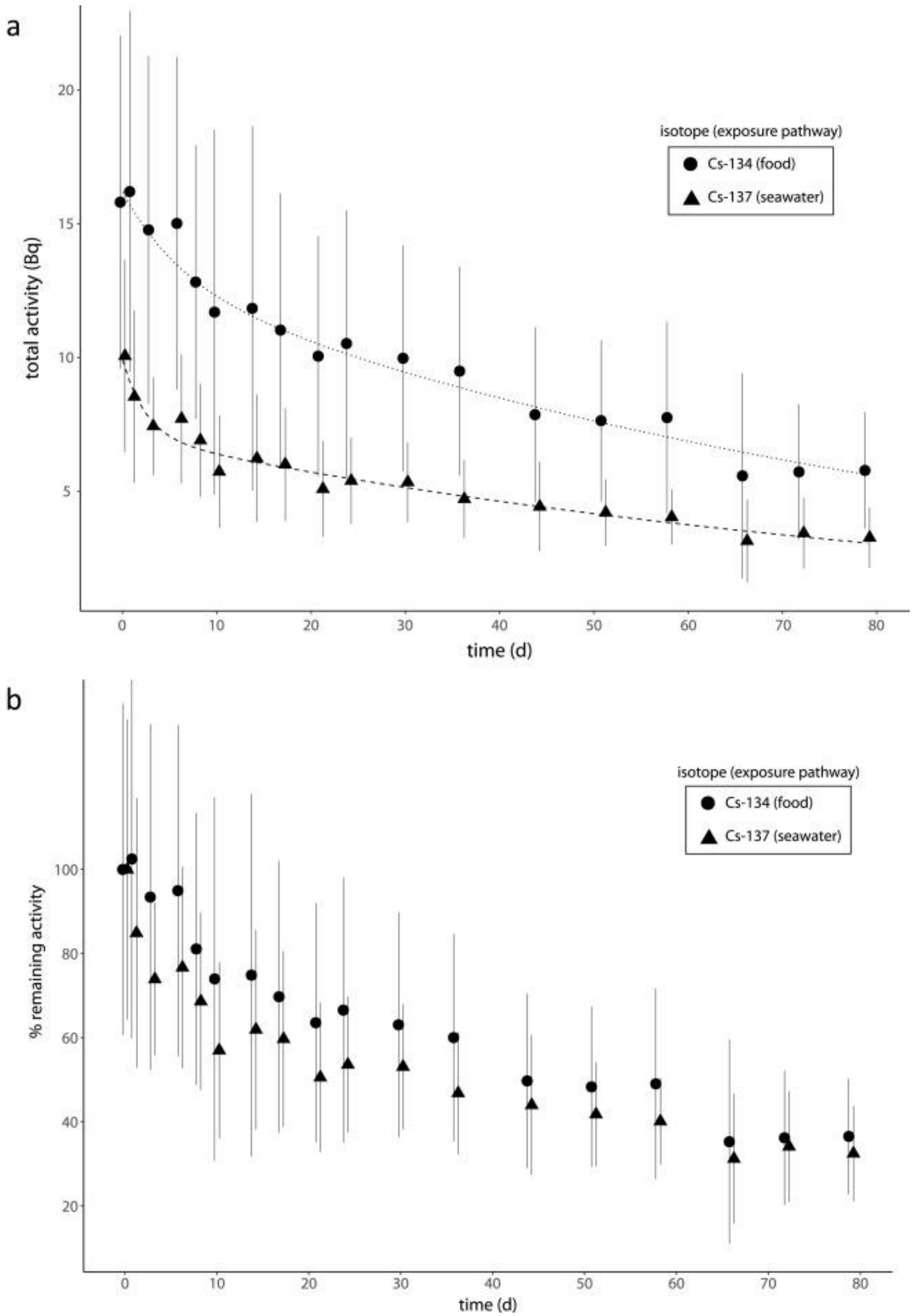
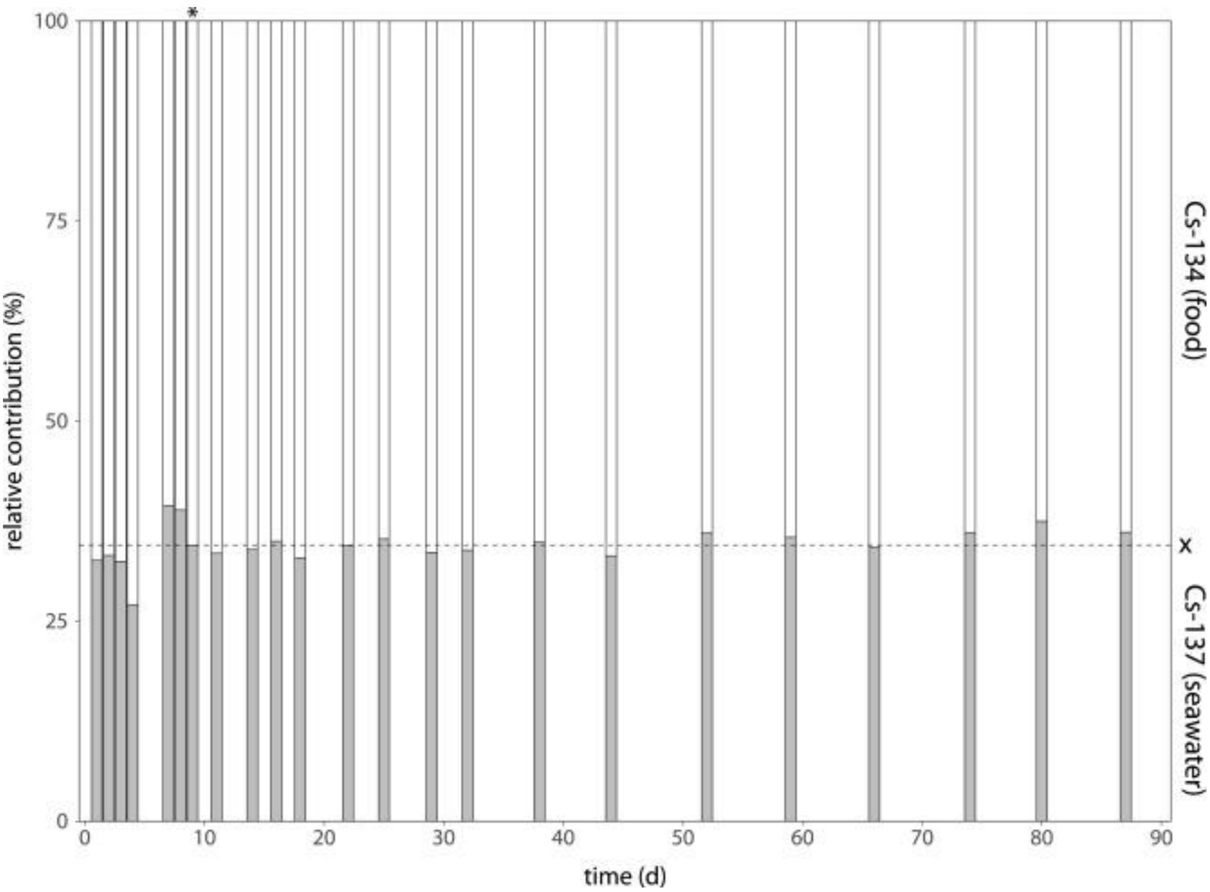

a
total activity (Bq)
time (d)
isotope (exposure pathway)
Cs-134 (food)
Cs-137 (seawater)
b
% remaining activity
time (d)
isotope (exposure pathway)
Cs-134 (food)
Cs-137 (seawater)

Figure 3



100
75
50
25
0
relative contribution (%)
0
10
20
30
40
50
60
70
80
90
time (d)
Cs-134 (food)
Cs-137 (seawater)
x
*

Figure 4

Tables

Table 1. Model parameters for the depuration kinetics of ^{134}Cs and ^{137}Cs in Japanese flatfish (*P. olivaceus*) exposed via food and seawater. A_{0s} and A_{0l}: activity (Bq) lost according to the short- and long-lived exponential component, respectively; $T_{b½}$: biological half-life (d) [$T_{b½} = \ln 2/k_e$]; ASE: asymptotic standard error; R^2: determination coefficient of kinetics. Probability (p) of each parameter estimation is indicated as follows: ^{NS}Not significant ($p > 0.05$), $^{*}p < 0.05$, $^{**}p < 0.001$.

Isotope	Exposure Pathway	A_{0s} ± ASE	$T_{b½s}$ ± ASE	A_{0l} ± ASE	$T_{b½l}$ ± ASE	R^2
Cs-134	Food	3.24 ± 2.81^{NS}	4.31 ± 6.10^{NS}	$12.94 \pm 2.58^{**}$	$65.63 \pm 27.74^{*}$	0.32
Cs-137	Seawater	$2.85 \pm 0.79^{**}$	1.68 ± 2.28^{NS}	$7.06 \pm 0.79^{**}$	$65.65 \pm 17.79^{**}$	0.49

Table 2. List of advantages and disadvantages of the double-tracer radioisotope approach used in this study.

Advantages	Disadvantages
saves time by running single concurrent experiment (also labour, lab resources)	requires purchasing two different radioactive sources, which implies an increasing cost
can evaluate simultaneous and/or compounding effects on single fish exposed by both pathways	potential analytical issues resolving both isotopes
	potential risk to not have full control of single tracer exposure (potential cross-contamination could occur such as seawater on food or leaching of labelled food into seawater)
	limited to two simultaneous exposure pathways studied per experiment