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1 **Interspecific comparison of radiocesium trophic transfer in two tropical fish species**

2

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21

22 **Abstract:**

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

35

36 Keywords: Experimental radiotracer study; ^{134}Cs ; Assimilation efficiency; Kinetics; Tissue
37 distribution

38

39 **1. Introduction**

40 Anthropogenic activities have resulted in various degrees of contamination of the world's seas
41 and oceans with radionuclides (Friedlander et al., 2005). Radionuclides such as ^{134}Cs are
42 waste products from industrial activities that can enter aquatic systems (Twining et al., 1996).
43 Scientific literature generally reports relatively low bioaccumulation of radiocesium (^{134}Cs
44 and ^{137}Cs) in fish (e.g. Heldal et al., 2003; Kasamatsu and Ishikawa, 1997; Topcuoğlu, 2001).
45 However, after the accident at the civilian Fukushima Daichii NPP, several field
46 investigations have shown the ability of different ecological groups of fish (such as pelagic or
47 benthic fish) to accumulate the released radiocesium (Iwata et al., 2013; Wada et al., 2013,
48 2016). The highest ^{134}Cs concentrations in marine fish (338 Bq kg^{-1}) were reported in seabass
49 caught in the surrounding of Hitachi, Japan (Chen, 2013). Until now, mechanisms for the
50 unusually high accumulation capacities reported have yet to be explained.

51 The determination of radionuclide bioaccumulation parameters under controlled laboratory
52 conditions can be key to better understanding the significance of field measurements (Warnau
53 and Bustamante, 2007). Indeed, an experimental radiotracer approach can provide
54 information about contamination pathways or uptake and depuration capacities of exposed
55 fish (e.g. Pan and Wang, 2016; Zhao et al., 2001). Such experiments allow accurately
56 assessing kinetic parameters in living fish, such as assimilation efficiency or uptake and
57 depuration rates that are crucial to understand Cs bioaccumulation patterns and useful for
58 modelling approaches (Doi et al., 2012; Thomann, 1981; Wang et al., 2000).

59 Previous experimental works have clearly demonstrated the importance of the food pathway
60 in the bioaccumulation of Cs by fish (e.g. Mathews and Fisher, 2009; Zhao et al., 2001).
61 Some studies have highlighted that concentration factors in prey and ingestion rate of the
62 predator are the dominant factors influencing the bioaccumulation of Cs in piscivorous fish
63 (Mathews and Fisher, 2009; Zhao et al., 2001). More recently, Pan and Wang (2016) have

64 shown that Cs bioaccumulation is affected by the trophic ecology of fish. However, there is
65 still a lack of knowledge regarding the ability of omnivorous tropical fish to accumulate Cs
66 from their food.

67 In this context, the present work investigated the trophic transfer of ^{134}Cs in two omnivorous
68 tropical fish, the silver moony *Monodactylus argenteus* and the spotted scat *Scatophagus*
69 *argus*. Although both species are omnivorous, their food habits are slightly different: the
70 silver moony feeds on plankton and detritus while the spotted scat feeds on worms,
71 crustaceans, insects and plant matter (Froese and Pauly, 2017). The choice of these species
72 has been driven by the need for more data regarding the radionuclide transfer in species from
73 tropical areas (e.g. Fowler and Fisher, 2005), where previous radioactive contamination
74 events have occurred (e.g. Mittelstaedt et al., 1999) and the assumption that tropical
75 organisms have a greater ability to accumulate radionuclides (e.g. Ke et al., 2000; Metian et
76 al., 2016). In this study, two levels of biological organization were considered; i.e. the whole
77 organism and the different organs and tissues, in order to determine the biokinetic parameters
78 of ^{134}Cs depuration from the fish and to characterize transfer dynamics of this radionuclide to,
79 among, and from the body compartments throughout the depuration phase.

80

81 **2. Materials and Methods**

82 2.1. Acclimation of fish

83 Wild juveniles of silver moony *M. argenteus* and spotted scat *S. argus* from Southeast Asia
84 were purchased from a French fish wholesaler and shipped to the IAEA-Environment
85 Laboratories premises in the Principality of Monaco. Fish were acclimated for 3 months to
86 laboratory conditions (700-L aquarium for juveniles; open circuit: 200 L h⁻¹ in each tank;
87 0.45- μm filtered seawater; salinity: 35 p.s.u.; temperature: $25 \pm 0.3^\circ\text{C}$; pH: 8.1 ± 0.1 ;
88 light/dark: 12h/12h). During the acclimation phase, fish were fed 3 to 4 times per day with

89 adult brine shrimp (*Artemia salina*) at a daily ration of 0.1-0.3g wet weight (wwt) per
90 individual. No mortality was observed during the acclimation period.

91

92 2.2. Experimental procedure

93 2.2.1. Radiolabelling of brine shrimp

94 In order to investigate the trophic transfer of ^{134}Cs in *M. argenteus* and *S. argus*,
95 radiolabelling of brine shrimp was carried out as described by Pouil et al. (2017). Radiotracer
96 of high specific activity in aqueous solution was purchased from Amersham, UK (^{134}Cs , $[t_{1/2}]$
97 = 2.1 years, specific activity: 37-370 MBq mg Cs^{-1}). The experimental seawater was spiked
98 with ^{134}Cs (nominal activity of 5 kBq L^{-1}), no change in pH was detectable after the tracer
99 addition. At the end of the exposure period, average ^{134}Cs activity in the brine shrimp was 9
100 Bq g^{-1} wwt. Briefly, preparation of the radiolabelled brine shrimp was carried out by
101 exposing them for 18 h in aerated 20-L aquaria (approx. 300 g wwt of adult brine shrimp per
102 aquarium). At the end of the exposure period, radiolabelled brine shrimp were stored at -20°C
103 before their use.

104

105 2.2.2. Exposure of tropical fish

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

110 The experiment consisted in a single-feeding exposure with thawed ^{134}Cs -radiolabelled brine
111 shrimp (i.e. 1 to 12 Bq/individual; see Pouil et al., 2016, 2017 for details). Two hours after the
112 single feeding, all fish were whole-body γ -counted alive. After counting, 21 individuals of
113 each species were transferred into two new 20-L aquaria containing clean, flowing seawater

114 (parameters as previously described). The remaining 9 fish of each species were returned into
115 the initial 70-L aquaria. No regurgitation of the ingested radiolabelled brine shrimp was
116 observed.

117 The 21 individuals were γ -counted alive (see Section 2.3) at different time intervals over 45 d
118 to follow the whole-body ^{134}Cs depuration kinetics. The batch of 9 fish were sampled at
119 different times (after 3, 7 and 14 days of depuration, $n=3$) during the depuration phase,
120 anesthetized, dissected in 7 body compartments (digestive tract, gills, kidney, liver, muscles,
121 skeleton and all remaining tissues), and then γ -counted to determine the body distribution of
122 ^{134}Cs .

123

124 2.3. Gamma counting

125 ^{134}Cs activity was counted in living fish and dissected tissues and organs using a high-
126 resolution γ -spectrometer system composed of three Germanium (N- or P-type) detectors
127 (EGNC 33-195-R, Canberra® and Eurysis®) connected to a multi-channel analyzer and a
128 computer equipped with a spectra analysis software (Interwinner® 6). The radioactivity was
129 determined by comparison with standards of known activity and of appropriate geometry
130 (Cresswell et al., 2017). Measurements were corrected for counting efficiency and physical
131 radioactive decay. The counting time was adjusted to obtain a propagated counting error less
132 than 5% (Rodriguez y Baena et al., 2006) and to ensure well-being of counted fish (see Pouil
133 et al., 2017).

134

135 2.5. Data treatment

136 Whole-body depuration kinetics were fitted using nonlinear regression routines with iterative
137 adjustment (Statistica® 7) and statistical methods described by Warnau et al. (1996). Briefly,

138 the depuration kinetics of the ^{134}Cs were best fitted using a two-component exponential
139 model:

$$140 \quad A_t = A_{0s} \cdot e^{-k_{es}t} + A_{0l} \cdot e^{-k_{el}t} \quad (1)$$

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

151 To assess the biomagnification potential of ^{134}Cs following dietary exposure, trophic transfer
152 factors (TTFs) were also calculated for a specific link in the food chain in which a predator
153 consumes metal in prey as follows:

$$154 \quad TTF = \frac{AE \times IR}{k_{el}} \quad (2)$$

155 Where AE is the assimilation efficiency of the ingested metal in the fish, IR is the weight-
156 specific ingestion rate of prey ($\text{g g}^{-1} \text{d}^{-1}$) and k_{el} is the depuration rate constant (d^{-1}) of the
157 radionuclide out of the predator (see Mathews et al., 2008; Zhao et al., 2001). A $TTF > 1$
158 suggests that biomagnification is possible, and $TTF < 1$ suggests that biomagnification is
159 unlikely (Reinfelder et al., 1998). For these TTF calculations we considered a range of
160 ingestion rates (IR) by fish likely to be encountered under natural conditions (0.02 to 0.10 g g^{-1}
161 d^{-1} ; Zhao et al., 2001). Significant differences for remaining activities in the two species
162 were tested using a Student t-test. The data were arcsin-transformed, and tested for

163 homogeneity of variance and normal distribution before the statistical analysis (Zar, 1996).
164 For ^{134}Cs organotropism, significant differences between the two species were tested using
165 Wilcoxon-Mann-Whitney non-parametric test (Zar, 1996). The level of significance was
166 always set at $\alpha = 0.05$.

167

168 **3. Results and discussion**

169 Experimental works have provided evidence that the food pathway can be the main uptake
170 route of radiocesium for different taxa (Metian et al., 2016; Pouil et al., 2015; Zhao et al.,
171 2001). Surprisingly, compared to other radionuclides, limited information is available on the
172 trophic transfer of cesium in tropical areas (Metian et al. 2016), and especially in fish,
173 including species that are consumed by humans. This is why the present study examined the
174 dietary transfer of Cs in tropical fish.

175 The whole-body depuration kinetics of ^{134}Cs following a single-feeding on radiolabelled
176 *Artemia salina* by the juveniles of silver moony *Monodactylus argenteus* and the spotted scat
177 *Scatophagus argus* were best described by a two-component exponential model (see Table 1
178 and Fig. 1). All the model parameters were significantly resolved ($p < 0.01$). ^{134}Cs was
179 efficiently assimilated ($\text{AE} > 50\%$; Fig. 1) by both species of fish, without significant
180 interspecific differences ($p > 0.05$). In both species, ^{134}Cs was slowly eliminated, with long-
181 term biological half-life ($T_{b1/2}$) values of 26 ± 5 d for *S. argus* and 40 ± 11 d for *M. argenteus*.
182 With AEs ranging from 51 to 95% (Table 1; Mathews et al., 2008; Mathews and Fisher, 2008;
183 Pan and Wang, 2016; Zhao et al., 2001) Cs is among the most efficiently assimilated non-
184 essential elements in fish (Pouil et al., 2018) and is possibly due to the predominant presence
185 of this element in the cytosolic fraction of the prey, which is considered as readily
186 bioavailable to higher trophic levels (Wallace and Lopez, 1996; Wallace and Luoma, 2003;
187 Zhang and Wang, 2006).

188 Although the mechanisms underlying the trophic transfer of Cs in fish are unclear, Rowan and
189 Rasmussen (1994) estimated a bioaccumulation of radiocesium generally 2 to 3 times higher
190 in piscivorous fish compared to planktivorous and benthivorous fish. Comparison between the
191 AE estimated in the present study in the omnivorous species tested (trophic level of 3.0;
192 Froese and Pauly, 2017) and AE available for piscivorous fish (Table 1) highlighted that the
193 Rowan and Rasmussen's assumption is generally true, especially for tropical species.
194 Calculated trophic transfer factors (TTFs) are ranging from 0.5 to 2.7 and 0.4 to 2.0 in *M.*
195 *argenteus* and *S. argus*, respectively. These ranges suggest possible biomagnification for both
196 fish when the ingestion rate (IR) is close to the median level (i.e. 0.04-0.05 g g⁻¹ d⁻¹). This
197 finding strongly suggests that biomagnification can occur in natural coastal environments as
198 has been proposed in previous studies (see Table 1).

199 In parallel to kinetic determination, organotropism data provide useful information for
200 assessing ¹³⁴Cs accumulation in aquatic food webs (Reinfelder and Fisher, 1994). During the
201 45-d depuration phase, dissections were performed after 3, 7 and 14 days (Fig. 2). After 2
202 weeks of depuration, ¹³⁴Cs activity was too low to be accurately measured in most of the
203 dissected samples. Figure 2 describes the distribution of ¹³⁴Cs in the 7 body compartments.
204 Statistical analysis revealed no difference between ¹³⁴Cs organotropism in the two species
205 during the depuration period. Furthermore, it is noteworthy that, except for muscles where the
206 proportion of ¹³⁴Cs tends to increase gradually throughout the depuration, the Cs distribution
207 in the other tissues is quite similar from day 3 to day 14 (Fig 2). Similar results have been
208 recently demonstrated for another radionuclide (⁶⁵Zn) in the same fish species (Pouil et al.,
209 2017).

210 Excluding the "remaining tissues" (18-24% of the body-burden), ¹³⁴Cs distribution among the
211 other body compartments was systematically ranked in the following decreasing order (Fig.
212 2): muscles (27-49%) > skeleton (21-26%) > digestive tract (5-17%) > gills (2-9%) >> liver

213 (1-3%) > kidney (<1%). This suggests that ^{134}Cs is strongly retained by the muscles and the
214 skeleton even after 3 days of depuration where more than 50% of the ingested ^{134}Cs is
215 distributed in these compartments. These findings can be related to the analogous behavior of
216 Cs^+ and K^+ (Ke et al., 2000; Smith et al., 2002). Indeed, K^+ is an essential element for fish and
217 is the major cation in intracellular fluid (Tacon, 1987). This element plays a role in the
218 maintenance of the integrity of the bone and is involved in muscle contractions (Lanham-
219 New, 2008; Lee, 2015). Thus, it is not surprising to find rapidly after the feeding a substantial
220 proportion of ^{134}Cs in the muscles and skeleton of juvenile fish, a physiological stage
221 characterized by a high growth rate with an increase in the muscular and bone masses.

222

223 **4. Conclusion**

224 The present study provides an interspecific comparison of the trophic transfer of ^{134}Cs in
225 tropical fish and useful information to understand and model the transfer of ^{134}Cs through
226 tropical aquatic ecosystems, where data regarding the radionuclide transfer are still scarce.
227 Furthermore, our data regarding organotropism show that ^{134}Cs is rapidly distributed in
228 muscles of fish and is thus potentially available for human consumers which can be key in a
229 risk-assessment perspective.

230

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344 **Captions to figures**

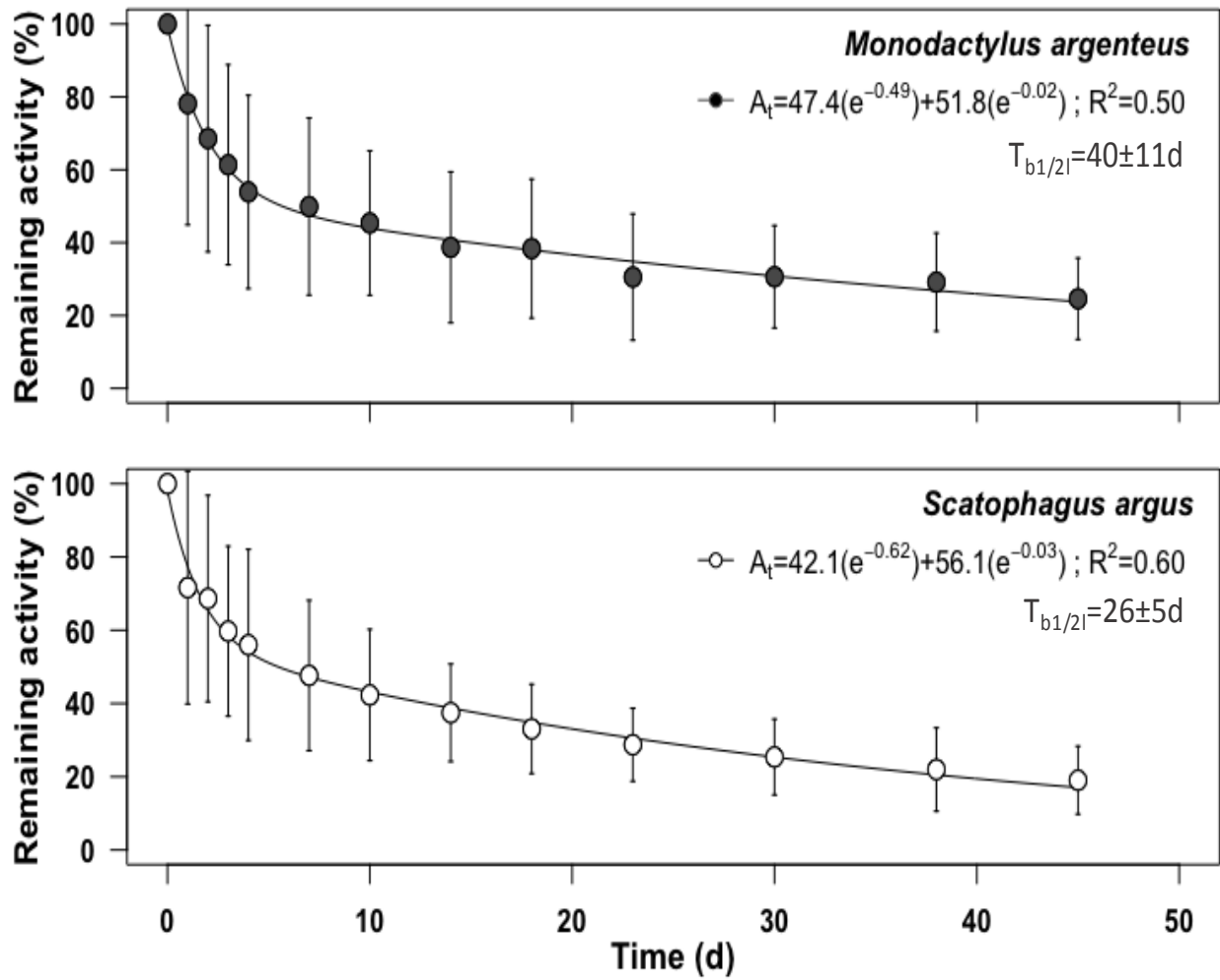
345

346 Figure 1. Whole-body depuration of ^{134}Cs in juvenile silver moony (*Monodactylus argenteus*)
347 and spotted scat (*Scatophagus argus*) after a single-feeding with radiolabelled brine shrimp
348 expressed as percentage of remaining activities (Means \pm SD, n = 21).

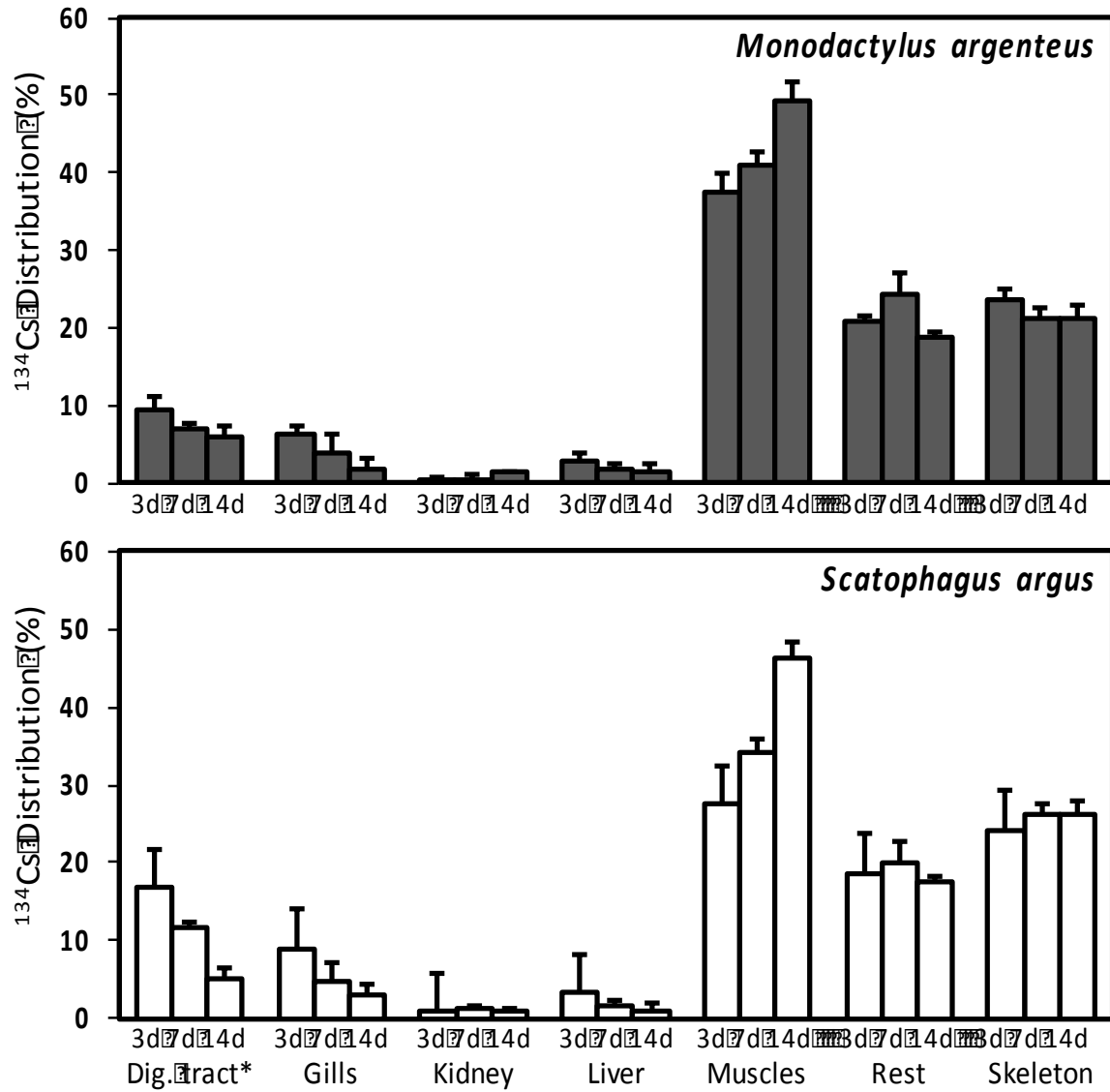
349

350 Figure 2. ^{134}Cs distribution (%) in body compartments of juvenile silver moony
351 (*Monodactylus argenteus*) and juvenile spotted scat (*Scatophagus argus*) after 3, 7 and 14
352 days of depuration following a single-feeding with radiolabelled brine shrimp. All values are
353 expressed as percentage of the whole-body activity (Means + SD, n=3).

354 *Digestive tract.



355
 356 Figure 1



357

358 Figure 2

359 Table 1. Comparison of radiocesium (^{134}Cs & ^{137}Cs) assimilation efficiencies (AEs) and
 360 trophic transfer factors (TTF) in temperate and tropical fish. Trophic level (TL) from Froese
 361 and Pauly (2017) is indicated for each species.

Fish	Prey	TL	AE (%)	TTF	IR ($\text{g g}^{-1} \text{d}^{-1}$)*	References
Temperate species						
<i>Scophthalmus maximus</i>	Fish	4.4 ± 0.0	63 ± 2	0.7 - 3.3**	0.02-0.1	Mathews et al., 2008
<i>Scyliorhinus canicula</i>	Fish	3.8 ± 0.3	73 ± 4	1.8 - 9.0**	0.02-0.1	Mathews et al., 2008
<i>Sparus aurata</i>	Brine shrimp Fish	3.7 ± 0.0	84 ± 4 74 ± 3	0.3 - 1.7** 0.9 - 4.4**	0.02-0.1	Mathews and Fisher, 2008 Mathews et al., 2008
<i>Dicentrarchus labrax</i>	Fish	3.5 ± 0.5	79 ± 3	0.5 - 2.6**	0.02-0.1	Mathews and Fisher, 2008
Tropical species						
<i>Terapon jarbua</i>	Bivalve (mussel) Bivalve (oyster) Bivalve (scallop)	3.9 ± 0.5	74 ± 9 73 ± 8 75 ± 8	1.4 - 4.3	0.05-0.15	Pan and Wang, 2016
<i>Chanos chanos</i>	Brine shrimp	2.4 ± 0.2	37 ± 2 35 ± 2	<0.2**	0.02-0.1	Prihatiningsih et al., 2016
<i>Sebastiscus marmoratus</i>	Bivalve (mussel) Bivalve (oyster) Bivalve (scallop)	3.7 ± 0.5	70 ± 8 71 ± 9 79 ± 4	1.4 - 4.2	0.05-0.15	Pan and Wang, 2016
<i>Lutjanus argentimaculatus</i>	Bivalve Brine shrimp Copepod Fish	3.6 ± 0.5	95 ± 4 78 ± 12 88 ± 2 90 ± 7	0.9 - 4.4	0.02-0.1	Zhao et al., 2001
<i>Scatophagus argus</i>	Brine shrimp	3.0 ± 0.4	56 ± 6	0.4 - 2.0	0.02-0.1	Present study
<i>Monodactylus argenteus</i>	Brine shrimp	3.0 ± 0.3	52 ± 6	0.5 - 2.7	0.02-0.1	Present study
<i>Siganus fuscescens</i>	Bivalve (mussel) Bivalve (oyster) Green alga Red alga	2.0 ± 0.1	55 ± 5 53 ± 13 51 ± 6 55 ± 12	1.0 - 3.1	0.05-0.15	Pan and Wang, 2016

362 *IR = Ingestion Rate

363 **Calculated from the kinetic data (average values of each parameter) available in the

364 corresponding reference