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

3 Roberta L. Hansman, Marc Metian, François Oberhänsli, Jean-Louis Teyssié, and Peter W. Swarzenski
4 *International Atomic Energy Agency – Environment Laboratories, Radioecology Laboratory; 4a Quai*
5 *Antoine 1er, MC-98000 Principality of Monaco*

6

7 **Abstract**

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

19 **1. Introduction**

20 As a consequence of the accident at the Tokyo Electric Power Company (TEPCO) Fukushima Dai-ichi
21 Nuclear Power Plant (FDNPP; IAEA, 2015), large amounts of radioactive caesium [estimates for ^{137}Cs
22 vary from 3.5 PBq according to Tsumune et al. (2012) to 27 PBq reported by Bailly du Bois et al.
23 (2012)] had been released into the ocean. This radioactive release was predominantly transported
24 southward (Aoyama et al., 2012; Tsumune et al., 2012), and relatively high concentrations of
25 radioactive caesium [both ^{134}Cs (half-life of 2.065 y) and ^{137}Cs (30.167 y)] were detected in a variety
26 of marine organisms around the southern coast of Fukushima Prefecture after the accident
27 (Arakawa et al., 2015; Shigenobu et al., 2014). Approximately 6 years have passed since the accident
28 occurred, and the radioactive caesium concentrations in seawater off the coast of Fukushima
29 Prefecture have now dropped so that they are close to pre-accident levels (0.001–0.002 Bq L⁻¹)
30 (Kusakabe et al., 2013; Oikawa et al., 2013). Concentration reductions have also been observed in
31 seaweed, cephalopods, shellfish, and crustaceans; however, the rates of reduction have varied
32 among taxonomic groups. Radiocaesium concentrations have also declined in fish species that were
33 significantly contaminated [e.g., Japanese rockfish (*Sebastes cheni*), fat greenling (*Hexagrammos*

34 *otakii*), and marbled sole (*Pleuronectes yokohamae*] (Iwata et al., 2013; Sohtome et al., 2014; Wada
35 et al., 2013).

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

46 Studies have focused on the differences in the bioaccumulation of radionuclides in marine organisms
47 depending on the particular contaminant pathway, be it through aqueous, dietary, sedimentary, or
48 maternal exposure routes. The uptake and depuration of radionuclides by marine organisms is
49 variable depending on species, element, and environmental conditions. Some studies have been
50 able to demonstrate that radiocaesium concentrations increase with increasing trophic levels
51 (Kasamatsu and Ishikawa, 1997; Mathews and Fisher, 2008), providing evidence for bioaccumulation
52 and suggesting biomagnification (Mathews and Fisher, 2008; Pan and Wang, 2016; Zhao et al.,
53 2001).

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

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

70 **2. Material and methods**

71 *2.1 Experimental organisms*

72 Japanese aquaculture juvenile fish *Paralichthys olivaceus* were obtained from a fish wholesaler
73 (Tropic Nguyen, France). They were acclimated to laboratory conditions for 4 weeks in an open
74 circuit 500-L aquarium; flux: 50 L h⁻¹ of 1- μ m filtered seawater; salinity: 38 g L⁻¹; temperature:
75 20.5 \pm 0.5 $^{\circ}$ C; pH: 8.0 \pm 0.1; light/dark cycle: 12 h/12 h. During this period fish were fed daily with
76 frozen *Artemia salina* and *Euphasia pacifica*.

77 *2.2 Radiotracers and counting*

78 The uptake and depuration of radiocaesium in *P. olivaceus* were determined using radiotracers
79 purchased from Polatom (¹³⁴CsCl in aqueous solution) and Areva Cerva Lee (¹³⁷CsCl in 0.1 N HCl).
80 ¹³⁴Cs and ¹³⁷Cs were counted using a high-resolution γ -spectrometer system composed of four
81 high-purity germanium (HPGe) detectors (efficiency = 50%) connected to a multi-channel analyzer
82 and a computer equipped with spectra analysis software Interwinner 6. Precise activities of ¹³⁴Cs
83 (605, 796 keV) and ¹³⁷Cs (662 keV) were determined using standards (i.e., phantoms, as described in
84 Cresswell et al., 2017) of known activity and appropriate geometries, and measurements were
85 subsequently corrected for counting efficiencies and radioactive decay (Cresswell et al., 2017).
86 Counting times ranged from 20 to 73 min with an average of 50 min. The counting times were
87 adjusted to obtain propagated counting errors generally less than 5%, although a few samples with
88 very low activities had counting errors up to 15%.

89 *2.3 Experimental procedure*

90 A single experiment was conducted to investigate Cs bioaccumulation in the Japanese flatfish
91 simultaneously through seawater and dietary exposure pathways over a long period (87 d total
92 consisting of 8 d of uptake followed by 79 d of depuration). The experiment was conducted using
93 eleven *P. olivaceus* fish (mean initial weight 5.19 \pm 1.85 g) in 70-L closed-circuit aquaria constantly
94 aerated with an aquarium water pump under the following conditions: salinity = 38 g L⁻¹,
95 temperature = 20.5 \pm 0.5 $^{\circ}$ C, pH = 8.0 \pm 0.1, light/dark cycle = 12 h/12 h. All 11 organisms were
96 exposed for 8 d to seawater spiked with ¹³⁷Cs dissolved in 1 μ m-filtered seawater (1 Bq mL⁻¹), and 10

97 of these were fed food labelled with ^{134}Cs to allow for one single-exposed (^{137}Cs via seawater)
98 control.

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

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

116 1.4 Data analyses

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

$$121 \quad (1) \quad CF_t = k_u t$$

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

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

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

126 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)
127 and 0, respectively; 's' and 'l' subscripts denote the short- and long-lived exponential components.
128 Biological half-lives ($T_{b_{1/2s}}$ and $T_{b_{1/2l}}$) were calculated from the corresponding depuration rate constant
129 (k_{es} and k_{el} , respectively) according to the relation $T_{b_{1/2}} = \ln 2/k_e$ as in Whicker and Schultz (1982).
130 Model constants and statistics were estimated by iterative adjustment of the model using the non-
131 linear curve fitting routines in the Statistica software package (StatSoft, Inc., 2004) and statistical
132 methods as in Warnau et al. (1996a, 1996b) and Metian et al. (2011). Additional statistical analyses
133 were performed using R (R Core Team, 2016).

134 The percentage of ^{134}Cs food activity assimilated was calculated by dividing the total ^{134}Cs activity
135 measured in the fish each day during the uptake phase by the total cumulative ^{134}Cs activity in the
136 food given (as ^{134}Cs -labelled *A. salina*). The relative contribution of ^{134}Cs (food) and ^{137}Cs (seawater)
137 to total activity was calculated as the proportion of the mean activity of each radioisotope (^{134}Cs or
138 ^{137}Cs in Bq) to the mean total activity (i.e., $^{134}\text{Cs} + ^{137}\text{Cs}$ in Bq) in the fish each day measurements
139 were taken during the experiment.

140 3. Results

141 3.1 Uptake

142 The simultaneous uptake of $^{134,137}\text{Cs}$ by *P. olivaceus* through both aqueous and dietary exposure
143 pathways is shown in Fig. 1 as total activity (Bq) over time (d). Multiple feedings of ^{134}Cs -labelled
144 food resulted in higher total activities in the fish than through seawater exposure. Over the initial
145 four days, the rate of accumulation was more than double for dietary uptake of Cs than seawater
146 exposure (3.441 Bq d^{-1} vs. 1.216 Bq d^{-1} ; $R^2 = 0.992$ and 0.971 for linear regression, respectively).
147 Although some depuration occurred during the two-day pause in ^{134}Cs -labelled feedings, the total
148 activity increased over the entire exposed period and $11.5 \pm 1.0\%$ of the total food ^{134}Cs activity
149 given to the fish was assimilated (Fig. 2). As seawater ^{137}Cs exposure continued during the two-day
150 pause in feeding and counting, total activity in fish for ^{137}Cs increased linearly over the entire
151 exposure period (1.225 Bq d^{-1} , $R^2 = 0.996$). While the multiple feeding strategy utilized in this
152 experiment does not allow for the calculation of assimilation efficiency (AE) as in single feeding
153 studies, the calculated concentration factor (CF) for seawater exposure reached a value of
154 1.61 ± 0.47 at the end of the exposure (day 8) with an uptake rate constant (k_u) of 0.205 d^{-1} .

155 3.2 Depuration

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

167 3.3 Global bioaccumulation

168 The relative contribution of ^{134}Cs vs. ^{137}Cs to total activity over the course of the entire experiment is
169 shown in Fig. 4. The average contribution of Cs activity from seawater exposure over all 87 d was
170 $34.6 \pm 2.5\%$ (\pm one standard deviation), and though slightly more variable during the uptake period,
171 there was no significant difference in relative contribution when compared to the loss phase
172 ($33.9 \pm 4.6\%$ and $34.7 \pm 1.2\%$, respectively; $p > 0.05$). Approximately two-thirds of the total Cs
173 radioactivity in *P. olivaceus* during both uptake and depuration is due to consumption of Cs-
174 contaminated food.

175 4. Discussion

176 The olive flounder is a commercially important fishery that was essentially closed in the waters
177 around Fukushima following the accident due to observed increased levels of radiocaesium
178 contamination above the Japanese standard limit for food safety of 100 Bq kg^{-1} wet weight enforced
179 in April 2012 (Wada et al., 2013). Concentrations of $^{134}\text{Cs} + ^{137}\text{Cs}$ in the surrounding seawater
180 immediately after the accident were initially very high but decreased rapidly (Aoyama et al., 2016),
181 yet concentrations in *P. olivaceus* tissues remained high and could be found in excess of the limit up
182 to 3 years later (up to 230 Bq kg^{-1} ; Kurita et al., 2015). By these standards, both dietary and aqueous
183 exposure to radiocaesium at the concentrations used in the present experiment led to
184 contamination levels in *P. olivaceus* within one day. During depuration, concentrations of
185 radiocaesium did not fall below the food safety limit by the end of the experiment 79 d after
186 exposure as final average concentrations were 569 ± 211 and $288 \pm 76 \text{ Bq kg}^{-1}$ for ^{134}Cs and ^{137}Cs ,
187 respectively. This accounted for 19.2% and 16.9% of the maximum ^{134}Cs and ^{137}Cs concentrations
188 at the beginning of the depuration period, respectively. This direct comparison from our laboratory

189 experiment and the field should be taken in context however, as the juvenile fish used here can have
190 different uptake and depuration biokinetics than commercial-sized adult flounder (e.g., Suzuki et al.,
191 1992). Nonetheless, it is still useful to make intermediate connections between laboratory and field
192 measurements with the goal of further understanding contamination pathways in the marine
193 environment.

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

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

222 In fish, the trophic transfer of radionuclides can be best assessed experimentally by two main
223 methods: (1) the “single-feeding” approach where fish are fed radiolabelled food for a unique pulse-
224 chase feeding [as described by Wang and Fisher (1999)], and (2) the “multi-feeding” approach where
225 fish are regularly exposed to radiolabelled food (e.g., Pouil et al., 2017). The latter has the advantage
226 of tracking more similarly to marine organisms consuming contaminated food over a period of time
227 as would be expected in natural systems with prolonged sources of Cs contamination. However, the
228 “multiple feeding” approach utilized in this experiment does not allow for the calculation of
229 assimilation efficiency (AE; see the review of Pouil et al., 2018). Nevertheless, an analogous
230 parameter to AE is the percentage of remaining ^{134}Cs activity when the data plateau after
231 approximately 60 d of depuration, which was $36.0 \pm 17.8\%$ for *P. olivaceus* in this experiment (Fig.
232 3B). Comparing this to calculated AEs from other single-feeding studies, juvenile cuttlefish displayed
233 an AE of $29.2 \pm 3.6\%$ and a similar long-lived biological half-life $T_{b1/2}$ of 66 d after a single feeding of
234 ^{134}Cs -contaminated *A. salina*, though depuration biological half-lives following seawater exposure
235 and dietary exposure in adults were much different ($T_{b1/2} = 6.1$ and 16 d, respectively) than for *P.*
236 *olivaceus* (Bustamante et al., 2006). From the same flatfish order as *P. olivaceus* (Pleuronectiformes),
237 the turbot *Psetta maxima* had a higher AE of $63 \pm 2\%$ and $T_{b1/2}$ of 36.5 d following consumption of
238 ^{134}Cs -contaminated prey (Mathews et al., 2008). An even greater AE of $79.6 \pm 8.6\%$ with a $T_{b1/2}$ of
239 13.9 d was determined in the killifish *Fundulus heteroclitus* after consuming ^{137}Cs -contaminated
240 blackworms (Wang et al., 2016).

241 Although the assimilation of Cs is very variable among fish species (from 50 to 95%; Pouil et al.,
242 2018), the remaining activity values in the present study are still considered low compared to AEs of
243 other high predatory species such as the seabass *Dicentrarchus labrax* (Mathews and Fisher, 2008)
244 and the false kelpfish *Sebastes marmoratus* (Pan and Wang, 2016). It is generally assumed that
245 AEs of Cs are higher in predator fish compared to planktivorous and herbivorous species (Pan and
246 Wang, 2016; Rowan and Rasmussen, 1994). In the present study, the amount of remaining activity
247 suggests that this statement is not always true. In fish, the mechanisms underlying species-
248 dependent AE of radionuclides are unclear though Chan et al. (2003) attributed the differences of
249 radionuclide AEs between the mudskipper *Periophthalmus modestus* and the rabbitfish *Siganus*
250 *canaliculatus* to the gut passage time (GPT), with a longer GPT corresponding to a higher AE.

251 In many studies considering the trophic transfer of Cs in fish, emphasis is on the potential for
252 biomagnification of this radionuclide in marine food chains (e.g., Pan and Wang, 2016; Zhao et al.,
253 2001). To determine this potential, the most common approach consists of calculating the trophic
254 transfer factor (TTF; Reinfelder et al., 1998) from the kinetic parameters (AE and k_e) and the

255 ingestion rate (IR). When TTF >1, it indicates a potential Cs biomagnification; when TTF <1,
256 biomagnification is unlikely (Mathews et al., 2008; Reinfelder et al., 1998). Several studies have
257 concluded that biomagnification of Cs can occur in the marine environment. In our study we cannot
258 calculate the TTF since we have not adopted an approach allowing for the proper measurement of
259 the required kinetic parameters; however, the “multi-feeding” approach carried out here can be
260 used to characterize when biomagnification is effective (i.e., when Cs concentrations are higher in
261 fish than in food). As such, based on the first 4 days of feeding with radiolabelled brine shrimp where
262 concentrations of Cs in fish were multiplied by approximately 4.5 (Fig. 2) and assuming a linear
263 increase in Cs concentrations in *P. olivaceus* (Fig. 1), biomagnification could occur in less than one
264 month. These preliminary results raise the interest of using the multiple feeding approach to confirm
265 experimentally previous results obtained by modelling.

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

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

287 **5. Conclusions**

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

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446 **Figure Captions**

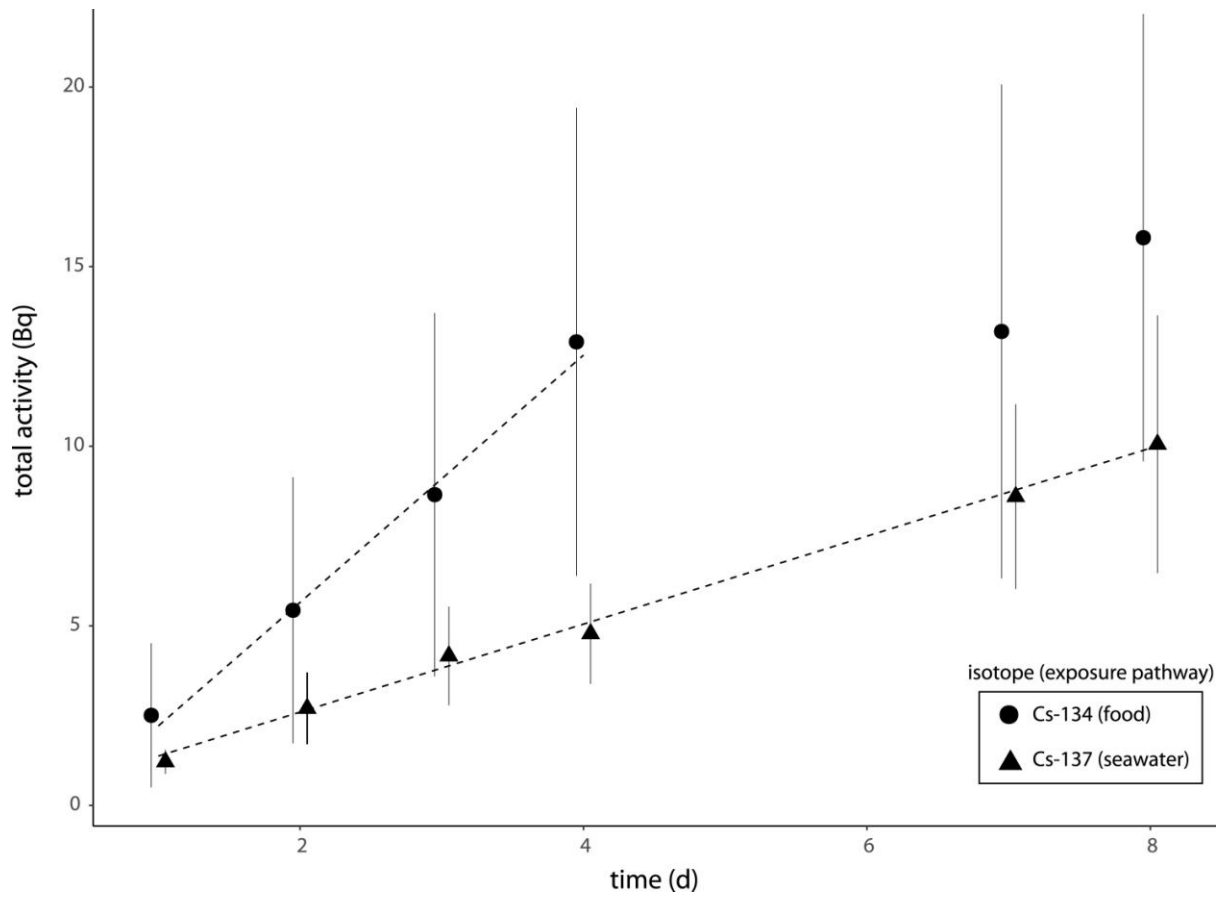
447 **Figure 1.** Uptake of ^{134}Cs via food and ^{137}Cs via seawater in Japanese flatfish (*P. olivaceus*) over 8 d.
448 Values are means \pm one standard deviation (n = 9-11).

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

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

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

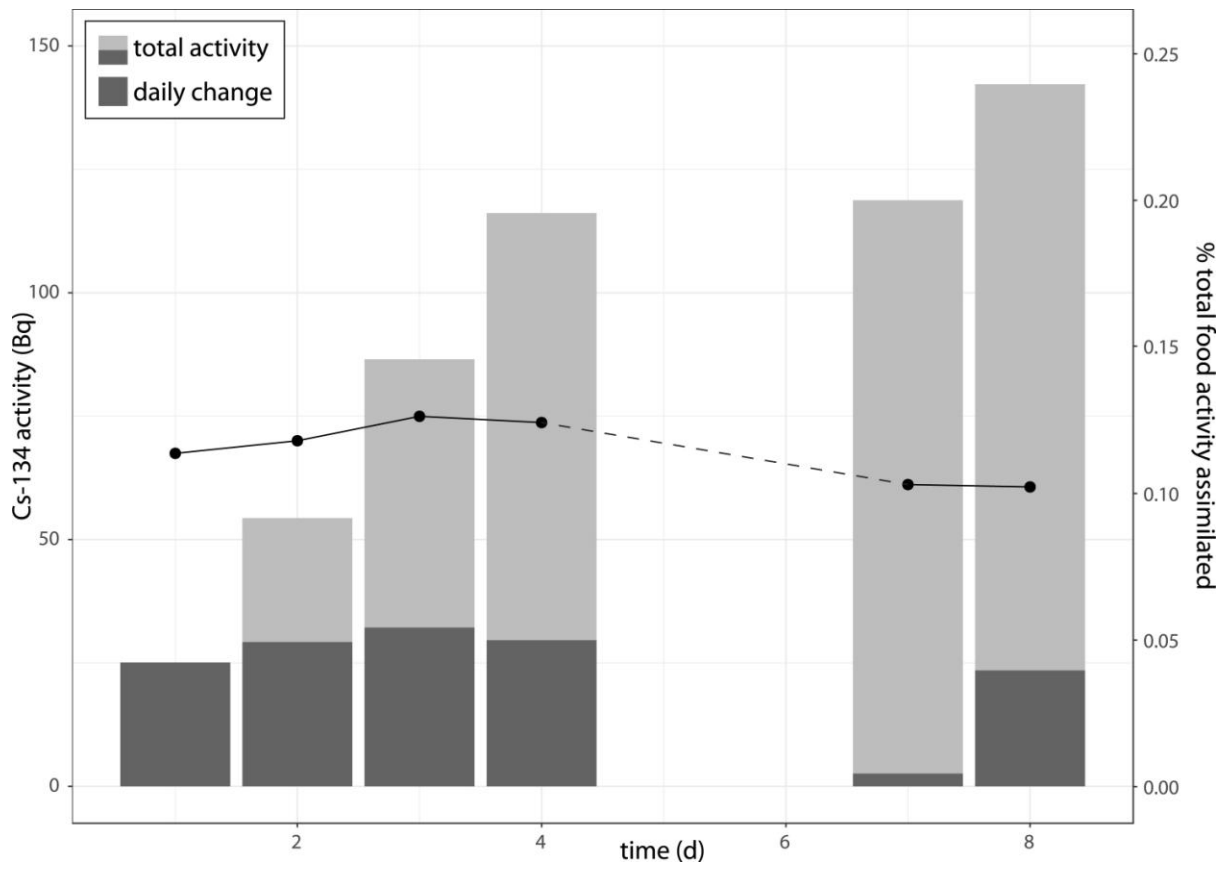
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459 **Figure 1**

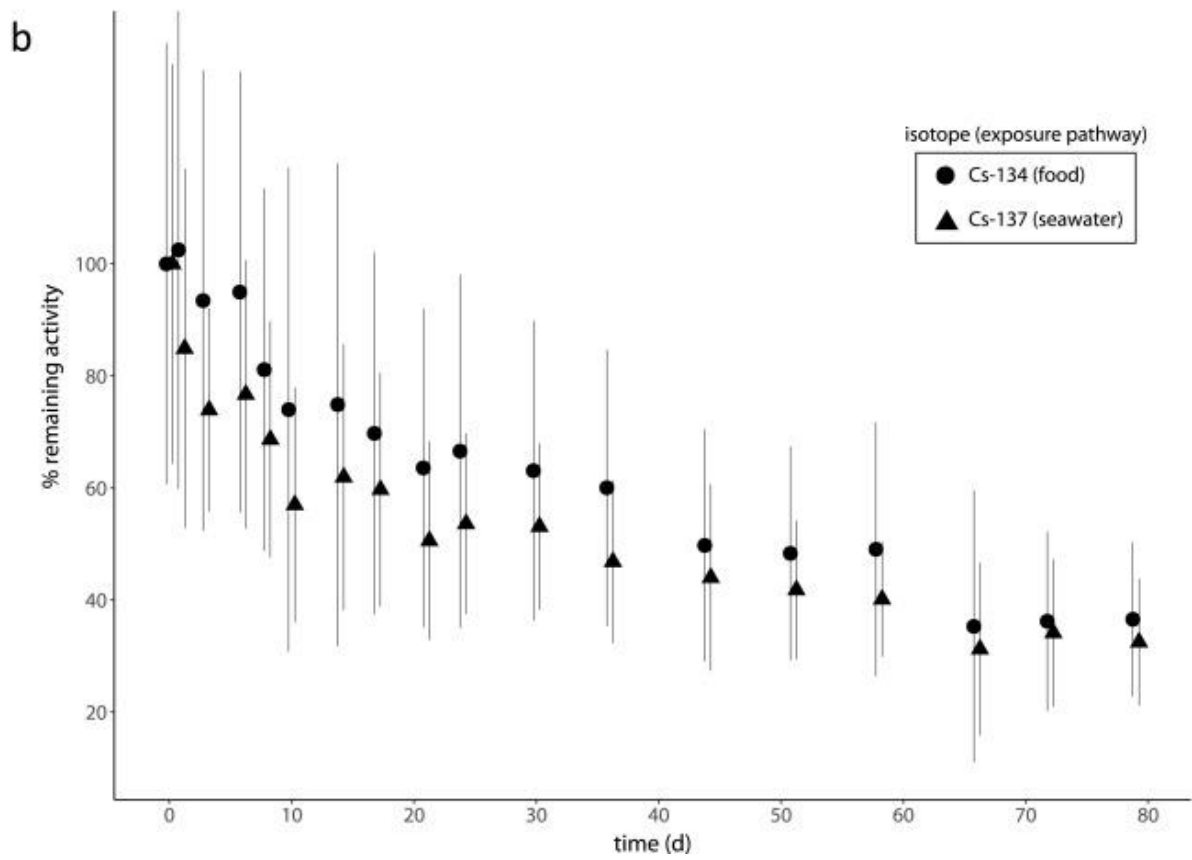
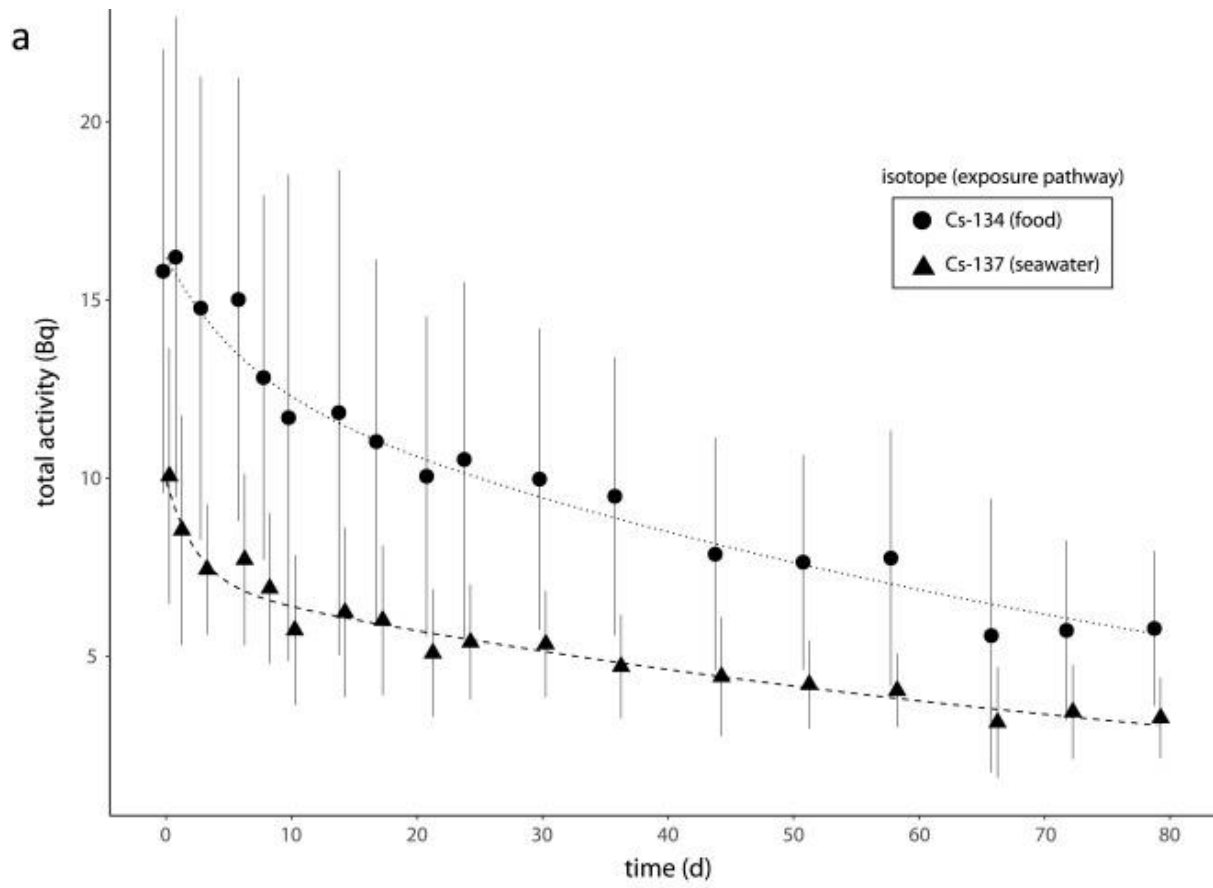
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462 **Figure 2**

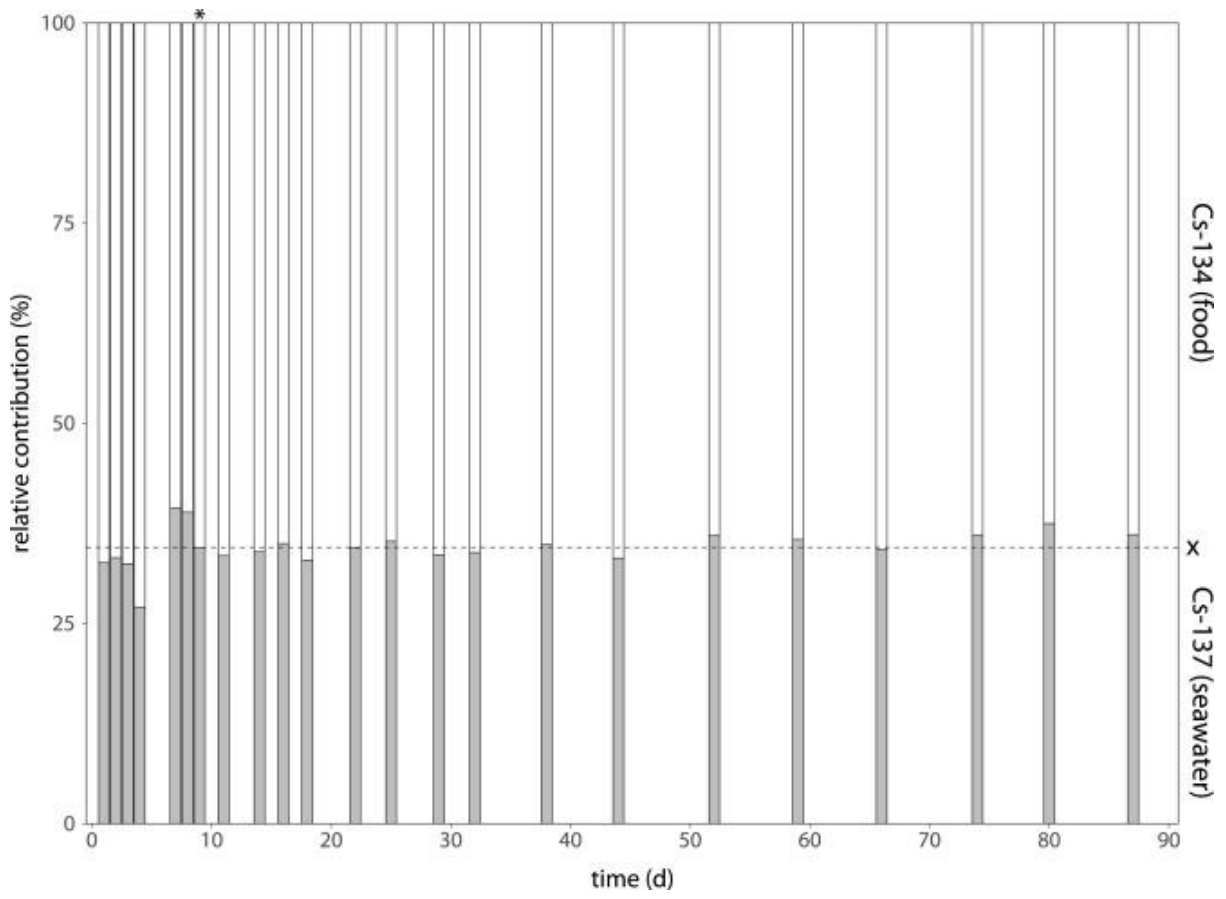
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465 **Figure 3**

466



467

468 **Figure 4**

469 **Tables**

470 **Table 1.** Model parameters for the depuration kinetics of ¹³⁴Cs and ¹³⁷Cs in Japanese flatfish (*P.*
 471 *olivaceus*) exposed via food and seawater. A_{0s} and A_{0l}: activity (Bq) lost according to the short- and
 472 long-lived exponential component, respectively; T_{b½}: biological half-life (d) [T_{b½} = ln2/k_e]; ASE:
 473 asymptotic standard error; R²: determination coefficient of kinetics. Probability (p) of each
 474 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 ± 2.58 ^{**}	65.63 ± 27.74 [*]	0.32
Cs-137	Seawater	2.85 ± 0.79 ^{**}	1.68 ± 2.28 ^{NS}	7.06 ± 0.79 ^{**}	65.65 ± 17.79 ^{**}	0.49

475

476 **Table 2.** List of advantages and disadvantages of the double-tracer radioisotope approach used in
 477 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

478